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**UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY**

HORIZON ORPHAN LLC, HORIZON THERAPEUTICS USA, INC., and THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, Plaintiffs, v. TEVA PHARMACEUTICALS, INC., Defendant.	**Civil Action No. __________________** **COMPLAINT FOR PATENT INFRINGEMENT** **(Filed Electronically)**

Plaintiffs Horizon Orphan LLC ("Horizon Orphan"), Horizon Therapeutics USA, Inc. ("Horizon USA") (together, "Horizon"), and The Regents of the University of California ("UC") (collectively, "Plaintiffs"), for their Complaint against Teva Pharmaceuticals, Inc. ("Teva" or "Defendant"), hereby allege as follows:

THE PARTIES

1. Horizon Orphan is a corporation organized and existing under the laws of the State of Delaware, having its principal place of business at 1 Horizon Way, Deerfield, Illinois 60015.

2. Horizon USA is a corporation organized and existing under the laws of the State of Delaware, having its principal place of business at 1 Horizon Way, Deerfield, Illinois 60015.

3. UC is a California non-profit constitutional corporation and the governing body of an educational institution, having its principal place of business at 1111 Franklin Street, Oakland, California 94607.

4. Upon information and belief, defendant Teva is a corporation organized and existing under the laws of the state of Delaware, having its principal place of business at 400 Interpace Parkway, Parsippany, New Jersey 07054. Upon information and belief, Teva was incorporated in Delaware on October 23, 2020, under File Number 3960741.

NATURE OF THE ACTION

5. This is a civil action for infringement of United States Patent No. 8,026,284 (“the ’284 patent”), United States Patent No. 9,192,590 (“the ’590 patent”), United States Patent No. 9,198,882 (“the ’882 patent”), United States Patent No. 9,173,851 (“the ’851 patent”), United States Patent No. 9,233,077 (“the ’077 patent”), and United States Patent No. 10,143,665 (“the ’665 patent”) (collectively, “the patents-in-suit”). This action arises under the patent laws of the United States, 35 U.S.C. §§ 100 *et seq.*, as well as the Declaratory Judgment Act, 28 U.S.C. §§ 2201-02.

JURISDICTION AND VENUE

6. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331 and 1338(a) and the Declaratory Judgment Act, 28 U.S.C. §§ 2201-02.

7. This Court may declare the rights and other legal relations of the parties pursuant to 28 U.S.C. §§ 2201-02 because this case is an actual controversy within the Court's jurisdiction.

8. This Court has personal jurisdiction over Teva because, *inter alia*, Teva has committed, aided, abetted, contributed to, and/or participated in the commission of a tortious act of patent infringement that has led to and/or will lead to foreseeable harm and injury to Plaintiffs, including in New Jersey. Upon information and belief, following approval of Teva's Abbreviated New Drug Application ("ANDA") Nos. 215410 and 216771, Teva will make, use, import, sell, and/or offer for sale its proposed generic versions of PROCYSBI® brand products in the United States, including in New Jersey, prior to the expiration of the patents-in-suit. This Court has personal jurisdiction over Teva for the additional reasons set forth below and for other reasons that will be presented to the Court if jurisdiction is challenged.

9. This Court also has personal jurisdiction over Teva by virtue of, *inter alia*, the fact that it has availed itself of the rights and benefits of the laws of New Jersey by engaging in systematic and continuous contacts with New Jersey. Upon information and belief, Teva maintains a physical presence in New Jersey with a manufacturing and research and development facility located in New Jersey, and is registered to do business in New Jersey. Upon information and belief, Teva regularly and continuously transacts business within New Jersey, including by developing, manufacturing, marketing, and selling generic pharmaceutical products. Upon information and belief, Teva derives substantial revenue from the sale of those

products in New Jersey and has availed itself of the privilege of conducting business within New Jersey.

10. Upon information and belief, Teva filed or caused to be filed ANDA No. 215410 with the FDA.

11. Upon information and belief, Teva filed or caused to be filed ANDA No. 216771 with the FDA.

12. Upon information and belief, Teva has continuously placed its products into the stream of commerce for distribution and consumption in the State of New Jersey and throughout the United States, and thus has engaged in the regular conduct of business within this Judicial District.

13. Venue is proper in this Judicial District as to Teva pursuant to 28 U.S.C. §§ 1391(b), (c), and/or (d), and 1400(b) because Teva has committed and will commit further acts of infringement in this Judicial District. Venue is also proper in this Judicial District as to Teva because Teva has a regular and established place of business in New Jersey, and for other reasons that will be presented to the Court if such venue is challenged.

THE PATENTS-IN-SUIT

14. On September 27, 2011, the ’284 patent, titled “Enterically Coated Cystamine, Cysteamine and derivatives thereof,” was duly and legally issued. UC is the owner of the ’284 patent. A copy of the ’284 patent is attached as Exhibit A.

15. Horizon is an exclusive licensee of the ’284 patent.

16. On November 24, 2015, the ’590 patent, titled “Enterically Coated Cysteamine, Cystamine and derivatives thereof,” was duly and legally issued. UC is the owner of the ’590 patent. A copy of the ’590 patent is attached as Exhibit B.

17. Horizon is an exclusive licensee of the ’590 patent.

18. On December 1, 2015, the ’882 patent, titled “Enterically Coated Cysteamine, Cystamine and derivatives thereof,” was duly and legally issued. UC is the owner of the ’882 patent. A copy of the ’882 patent is attached as Exhibit C.

19. Horizon is an exclusive licensee of the ’882 patent.

20. On November 3, 2015, the ’851 patent, titled “Delayed Release Cysteamine Bead Formulation, and Methods of Making and Using Same,” was duly and legally issued. Horizon Orphan and UC are co-owners of the ’851 patent. A copy of the ’851 patent is attached as Exhibit D.

21. Horizon USA is an exclusive licensee of the ’851 patent.

22. On January 12, 2016, the ’077 patent, titled “Delayed Release Cysteamine Bead Formulation, and Methods of Making and Using Same,” was duly and legally issued. Horizon Orphan and UC are co-owners of the ’077 patent. A copy of the ’077 patent is attached as Exhibit E.

23. Horizon USA is an exclusive licensee of the ’077 patent.

24. On December 4, 2018, the ’665 patent, titled “Methods for Storing Cysteamine Formulations and Related Methods of Treatment,” was duly and legally issued. Horizon Orphan is the owner of the ’665 patent. A copy of the ’665 patent is attached as Exhibit F.

25. Horizon USA is the exclusive licensee of the ’665 patent.

ACTS GIVING RISE TO THIS ACTION

26. Horizon USA holds approved New Drug Application (“NDA”) No. 203389 for cysteamine bitartrate delayed release capsules, which it markets and sells in the United States under the brand name “PROCYSBI®.”

27. Horizon USA holds approved NDA No. 213491 for cysteamine bitartrate delayed release oral granules, which it markets and sells in the United States under the brand name "PROCYSBI®."

28. Pursuant to 21 U.S.C. § 355(b)(l), the patents-in-suit are listed in the United States Food and Drug Administration ("FDA") publication titled, "Approved Drug Products with Therapeutic Equivalence Evaluations" (also known as the "Orange Book") as covering PROCYSBI® brand cysteamine bitartrate delayed release capsules or their use.

29. Pursuant to 21 U.S.C. § 355(b)(l), the patents-in-suit are listed in the United States Food and Drug Administration ("FDA") publication titled "Approved Drug Products with Therapeutic Equivalence Evaluations" (also known as the "Orange Book") as covering PROCYSBI® brand cysteamine bitartrate delayed release oral granules or their use.

30. Upon information and belief, Teva submitted ANDA No. 215410 to the FDA under § 505(j) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355(j)) seeking the FDA approval necessary to engage in the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States, including the State of New Jersey, of generic 25 mg and 75 mg cysteamine bitartrate delayed release capsules ("the Teva Generic Capsule Product") prior to the expiration of certain Orange Book-listed patents for the treatment of nephropathic cystinosis.

31. Upon information and belief, Teva submitted ANDA No. 216771 to the FDA under § 505(j) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355(j)) seeking the FDA approval necessary to engage in the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States, including the State of New Jersey, of generic 75 mg and 300 mg cysteamine bitartrate delayed release oral granules in packets ("the Teva Generic Oral Granules Product") (together with the Teva Generic Capsule Product, "the Teva Generic

Products") prior to the expiration of certain Orange Book-listed patents for the treatment of nephropathic cystinosis.

32. Plaintiffs commenced this action within 45 days of the date of receipt of Teva's written notification of ANDA No. 215410 and its accompanying § 505(j)(2)(A)(vii)(IV) certification dated February 15, 2022 ("Teva's ANDA No. 215410 Notice Letter").

33. Plaintiffs commenced this action within 45 days of the date of receipt of Teva's written notification of ANDA No. 216771 and its accompanying § 505(j)(2)(A)(vii)(IV) certification dated February 1, 2022 ("Teva's ANDA No. 216771 Notice Letter") (together with Teva's ANDA No. 215410 Notice Letter, "Teva's ANDA Notice Letters").

34. Teva's ANDA Notice Letters contained an Offer of Confidential Access ("OCA") to certain confidential information regarding the Teva Generic Products. Plaintiffs submitted markups of the OCA in an attempt to negotiate the terms for confidential access. As of the filing of this Complaint, however, Plaintiffs have not received any response from Teva.

35. To date, Teva has not provided Plaintiffs with a copy of any portions of ANDA Nos. 215410 or 216771 or any information regarding the Teva Generic Products, beyond the information set forth in Teva's ANDA Notice Letters. The limited information relating to the Teva Generic Products that was provided in Teva's ANDA Notice Letters does not demonstrate that the Teva Generic Products, which Teva has asked the FDA to approve for sale in the U.S., will not fall within the scope of issued claims of the patents-in-suit.

COUNT I – INFRINGEMENT OF THE '284 PATENT BY TEVA'S ANDA NO. 215410

36. Plaintiffs re-allege paragraphs 1-35 as if fully set forth herein.

37. Upon information and belief, ANDA No. 215410 specifically seeks FDA approval to market a generic version of PROCYSBI® brand 25 mg and 75 mg cysteamine bitartrate delayed release capsules prior to the expiration of the '284 patent.

38. Upon information and belief, ANDA No. 215410 includes a Paragraph IV Certification that the claims of the ’284 patent are invalid and/or would not be infringed by the commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Capsule Product.

39. Teva’s submission to the FDA of ANDA No. 215410, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the ’284 patent under 35 U.S.C. § 271.

40. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Capsule Generic Product—if approved by the FDA, prior to the expiration of the ’284 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of the ’284 patent.

41. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand products into the United States, and/or induces or contributes to such conduct, Teva will infringe the ’284 patent under 35 U.S.C. § 271.

42. Plaintiffs will be irreparably harmed by Teva’s infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

43. Upon information and belief, Teva was aware of the existence of the ’284 patent and was aware that the filing of ANDA No. 215410 and the certification with respect to the ’284 patent constituted an act of infringement of that patent.

COUNT II – INFRINGEMENT OF THE ’590 PATENT BY TEVA’S ANDA NO. 215410

44. Plaintiffs re-allege paragraphs 1-43 as if fully set forth herein.

45. Upon information and belief, ANDA No. 215410 specifically seeks FDA approval to market a generic version of PROCYSBI® brand 25 mg and 75 mg cysteamine bitartrate delayed release capsules prior to the expiration of the ’590 patent.

46. Upon information and belief, ANDA No. 215410 includes a Paragraph IV Certification that the claims of the ’590 patent are invalid and/or would not be infringed by the commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Capsule Product.

47. Teva’s submission to the FDA of ANDA No. 215410, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the ’590 patent under 35 U.S.C. § 271.

48. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Generic Capsule Product—if approved by the FDA, prior to the expiration of the ’590 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of the ’590 patent.

49. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand products into the United States, and/or induces or contributes to such conduct, Teva will infringe the ’590 patent under 35 U.S.C. § 271.

50. Plaintiffs will be irreparably harmed by Teva’s infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

51. Upon information and belief, Teva was aware of the existence of the ’590 patent and was aware that the filing of ANDA No. 215410 and the certification with respect to the ’590 patent constituted an act of infringement of that patent.

**COUNT III – INFRINGEMENT
OF THE ’882 PATENT BY TEVA’S ANDA NO. 215410**

52. Plaintiffs re-allege paragraphs 1-51 as if fully set forth herein.

53. Upon information and belief, ANDA No. 215410 specifically seeks FDA approval to market a generic version of Horizon’s PROCYSBI® brand 25 mg and 75 mg cysteamine bitartrate delayed release capsules prior to the expiration of the ’882 patent.

54. Upon information and belief, ANDA No. 215410 includes a Paragraph IV Certification that the claims of the ’882 patent are invalid and/or would not be infringed by the commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Capsule Product.

55. Teva’s submission to the FDA of ANDA No. 215410, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the ’882 patent under 35 U.S.C. § 271.

56. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Generic Capsule Product—if approved by the FDA, prior to the expiration of the ’882 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of the ’882 patent.

57. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand

products into the United States, and/or induces or contributes to such conduct, Teva will infringe the ’882 patent under 35 U.S.C. § 271.

58. Plaintiffs will be irreparably harmed by Teva’s infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

59. Upon information and belief, Teva was aware of the existence of the ’882 patent and was aware that the filing of ANDA No. 215410 and the certification with respect to the ’882 patent constituted an act of infringement of that patent.

COUNT IV – INFRINGEMENT
OF THE ’851 PATENT BY TEVA’S ANDA NO. 215410

60. Plaintiffs re-allege paragraphs 1-59 as if fully set forth herein.

61. Upon information and belief, ANDA No. 215410 specifically seeks FDA approval to market a generic version of PROCYSBI® brand 25 mg and 75 mg cysteamine bitartrate delayed release capsules prior to the expiration of the ’851 patent.

62. Upon information and belief, ANDA No. 215410 includes a Paragraph IV Certification that the claims of the ’851 patent are invalid and/or would not be infringed by the commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Capsule Product.

63. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Generic Capsule Product—if approved by the FDA, prior to the expiration of the ’851 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of certain additional claims of the ’851 patent.

64. Teva’s submission to the FDA of ANDA No. 215410, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the ’851 patent under 35 U.S.C. § 271.

65. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand products into the United States, and/or induces or contributes to such conduct, Teva will infringe the ’851 patent under 35 U.S.C. § 271.

66. Plaintiffs will be irreparably harmed by Teva’s infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

67. Upon information and belief, Teva was aware of the existence of the ’851 patent and was aware that the filing of ANDA No. 215410 and the certification with respect to the ’851 patent constituted an act of infringement of that patent.

COUNT V – INFRINGEMENT OF THE ’077 PATENT BY TEVA’S ANDA NO. 215410

68. Plaintiffs re-allege paragraphs 1-67 as if fully set forth herein.

69. Upon information and belief, ANDA No. 215410 specifically seeks FDA approval to market a generic version of PROCYSBI® brand 25 mg and 75 mg cysteamine bitartrate delayed release capsules prior to the expiration of the ’077 patent.

70. Upon information and belief, ANDA No. 215410 includes a Paragraph IV Certification that the claims of the ’077 patent are invalid and/or would not be infringed by the commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Capsule Product.

71. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Generic Capsule Product—if approved by the FDA, prior to the expiration of the ’077 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of certain additional claims of the ’077 patent.

72. Teva's submission to the FDA of ANDA No. 215410, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the '077 patent under 35 U.S.C. § 271.

73. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand products into the United States, and/or induces or contributes to such conduct, Teva will infringe the '077 patent under 35 U.S.C. § 271.

74. Plaintiffs will be irreparably harmed by Teva's infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

75. Upon information and belief, Teva was aware of the existence of the '077 patent and was aware that the filing of ANDA No. 215410, and the certification with respect to the '077 patent constituted an act of infringement of that patent.

COUNT VI – INFRINGEMENT
OF THE '665 PATENT BY TEVA'S ANDA NO. 215410

76. Plaintiffs re-allege paragraphs 1-75 as if fully set forth herein.

77. Upon information and belief, ANDA No. 215410 specifically seeks FDA approval to market a generic version of PROCYSBI® brand 25 mg and 75 mg cysteamine bitartrate delayed release capsules prior to the expiration of the '665 patent.

78. Upon information and belief, ANDA No. 215410 includes a Paragraph IV Certification that the claims of the '665 patent are invalid and/or would not be infringed by the commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Capsule Product.

79. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Generic Capsule Product—if

approved by the FDA, prior to the expiration of the ’665 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of certain additional claims of the ’665 patent.

80. Teva’s submission to the FDA of ANDA No. 215410, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the ’665 patent under 35 U.S.C. § 271.

81. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand products into the United States, and/or induces or contributes to such conduct, Teva will infringe the ’665 patent under 35 U.S.C. § 271.

82. Plaintiffs will be irreparably harmed by Teva’s infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

83. Upon information and belief, Teva was aware of the existence of the ’665 patent and was aware that the filing of ANDA No. 215410 and the certification with respect to the ’665 patent constituted an act of infringement of that patent.

COUNT VII – INFRINGEMENT OF THE ’284 PATENT BY TEVA’S ANDA NO. 216771

84. Plaintiffs re-allege paragraphs 1-83 as if fully set forth herein.

85. Upon information and belief, ANDA No. 216771 specifically seeks FDA approval to market a generic version of PROCYSBI® brand 75 mg and 300 mg cysteamine bitartrate delayed release oral granules in packets, prior to the expiration of the ’284 patent.

86. Upon information and belief, ANDA No. 216771 includes a Paragraph IV Certification that the claims of the ’284 patent are invalid and/or would not be infringed by the

commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Oral Granules Product.

87. Teva's submission to the FDA of ANDA No. 216771, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the '284 patent under 35 U.S.C. § 271.

88. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Generic Oral Granules Product—if approved by the FDA, prior to the expiration of the '284 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of the '284 patent.

89. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand products into the United States, and/or induces or contributes to such conduct, Teva will infringe the '284 patent under 35 U.S.C. § 271.

90. Plaintiffs will be irreparably harmed by Teva's infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

91. Upon information and belief, Teva was aware of the existence of the '284 patent and was aware that the filing of ANDA No. 216771 and the certification with respect to the '284 patent constituted an act of infringement of that patent.

COUNT VIII – INFRINGEMENT OF THE '590 PATENT BY TEVA'S ANDA NO. 216771

92. Plaintiffs re-allege paragraphs 1-91 as if fully set forth herein.

93. Upon information and belief, ANDA No. 216771 specifically seeks FDA approval to market a generic version of PROCYSBI® brand 75 mg and 300 mg cysteamine bitartrate delayed release oral granules in packets, prior to the expiration of the ’590 patent.

94. Upon information and belief, ANDA No. 216771 includes a Paragraph IV Certification that the claims of the ’590 patent are invalid and/or would not be infringed by the commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Oral Granules Product.

95. Teva’s submission to the FDA of ANDA No. 216771, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the ’590 patent under 35 U.S.C. § 271.

96. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Generic Oral Granules Product—if approved by the FDA, prior to the expiration of the ’590 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of the ’590 patent.

97. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand products into the United States, and/or induces or contributes to such conduct, Teva will infringe the ’590 patent under 35 U.S.C. § 271.

98. Plaintiffs will be irreparably harmed by Teva’s infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

99. Upon information and belief, Teva was aware of the existence of the ’590 patent and was aware that the filing of ANDA No. 216771 and the certification with respect to the ’590 patent constituted an act of infringement of that patent.

COUNT IX – INFRINGEMENT
OF THE ’882 PATENT BY TEVA’S ANDA NO. 216771

100. Plaintiffs re-allege paragraphs 1-99 as if fully set forth herein.

101. Upon information and belief, ANDA No. 216771 specifically seeks FDA approval to market a generic version of Horizon’s PROCYSBI® brand 75 mg and 300 mg cysteamine bitartrate delayed release oral granules in packets prior to the expiration of the ’882 patent.

102. Upon information and belief, ANDA No. 216771 includes a Paragraph IV Certification that the claims of the ’882 patent are invalid and/or would not be infringed by the commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Oral Granules Product.

103. Teva’s submission to the FDA of ANDA No. 216771, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the ’882 patent under 35 U.S.C. § 271.

104. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Generic Oral Granules Product—if approved by the FDA, prior to the expiration of the ’882 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of the ’882 patent.

105. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand

products into the United States, and/or induces or contributes to such conduct, Teva will infringe the ’882 patent under 35 U.S.C. § 271.

106. Plaintiffs will be irreparably harmed by Teva’s infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

107. Upon information and belief, Teva was aware of the existence of the ’882 patent and was aware that the filing of ANDA No. 216771 and the certification with respect to the ’882 patent constituted an act of infringement of that patent.

COUNT X – INFRINGEMENT
OF THE ’851 PATENT BY TEVA’S ANDA NO. 216771

108. Plaintiffs re-allege paragraphs 1-107 as if fully set forth herein.

109. Upon information and belief, ANDA No. 216771 specifically seeks FDA approval to market a generic version of PROCYSBI® brand 75 mg and 300 mg cysteamine bitartrate delayed release oral granules in packets prior to the expiration of the ’851 patent.

110. Upon information and belief, ANDA No. 216771 includes a Paragraph IV Certification that the claims of the ’851 patent are invalid and/or would not be infringed by the commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Capsule Product.

111. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Generic Oral Granules Product—if approved by the FDA, prior to the expiration of the ’851 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of certain additional claims of the ’851 patent.

112. Teva’s submission to the FDA of ANDA No. 216771, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the ’851 patent under 35 U.S.C. § 271.

113. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand products into the United States, and/or induces or contributes to such conduct, Teva will infringe the ’851 patent under 35 U.S.C. § 271.

114. Plaintiffs will be irreparably harmed by Teva’s infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

115. Upon information and belief, Teva was aware of the existence of the ’851 patent and was aware that the filing of ANDA No. 216771 and the certification with respect to the ’851 patent constituted an act of infringement of that patent.

COUNT XI – INFRINGEMENT
OF THE ’077 PATENT BY TEVA’S ANDA NO. 216771

116. Plaintiffs re-allege paragraphs 1-115 as if fully set forth herein.

117. Upon information and belief, ANDA No. 216771 specifically seeks FDA approval to market a generic version of PROCYSBI® brand 75 mg and 300 mg cysteamine bitartrate delayed release oral granules in packets prior to the expiration of the ’077 patent.

118. Upon information and belief, ANDA No. 216771 includes a Paragraph IV Certification that the claims of the ’077 patent are invalid and/or would not be infringed by the commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Capsule Product.

119. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Generic Oral Granules Product—if approved by the FDA, prior to the expiration of the ’077 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of certain additional claims of the ’077 patent.

120. Teva's submission to the FDA of ANDA No. 216771, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the '077 patent under 35 U.S.C. § 271.

121. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand products into the United States, and/or induces or contributes to such conduct, Teva will infringe the '077 patent under 35 U.S.C. § 271.

122. Plaintiffs will be irreparably harmed by Teva's infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

123. Upon information and belief, Teva was aware of the existence of the '077 patent and was aware that the filing of ANDA No. 216771, and the certification with respect to the '077 patent constituted an act of infringement of that patent.

COUNT XII – INFRINGEMENT OF THE '665 PATENT BY TEVA'S ANDA NO. 216771

124. Plaintiffs re-allege paragraphs 1-123 as if fully set forth herein.

125. Upon information and belief, ANDA No. 216771 specifically seeks FDA approval to market a generic version of PROCYSBI® brand 75 mg and 300 mg cysteamine bitartrate delayed release oral granules in packets prior to the expiration of the '665 patent.

126. Upon information and belief, ANDA No. 216771 includes a Paragraph IV Certification that the claims of the '665 patent are invalid and/or would not be infringed by the commercial manufacture, use, sale, offer for sale, or importation into the United States of the Teva Generic Oral Granules Product.

127. Upon information and belief, the commercial manufacture, use, sale, offer for sale in, and/or importation into the United States of the Teva Generic Oral Granules

Product—if approved by the FDA, prior to the expiration of the ’665 patent, and for use in accordance with its proposed labeling—would infringe and/or induce and/or contribute to the infringement of certain additional claims of the ’665 patent.

128. Teva’s submission to the FDA of ANDA No. 216771, including the § 505(j)(2)(A)(vii)(IV) allegations, constitutes infringement of the ’665 patent under 35 U.S.C. § 271.

129. Plaintiffs are entitled to a declaration that, if Teva commercially manufactures, uses, offers for sale, or sells its proposed generic versions of PROCYSBI® brand products within the United States, imports its proposed generic versions of PROCYSBI® brand products into the United States, and/or induces or contributes to such conduct, Teva will infringe the ’665 patent under 35 U.S.C. § 271.

130. Plaintiffs will be irreparably harmed by Teva’s infringing activities unless those activities are enjoined by this Court. Plaintiffs do not have an adequate remedy at law.

131. Upon information and belief, Teva was aware of the existence of the ’665 patent and was aware that the filing of ANDA No. 216771 and the certification with respect to the ’665 patent constituted an act of infringement of that patent.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs request that:

A. A Judgment be entered declaring that Teva has infringed one or more claims of the ’284 patent by submitting ANDA No. 215410;

B. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 215410 be a date that is not earlier than the expiration date of the ’284 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

C. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release capsules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the '284 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

D. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release capsules identified in this Complaint prior to the expiration of the '284 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement, together with interest;

E. A Judgment be entered declaring that Teva has infringed one or more claims of the '590 patent by submitting ANDA No. 215410;

F. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 215410 be a date that is not earlier than the expiration date of the '590 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

G. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release capsules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the '590 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

H. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release capsules identified in this Complaint prior to the expiration of the ’590 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement together with interest;

I. A Judgment be entered declaring that Teva has infringed one or more claims of the ’882 patent by submitting ANDA No. 215410;

J. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 215410 be a date that is not earlier than the expiration date of the ’882 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

K. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release capsules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the ’882 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

L. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release capsules identified in this Complaint prior to the expiration of the ’882 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement together with interest;

M. A Judgment be entered declaring that Teva has infringed one or more claims of the ’851 patent by submitting ANDA No. 215410;

N. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 215410 be a date that is not earlier than the expiration date of the ’851 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

O. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release capsules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the ’851 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

P. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release capsules identified in this Complaint prior to the expiration of the ’851 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement together with interest;

Q. A Judgment be entered declaring that Teva has infringed one or more claims of the ’077 patent by submitting ANDA No. 215410;

R. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 215410 be a date that is not earlier than the expiration date of the ’077 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

S. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed

release capsules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the ’077 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

T. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release capsules identified in this Complaint prior to the expiration of the ’077 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement together with interest;

U. A Judgment be entered declaring that Teva has infringed one or more claims of the ’665 patent by submitting ANDA No. 215410;

V. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 215410 be a date that is not earlier than the expiration date of the ’665 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

W. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release capsules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the ’665 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

X. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release capsules identified in this Complaint prior to the expiration

of the ’665 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement together with interest;

Y. A Judgment be entered declaring that Teva has infringed one or more claims of the ’284 patent by submitting ANDA No. 216771;

Z. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 216771 be a date that is not earlier than the expiration date of the ’284 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

AA. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release oral granules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the ’284 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

BB. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release oral granules identified in this Complaint prior to the expiration of the ’284 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement, together with interest;

CC. A Judgment be entered declaring that Teva has infringed one or more claims of the ’590 patent by submitting ANDA No. 216771;

DD. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 216771 be a date that is not earlier than the expiration date of the ’590 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

EE. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release oral granules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the ’590 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

FF. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release oral granules identified in this Complaint prior to the expiration of the ’590 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement together with interest;

GG. A Judgment be entered declaring that Teva has infringed one or more claims of the ’882 patent by submitting ANDA No. 216771;

HH. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 216771 be a date that is not earlier than the expiration date of the ’882 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

II. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release oral granules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the ’882 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

JJ. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release oral granules identified in this Complaint prior to the expiration of the ’882 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement together with interest;

KK. A Judgment be entered declaring that Teva has infringed one or more claims of the ’851 patent by submitting ANDA No. 216771;

LL. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 216771 be a date that is not earlier than the expiration date of the ’851 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

MM. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release oral granules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the ’851 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

NN. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release oral granules identified in this Complaint prior to the expiration of the ’851 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement together with interest;

OO. A Judgment be entered declaring that Teva has infringed one or more claims of the ’077 patent by submitting ANDA No. 216771;

PP. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 216771 be a date that is not earlier than the expiration date of the ’077 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

QQ. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release oral granules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the ’077 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

RR. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release oral granules identified in this Complaint prior to the expiration of the ’077 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement together with interest;

SS. A Judgment be entered declaring that Teva has infringed one or more claims of the ’665 patent by submitting ANDA No. 216771;

TT. An Order be issued pursuant to 35 U.S.C. § 271(e)(4)(A) that the effective date of any approval of ANDA No. 216771 be a date that is not earlier than the expiration date of the ’665 patent, or any later expiration of exclusivity to which Plaintiffs are or become entitled;

UU. An Order be issued that Teva, its officers, agents, servants, and employees, and those persons in active concert or participation with them, are preliminarily and permanently enjoined from commercially manufacturing, using, offering for sale, importing, or selling the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed

release oral granules identified in this Complaint, and any other product that infringes or induces or contributes to the infringement of the ’665 patent, prior to its expiration, including any extensions to which Plaintiffs are or become entitled;

VV. If Teva engages in the commercial manufacture, use, offer for sale, sale, and/or importation into the United States of the proposed generic versions of PROCYSBI® brand cysteamine bitartrate delayed release oral granules identified in this Complaint prior to the expiration of the ’665 patent, a Judgment awarding damages to Plaintiffs resulting from such infringement together with interest;

WW. Plaintiffs be awarded attorneys’ fees, costs, and expenses that they incur in litigating this action;

XX. A Judgment be entered that this case is exceptional and that Plaintiffs are entitled to its reasonable attorneys’ fees pursuant to 35 U.S.C. § 285;

YY. Plaintiffs be awarded such other and further relief as this Court deems just and proper.

Dated: March 15, 2022

By: s/ Charles M. Lizza

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CERTIFICATION PURSUANT TO LOCAL CIVIL RULES 11.2 AND 40.1

Pursuant to Local Civil Rules 11.2 and 40.1, I hereby certify that the matter in controversy involves the same plaintiffs and some of the same patents, and that Teva is seeking FDA approval to market generic versions of PROCYSBI®, which was also at issue in the matter captioned *Horizon Orphan LLC, et al. v. Lupin Ltd., et al.*, Civil Action No. 20-10339 (MCA)(LDW)(D.N.J.), which was filed on August 11, 2020. This matter was dismissed by the Hon. Madeline Cox Arleo, U.S.D.J. on October 6, 2021.

I hereby certify that, to the best of my knowledge, the matter in controversy is not the subject of any other action pending in any court or of any pending arbitration or administrative proceeding.

Dated: March 15, 2022

By: s/ Charles M. Lizza

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EXHIBIT A

US008026284B2

(12) United States Patent
Dohil et al.

(10) Patent No.: US 8,026,284 B2
(45) Date of Patent: Sep. 27, 2011

(54) **ENTERICALLY COATED CYSTAMINE, CYSTEAMINE AND DERIVATIVES THEREOF**

(75) Inventors: **Ranjan Dohil**, San Diego, CA (US); **Jerry Schneider**, La Jolla, CA (US)

(73) Assignee: **The Regents of the University of California**, Oakland, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 239 days.

(21) Appl. No.: **11/990,869**

(22) PCT Filed: **Jan. 26, 2007**

(86) PCT No.: **PCT/US2007/002325**

§ 371 (c)(1), (2), (4) Date: **Nov. 13, 2008**

(87) PCT Pub. No.: **WO2007/089670**

PCT Pub. Date: **Aug. 9, 2007**

(65) **Prior Publication Data**

US 2009/0076166 A1 Mar. 19, 2009

Related U.S. Application Data

(60) Provisional application No. 60/762,715, filed on Jan. 27, 2006.

(51) **Int. Cl.**
A01N 33/08 (2006.01)

(52) **U.S. Cl.** **514/665**; 424/474; 424/477

(58) **Field of Classification Search** .... None
See application file for complete search history.

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(Continued)

Primary Examiner — Robert A Wax
Assistant Examiner — Hasan Ahmed
(74) *Attorney, Agent, or Firm* — Joseph R. Baker, Jr.; Gavrilovich Dodd & Lindsey LLP

(57) **ABSTRACT**

The disclosure provides oral cysteamine and cystamine formulations useful for treating cystinosis and neurodegenerative diseases and disorders. The formulations provide controlled release compositions that improve quality of life and reduced side-effects.

27 Claims, 4 Drawing Sheets

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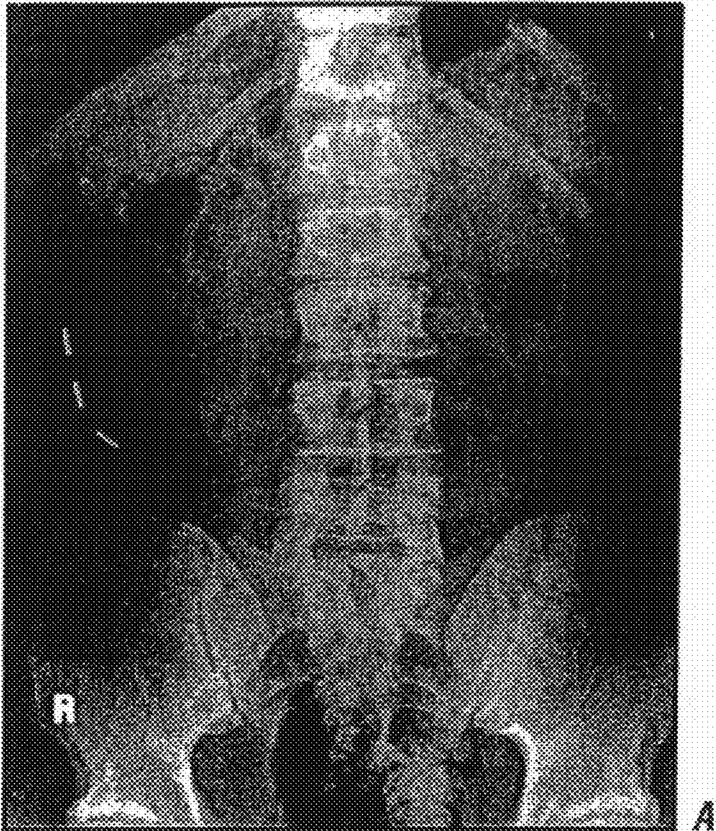

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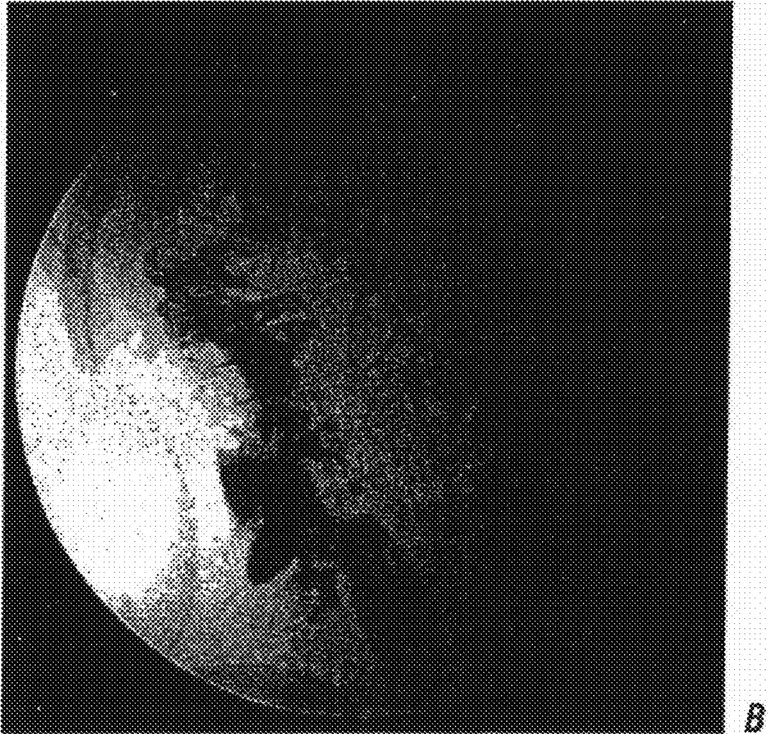

B

FIG. 1

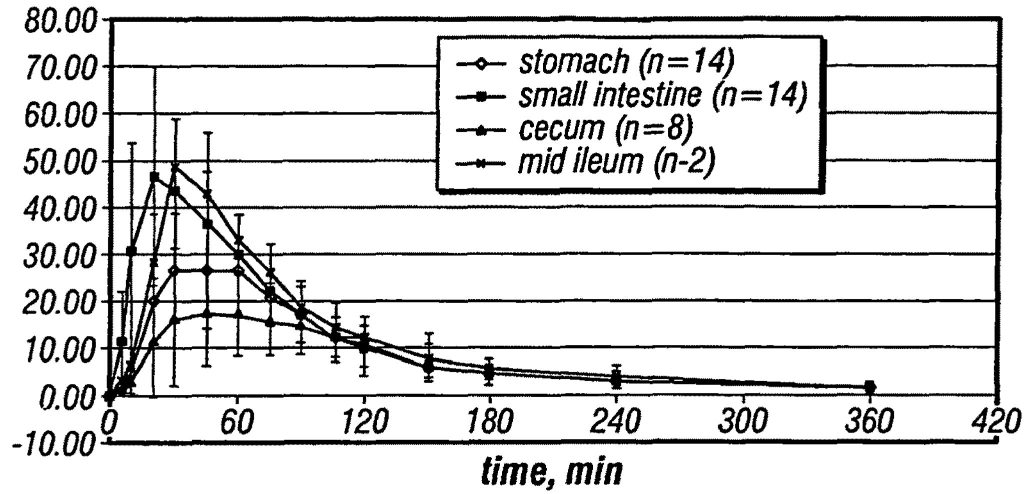

FIG. 2

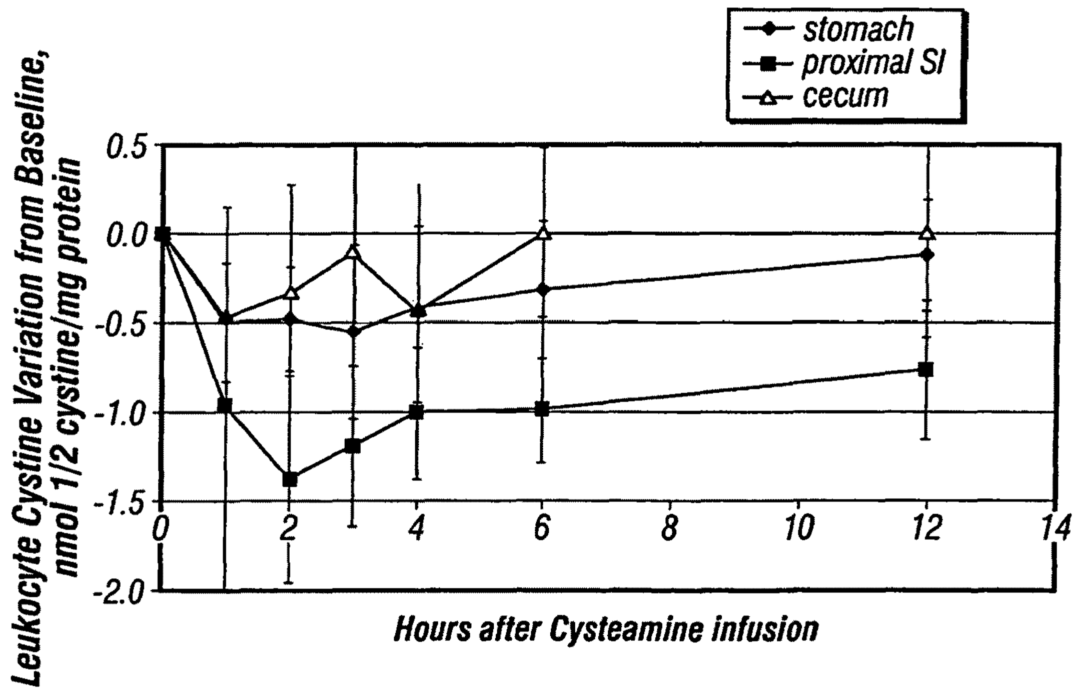

FIG. 3

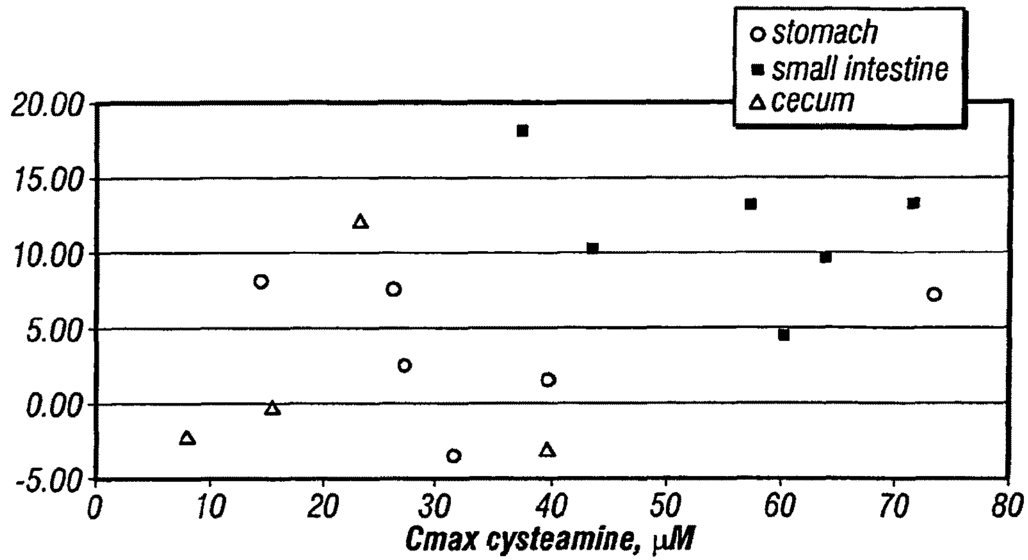

FIG. 4

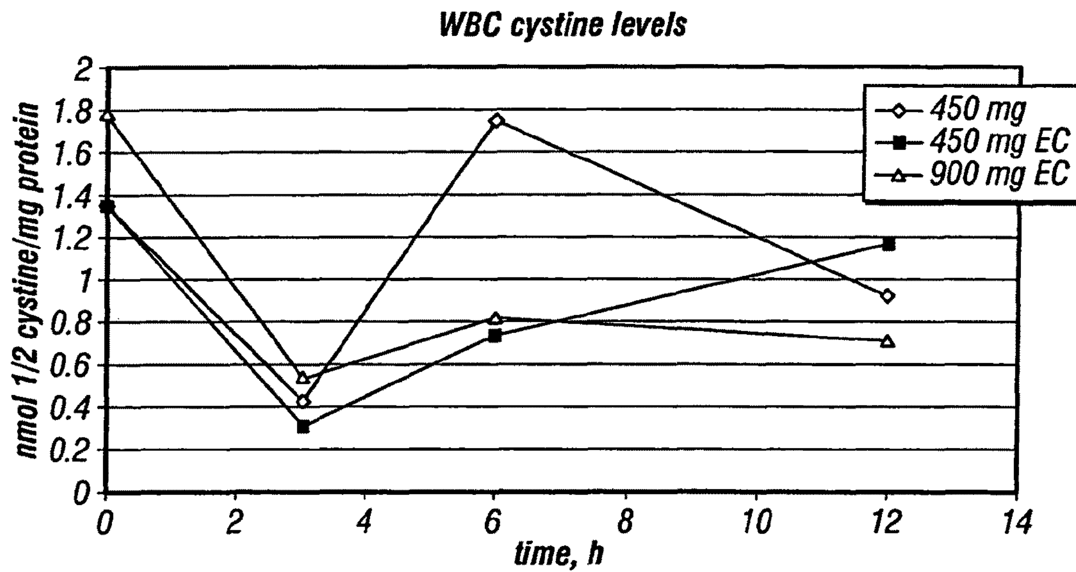

FIG. 5

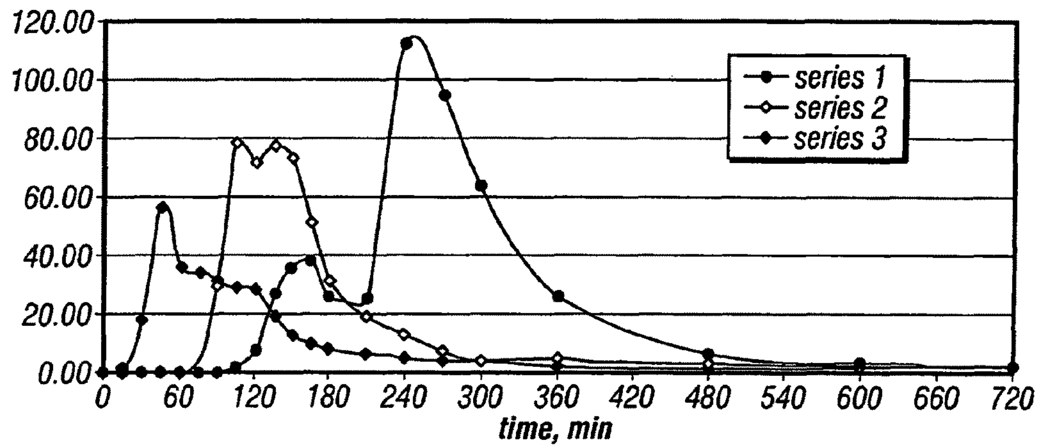

FIG. 6

ENTERICALLY COATED CYSTAMINE, CYSTEAMINE AND DERIVATIVES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is filed under 35 U.S.C. §371 and claims priority to International Application Serial No. PCT/US2007/002325, filed Jan. 26, 2007, which claims priority under 35 U.S.C. §119 to U.S. Provisional Application Ser. No. 60/762,715, filed Jan. 27, 2006, the disclosure of which is incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to methods, compositions and treatments for metabolic conditions and free radical damage. More specifically, the invention relates to methods and composition useful for treating Cystinosis and neurodegenerative diseases such as Huntington's, Alzheimer's and Parkinson's disease, as free radical and radioprotectants, and as heptoprotectant agents.

BACKGROUND

Cystinosis is a rare, autosomal recessive disease caused by intra-lysosomal accumulation of the amino acid cystine within various tissues, including the spleen, liver, lymph nodes, kidney, bone marrow, and eyes. Nephropathic cystinosis is associated with kidney failure that necessitates kidney transplantation. To date, the only specific treatment for nephropathic cystinosis is the sulfhydryl agent, cysteamine. Cysteamine has been shown to lower intracellular cystine levels, thereby reducing the rate of progression of kidney failure in children.

Cysteamine, through a mechanism of increased gastrin and gastric acid production, is ulcerogenic. When administered orally to children with cystinosis, cysteamine has also been shown to cause a 3-fold increase in gastric acid production and a 50% rise of serum gastrin levels. As a consequence, subjects that use cysteamine suffer gastrointestinal (GI) symptoms and are often unable to take cysteamine regularly or at full dose.

To achieve sustained reduction of leukocyte cystine levels, patients are normally required to take oral cysteamine every 6 hours, which invariably means having to awaken from sleep. However, when a single dose of cysteamine was administered intravenously the leukocyte cystine level remained suppressed for more than 24 hours, possibly because plasma cysteamine concentrations were higher and achieved more rapidly than when the drug is administered orally. Regular intravenous administration of cysteamine would not be practical. Accordingly, there is a need for formulations and delivery methods that would result in higher plasma, and thus intracellular, concentration as well as decrease the number of daily doses and therefore improve the quality of life for patients.

SUMMARY

The invention provides a composition comprising an enterically coated cystamine or cystamine derivative.

The invention also provides a composition comprising an enterically coated cysteamine or cysteamine derivative.

The invention further provides a composition comprising a coated cystinosis therapeutic agent that has increased uptake in the small intestine compared to a non-coated cystinosis therapeutic agent when administered orally. In one aspect, the coated cystinosis therapeutic agent comprises a cysteamine or cysteamine derivative.

The invention also provides a method of treating a subject with cystinosis, comprising administering to the subject a composition of the invention.

The invention also contemplates a method of treating a subject with a neurodegenerative disease or disorder comprising administering to the subject a composition of the invention comprising an enterically coated cystamine or cystamine derivative.

The invention provides a pharmaceutical formulation comprising a composition of the invention further including various pharmaceutically acceptable agents (e.g., flavorants, binders and the like) in a pharmaceutically acceptable carrier.

The invention provides a method of treating cystinosis or a neurodegenerative disease or disorder comprising administering a composition of the invention and a second therapeutic agent.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows enterocolonic tube. (A) Is an abdominal X-ray film showing the radiopaque weighted tip of the tube entering the ascending colon. (B) Is a contrast infused picture. The tube has passed through the small intestine and the tip is confirmed.

FIG. **2** shows mean plasma cysteamine levels taken from patients with cystinosis and control subjects after delivery of drug into various intestinal sites. Error bars are standard error of the mean. In 2 control subjects, most distal point of drug delivery was the mid-ileal region.

FIG. **3** shows the mean change in leukocyte cystine levels, compared with baseline levels, over a 12-hour period following delivery of cysteamine into varying intestinal sites. Negative levels signify increased leukocyte cystine depletion compared with baseline.

FIG. **4** shows a scatterplot of plasma cysteamine C_{max} vs. AOC of WBC Cystine changes from Baseline. Positive value means decrease from baseline. Negative value means increase from baseline. AOC change from baseline was affected by C_{max} for cysteamine ($P<0.001$).

FIG. **5** shows serial leukocyte cystine levels alter drug was given as normal Cystagon® and enteric-coated (EC) cysteamine on alternate days. These serial levels were taken during the inpatient phase of the study. Desired cystine levels are below 1 mmol ½ cystine/mg protein. Higher dose enteric-coated (yellow)) drug resulted in prolonged cystine suppression with 12 hour levels still within desired range.

FIG. **6** shows the blood cysteamine levels following a single 450 mg dose of Cystagon® (series 1), 450 mg EC-cysteamine (series 2) and 900 mg EC-cysteamine (series 3). The C_{max} is higher following EC drug. In addition, the time to C_{max} is longer following EC-drug, suggesting that the drug is released from the capsule within the small intestine rather than the stomach.

DETAILED DESCRIPTION

As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a derivative" includes a plurality of such derivatives and reference to "a subject" includes reference to one or more subjects known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood

to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein.

The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior disclosure.

Cystinosis is a metabolic disease characterized by an abnormal accumulation of the amino acid cystine in various organs of the body such as the kidney, eye, muscle, pancreas, and brain. Different organs are affected at different ages.

There are three clinical forms of cystinosis. Infantile (or nephropathic) cystinosis; late-onset cystinosis; and benign cystinosis. The latter form does not produce kidney damage. Infantile cystinosis is usually diagnosed between 6 and 18 months of age with symptoms of excessive thirst and urination, failure to thrive, rickets, and episodes of dehydration. These findings are caused by a disorder called renal tubulopathy or Fanconi syndrome. As a consequence important nutrients and minerals are lost in the urine. Children with cystinosis also have crystals in their eyes (after one year of age) which may lead to photosensitivity. They also have an increased level of cystine in their white blood cells without adverse effect but allowing the diagnosis to be ascertained. Without specific treatment, children with cystinosis develop end-stage renal failure, i.e., lose their kidney function, usually between 6 and 12 years of age. Without cysteamine treatment subjects can develop complications in other organs due to the continued accumulation of cystine throughout the body. These complications can include muscle wasting, difficulty swallowing, diabetes, and hypothyroidism.

Some symptoms include the inability of the kidneys to concentrate urine and allow important quantities of sodium, potassium, phosphorus, bicarbonate and substances like carnitine to be excreted in the urine. Treatment of symptoms compensates for these urinary losses. Subjects need to drink large quantities of water, because up to 2 to 3 liters of water are lost in the urine every day driving the feeling of being thirsty. In addition, the loss of urinary electrolytes (sodium, potassium, bicarbonate, phosphorus) must be compensated in the subject. It is often necessary to add a salt supplement in the form of sodium chloride. Children also lose bicarbonate and potassium in the urine, which can be compensated for by giving sodium bicarbonate and potassium bicarbonate.

Specific treatments of cystinosis aim to reduce cystine accumulation within the cells. Cystinosis is currently treated with cysteamine (Cystagon®). Cysteamine also improves growth of cystinosis children. Cysteamine is only active in a very short period of time not exceeding 5-6 hours, thus requiring administration of Cystagon® capsules 4 times a day, that is to say about every 6 hours. This treatment is also only effective if continued day after day, indefinitely in order to control the disease. About 1000 children require lifelong treatment to prolong their lives and prevent deterioration of kidney function. However, as mentioned above, cysteamine administration results in increased gastric secretions and is ulcerogenic. In addition, routes and timing of administration provide difficulty for subjects in need of such therapy. Recently, a similar drug called cystamine (the disulfide form of cysteamine) has been studied for neurodegenerative disorders including Huntington's and Parkinson's diseases. Cystamine has similar side-effects and dosing difficulties to that of cysteamine.

Cysteamine is a potent gastric acid-secretagogue that has been used in laboratory animals to induce duodenal ulceration; studies in humans and animals have shown that cysteamine-induced gastric acid hypersecretion is most likely mediated through hypergastrinemia. In previous studies performed in children with cystinosis who suffered regular upper gastrointestinal symptoms, a single oral dose of cysteamine (11-23 mg/kg) was shown to cause hypergastrinemia and a 2-to 3-fold rise in gastric acid-hypersecretion. Symptoms suffered by these individuals included abdominal pain, heartburn, nausea, vomiting, and anorexia. The disclosure demonstrates that cysteamine-induced hypergastrinemia arises, in part, as a local effect on the gastric antral-predominant G-cells in susceptible individuals. The data also suggest that this is also a systemic effect of gastrin release by cysteamine. Depending upon the route of administration, plasma gastrin levels usually peak after intragastric delivery within 30 minutes, whereas the plasma cysteamine levels peak later.

Subjects with cystinosis are required to ingest oral cysteamine (Cystagon®) every 6 hours, day and night. When taken regularly, cysteamine can deplete intracellular cystine by up to 90% (as measured in circulating white blood cells), and this has been shown to reduce the rate of progression to kidney failure/transplantation and also to obviate the need for thyroid replacement therapy. Unfortunately, because of the strict treatment regimen and the associated symptoms, non-adherence with cysteamine therapy remains a problem, particularly among adolescent and young adult patients. By reducing the frequency of required cysteamine dosing, adherence to a therapeutic regimen can be improved. The disclosure demonstrates that delivery of cysteamine to the small intestine reduces gastric distress and ulceration and improves bioavailability of cysteamine in the circulation. Delivery of cysteamine into the small intestine is useful due to improved absorption rate from the SI, greater surface area of the SI, and/or less cysteamine undergoing hepatic first pass elimination when absorbed through the small intestine. This disclosure shows a dramatic decrease in leukocyte cystine within an hour of cysteamine delivery.

In addition, sulfhydryl (SH) compounds such as cysteamine, cystamine, and glutathione are among the most important and active intracellular antioxidants. Cysteamine protects animals against bone marrow and gastrointestinal radiation syndromes. The rationale for the importance of SH compounds is further supported by observations in mitotic cells. These are the most sensitive to radiation injury in terms of cell reproductive death and are noted to have the lowest level of SH compounds. Conversely, S-phase cells, which are the most resistant to radiation injury using the same criteria, have demonstrated the highest levels of inherent SH compounds. In addition, when mitotic cells were treated with cysteamine, they became very resistant to radiation. It has also been noted that cysteamine may directly protect cells against induced mutations. The protection is thought to result from scavenging of free radicals, either directly or via release of protein-bound GSH. An enzyme that liberates cysteamine from coenzyme A has been reported in avian liver and hog kidney. Recently, studies have appeared demonstrating a protective effect of cysteamine against the hepatotoxic agents acetaminophen, bromobenzene, and phalloidine.

Cystamine, in addition, to its role as a radioprotectant, has been found to alleviate tremors and prolong life in mice with the gene mutation for Huntington's disease (HD). The drug may work by increasing the activity of proteins that protect nerve cells, or neurons, from degeneration. Cystamine appears to inactivate an enzyme called transglutaminase and thus results in a reduction of huntingtin protein (Nature Medi-

cine 8, 143-149, 2002). In addition, cystamine was found to increase the levels of certain neuroprotective proteins. However, due to the current methods and formulation of delivery of cystamine, degradation and poor uptake require excessive dosing.

The disclosure is not limited with respect to a specific cysteamine or cystamine salt or ester or derivative; the compositions of the disclosure can contain any cysteamine or cystamine, cysteamine or cystamine derivative, or combination of cysteamine or cystamines. The active agents in the composition, i.e., cysteamine or cystamine, may be administered in the form of a pharmacologically acceptable salt, ester, amide, prodrug or analog or as a combination thereof. Salts, esters, amides, prodrugs and analogs of the active agents may be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry and described, for example, by J. March, "Advanced organic Chemistry: Reactions, Mechanisms and Structure," 4th Ed. (New York: Wiley-Interscience, 1992). For example, basic addition salts are prepared from the neutral drug using conventional means, involving reaction of one or more of the active agent's free hydroxyl groups with a suitable base. Generally, the neutral form of the drug is dissolved in a polar organic solvent such as methanol or ethanol and the base is added thereto. The resulting salt either precipitates or may be brought out of solution by addition of a less polar solvent. Suitable bases for forming basic addition salts include, but are not limited to, inorganic bases such as sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, trimethylamine, or the like. Preparation of esters involves functionalization of hydroxyl groups which may be present within the molecular structure of the drug. The esters are typically acyl-substituted derivatives of free alcohol groups, i.e., moieties which are derived from carboxylic acids of the formula R—COOH where R is alkyl, and typically is lower alkyl. Esters can be reconverted to the free acids, if desired, by using conventional hydrogenolysis or hydrolysis procedures. Preparation of amides and prodrugs can be carried out in an analogous manner. Other derivatives and analogs of the active agents may be prepared using standard techniques known to those skilled in the art of synthetic organic chemistry, or may be deduced by reference to the pertinent literature.

The disclosure provides delivery methods and compositions that overcome the problems associated with cysteamine and cystamine delivery. The methods of compositions of the disclosure provide enteric-coated compositions that result in less frequent dosing (2×/day vs. 4×/day), increased patient compliance and fewer gastrointestinal side effects (e.g., pain, heartburn, acid production, vomiting) and other side effects (e.g., patients smell like rotten eggs —a particular compliance problem as subjects reach puberty). The disclosure provides enteric-coated cysteamine compositions (sulfhydryl/Cystagon®) and cystamine compositions.

The disclosure provides methods for the treatment of cystinosis, the treatment of neurodegenerative disease such as Alzheimer Disease, Huntington's and Parkinson's disease and free radical damage using enterically coated cysteamine and cystamine, respectively.

The disclosure provides composition comprising enterically formulated cysteamine and cystamine derivatives. Examples of cysteamine derivatives include hydrochloride, bitartrate and phosphocysteamine derivatives. Cystamine and cystamine derivatives include sulfated cysteamnine. Enteric coatings prolong release until the cystamine, cystamine derivative, or cysteamine derivative/Cystagon® reaches the intestinal tract, typically the small intestine. Because of the enteric coatings, delivery to the small intestine is improved

thereby improving uptake of active ingredient while reducing gastric side effects. This will result in a reduction in the need for frequent administration that currently is associated with Cystagon® therapy, cystamine and cysteamine therapy.

An "enterically coated" drug or tablet refers to a drug or tablet that is coated with a substance—i.e., with an "enteric coating"—that remains intact in the stomach but dissolves and releases the drug once the small intestine is reached.

As used herein "enteric coating", is a material, a polymer material or materials which encase the medicament core (e.g., cystamine, cysteamine, Cystagon®). Typically, a substantial amount or all of the enteric coating material is dissolved before the medicament or therapeutically active agent is released from the dosage form, so as to achieve delayed dissolution of the medicament core. A suitable pH-sensitive polymer is one which will dissolve in intestinal juices at a higher pH level (pH greater than 4.5), such as within the small intestine and therefore permit release of the pharmacologically active substance in the regions of the small intestine and not in the upper portion of the GI tract, such as the stomach.

The coating material is selected such that the therapeutically active agent will be released when the dosage form reaches the small intestine or a region in which the pH is greater than pH 4.5. The coating may be a pH-sensitive materials, which remain intact in the lower pH environs of the stomach, but which disintegrate or dissolve at the pH commonly found in the small intestine of the patient. For example, the enteric coating material begins to dissolve in an aqueous solution at pH between about 4.5 to about 5.5. For example, pH-sensitive materials will not undergo significant dissolution until the dosage form has emptied from the stomach. The pH of the small intestine gradually increases from about 4.5 to about 6.5 in the duodenal bulb to about 7.2 in the distal portions of the small intestine (ileum). In order to provide predictable dissolution corresponding to the small intestine transit time of about 3 hours (e.g., 2-3 hours) and permit reproducible release therein, the coating should begin to dissolve within the pH range of the duodenum, and continue to dissolve at the pH range within the small intestine. Therefore, the amount of enteric polymer coating should be sufficient to substantially dissolved during the approximate three hour transit time within the small intestine (e.g., the proximal and mid-small intestine).

Enteric coatings have been used for many years to arrest the release of the drug from orally ingestible dosage forms. Depending upon the composition and/or thickness, the enteric coatings are resistant to stomach acid for required periods of time before they begin to disintegrate and permit release of the drug in the lower stomach or upper part of the small intestines. Examples of some enteric coatings are disclosed in U.S. Pat. No. 5,225,202 which is incorporated by reference fully herein. As set forth in U.S. Pat. No. 5,225,202, some examples of coating previously employed are beeswax and glyceryl monostearate; beeswax, shellac and cellulose; and cetyl alcohol, mastic and shellac, as well as shellac and stearic acid (U.S. Pat. No. 2,809,918); polyvinyl acetate and ethyl cellulose (U.S. Pat. No. 3,835,221); and neutral copolymer of polymethacrylic acid esters (Eudragit L30D) (F. W. Goodhart et al., Pharm. Tech., pp. 64-71, April 1984); copolymers of methacrylic acid and methacrylic add methylester (Eudragits), or a neutral copolymer of polymethacrylic add esters containing metallic stearates (Mehta et al., U.S. Pat. Nos. 4,728,512 and 4,794,001). Such coatings comprise mixtures of fats and fatty acids, shellac and shellac derivatives and the cellulose acid phthlates, e.g., those having a free carboxyl content. See, Remington's at page 1590, and Zeitova at al. (U.S. Pat. No. 4,432,966), for descriptions of

suitable enteric coating compositions. Accordingly, increased adsorption in the small intestine due to enteric coatings of cystamine, cysteamine derivatives (including Cystagon®) can result in improvements in cystinosis as well as neurodegenerative diseases including, for example, Huntington's disease.

Generally, the enteric coating comprises a polymeric material that prevents cysteamine or cystamine release in the low pH environment of the stomach but that ionizes at a slightly higher pH, typically a pH of 4 or 5, and thus dissolves sufficiently in the small intestines to gradually release the active agent therein. Accordingly, among the most effective enteric coating materials are polyacids having a pK_a in the range of about 3 to 5. Suitable enteric coating materials include, but are not limited to, polymerized gelatin, shellac, methacrylic acid copolymer type C NF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers, typically formed from methyl acrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters (Eudragit NE, Eudragit RL, Eudragit RS). For example, the enterically coating can comprise Eudragit L30D, triethylcitrate, and hydroxypropylmethylcellulose (HPMC), Cystagon® (or other cysteamine derivative), wherein the coating comprises 10 to 13% of the final product.

By "pharmaceutically acceptable carrier" or "pharmaceutically acceptable vehicle" are meant materials that are suitable for oral administration and not biologically, or otherwise, undesirable, i.e., that may be administered to a subject along with an active ingredient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of a pharmaceutical composition in which it is contained.

Similarly, a "pharmaceutically acceptable" salt, ester or other derivative of an active agent comprise, for example, salts, esters or other derivatives which are not biologically or otherwise undesirable.

"Stabilizing agents" refer to compounds that lower the rate at which pharmaceutical degrades, particularly an oral pharmaceutical formulation under environmental conditions of storage.

By the terms "effective amount" or "therapeutically effective amount" of a enteric formulation of cysteamine or cystamine refers to a nontoxic but sufficient amount of the agent to provide the desired therapeutic effect. As will be pointed out below, the exact amount required will vary from subject to subject, depending on the age, weight, and general condition of the subject, the severity of the condition being treated, and the like. An appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

In one aspect of the disclosure there is provided a stabilized pharmaceutical composition for administration of an cysteamine or cystamine, wherein the cysteamine or cystamine is enterically coated.

The cysteamine or cystamine is present in the composition in a therapeutically effective amount; typically, the composition is in unit dosage form. The amount of cysteamine or cystamine administered will, of course, be dependent on the age, weight, and general condition of the subject, the severity of the condition being treated, and the judgment of the prescribing physician. Suitable therapeutic amounts will be known to those skilled in the art and/or are described in the pertinent reference texts and literature. In one aspect, the dose is administered twice per day at about 0.5-1.0 g/m^2 (e.g., 0.7-0.8 g/m^2) body surface area. Current non-enterically coated doses are about 1.35 g/m2 body surface area and are administered 4-5 times per day.

Theentericaly coated cysteamine or cystamine can comprise various excipients, as is well known in the pharmaceutical art, provided such excipients do not exhibit a destabilizing effect on any components in the composition. Thus, excipients such as binders, bulking agents, diluents, disintegrants, lubricants, fillers, carriers, and the like can be combined with the cysteamine or cystamine. For solid compositions, diluents are typically necessary to increase the bulk of a tablet so that a practical size is provided for compression. Suitable diluents include dicalcium phosphate, calcium sulfate, lactose, cellulose, kaolin, mannitol, sodium chloride, dry starch and powdered sugar. Binders are used to impart cohesive qualities to a tablet formulation, and thus ensure that a tablet remains intact after compression. Suitable binder materials include, but are not limited to, starch (including corn starch and pregelatinized starch), gelatin, sugars (including sucrose, glucose, dextrose and lactose), polyethylene glycol, waxes, and natural and synthetic gums, e.g., acacia sodium alginate, polyvinylpyrrolidone, cellulosic polymers (including hydroxypropyl cellulose, hydroxypropyl methylcellulose, methyl cellulose, hydroxyethyl cellulose, and the like), and Veegum. Lubricants are used to facilitate tablet manufacture; examples of suitable lubricants include, for example, magnesium stearate, calcium stearate, and stearic acid, and are typically present at no more than approximately 1 weight percent relative to tablet weight. Disintegrants are used to facilitate tablet disintegration or "breakup" after administration, and are generally starches, clays, celluloses, algins, gums or crosslinked polymers. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and the like. If desired, flavoring, coloring and/or sweetening agents may be added as well. Other optional components for incorporation into an oral formulation herein include, but are not limited to, preservatives, suspending agents, thickening agents, and the like. Fillers include, for example, insoluble materials such as silicon dioxide, titanium oxide, alumina, talc, kaolin, powdered cellulose, microcrystalline cellulose, and the like, as well as soluble materials such as mannitol, urea, sucrose, lactose, dextrose, sodium chloride, sorbitol, and the like.

A pharmaceutical composition may also comprise a stabilizing agent such as hydroxypropyl methylcellulose or polyvinylpyrrolidone, as disclosed in U.S. Pat. No. 4,301,146. Other stabilizing agents include, but are not limited to, cellulosic polymers such as hydroxypropyl cellulose, hydroxyethyl cellulose, methyl cellulose, ethyl cellulose, cellulose acetate, cellulose acetate phthalate, cellulose acetate trimellitate, hydroxypropyl methylcellulose phthalate, microcrystalline cellulose and carboxymethylcellulose sodium; and vinyl polymers and copolymers such as polyvinyl acetate, polyvinylacetate phthalate, vinylacetate crotonic acid copolymer, and ethylene-vinyl acetate copolymers. The stabilizing agent is present in an amount effective to provide the desired stabilizing effect; generally, this means that the ratio of cysteamine or cystamine to the stabilizing agent is at least about 1:500 w/w, more commonly about 1:99 w/w.

The tablets are manufactured by first enterically coating the cysteamine or cystamine. A method for forming tablets herein is by direct compression of the powders containing the enterically coated cysteamine or cystamine, optionally in combination with diluents, binders, lubricants, disintegrants, colorants, stabilizers or the like. As an alternative to direct compression, compressed tablets can be prepared using wet-granulation or dry-granulation processes. Tablets may also be molded rather than compressed, starting with a moist material containing a suitable water-soluble lubricant.

In an alternative embodiment, the enterically coated cysteamine or cystamine are granulated and the granulation is compressed into a tablet or filled into a capsule. Capsule materials may be either hard or soft, and are typically sealed, such as with gelatin bands or the like. Tablets and capsules for oral use will generally include one or more commonly used excipients as discussed herein.

For administration of the dosage form, i.e., the tablet or capsule comprising the enterically coated cysteamine or cystamine, a total weight in the range of approximately 100 mg to 1000 mg is used. The dosage form is orally administered to a patient suffering from a condition for which an cysteamine or cystamine would typically be indicated, including, but not limited to, cystinosis and neurodegenerative diseases such as Huntington's, Alzheimer's and Parkinson's disease.

The compositions of the disclosure can be used in combination with other therapies useful for treating cystinosis and neurodegenerative diseases and disorders. For example, indomethacin therapy (Indocid® or Endol®) is an anti-inflammatory used to treat rheumatoid arthritis and lumbago, but it can be used to reduce water and electrolyte urine loss. In children with cystinosis, indomethacin reduces the urine volume and therefore liquid consumption by about 30%, sometimes by half. In most cases this is associated with an appetite improvement. Indomethacin treatment is generally followed for several years.

Other therapies can be combined with the methods and compositions of the disclosure to treat diseases and disorders that are attributed or result from cystinosis. Urinary phosphorus loss, for example, entails rickets, and it may be necessary to give a phosphorus supplement. Carnitine is lost in the urine and blood levels are low. Carnitine allows fat to be used by the muscles to provide energy. Hormone supplementation is sometimes necessary. Sometimes the thyroid gland will not produce enough thyroid hormones. This is given as thyroxin (drops or tablets). Insulin treatment is sometimes necessary if diabetes appears, when the pancreas does not produce enough insulin. These treatments have become rarely necessary in children whom are treated with cysteamine, since the treatment protects the thyroid and the pancreas. Some adolescent boys require a testosterone treatment if puberty is late. Growth hormone therapy may be indicated if growth is not sufficient despite a good hydro electrolytes balance. Accordingly, such therapies can be combined with the enterically coated cysteamine and cystamine compositions and methods of the disclosure.

The effectiveness of a method or composition of the disclosure can be assessed by measuring leukocyte cystine concentrations. Dosage adjustment and therapy can be made by a medical specialist depending upon, for example, the severity of cystenosis and/or the concentration of cystine. Additional therapies including the use of omeprazole (Prilosec®) can reduce these symptoms.

In addition, various prodrugs can be "activated" by use of the enterically coated cysteamine. Prodrugs are pharmacologically inert, they themselves do not work in the body, but once they have been absorbed, the prodrug decomposes. The prodrug approach has been used successfully in a number of therapeutic areas including antibiotics, antihistamines and ulcer treatments. The advantage of using prodrugs is that the active agent is chemically camouflaged and no active agent is released until the drug has passed out of the gut and into the cells of the body. For example, a number of produgs use S-S bonds. Weak reducing agents, such as cysteamine, reduce these bonds and release the drug. Accordingly, the compositions of the disclosure are useful in combination with prodrugs for timed release of the drug. In this aspect, a pro-drug can be administered followed by administration of an enterically coated cysteamine compositions of the invention (at a desired time) to activate the pro-drug.

It is to be understood that while the invention has been described in conjunction with specific embodiments thereof, that the foregoing description as well as the examples which follow are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention

EXAMPLES

Subjects. Children with cystinosis, ≧12 years old, and taking regular cysteamine bitartrate (Cystagon®; Mylan, Morgantown, W. Va.) were recruited to the study (Table I). Adult control patients were recruited locally. Patients with cystinosis had a mean leukocyte cystine level of less than 2.0 nmol half-cystine/mg protein over the past year. Cysteamine therapy was discontinued 2 days before admission, and acid suppressants, antibiotics, nonsteroidal anti-inflammatory drugs, pro-kinetic agents, and antihistamines were discontinued 2 weeks before admission. None of the patients had undergone kidney transplantation. Baseline chemistry, *Helicobacter pylori* serologic study, complete blood count, and urinalysis were performed.

TABLE I

Cystinosis patient data

Patient	Age (yrs.)	Sex	Weight (kg)	Cysteamine dose (mg) *	Serum creatinine (mg/dL)
1	16	Male	61.5	500	1.0
2	14	Male	39.4	406	1.2
3	13	Female	39.1	406	1.5
4	19	Female	38.1	406	1.4
5	13	Female	50.1	500	1.0
6	16	Male	58.7	500	3.1

* Dose of cysteamine base delivered into varying delivery sites

Cysteamine bitartrate delivery. Cysteamine was infused through a silicone rubber nasoenteric tube (Dentsleeve Pty Ltd, Australia), 3 mm in diameter and 4.5 meters long. The tube, specifically made for this study, had a tungsten-weighted tip, and immediately proximal to this was an inflatable balloon (5-mL capacity). Immediately proximal to the balloon was an infusion port (1 mm diameter) through which the drug was delivered. After an overnight fast (except for water), the dose of cysteamine bitartrate (10 mg/kg/dose of base, maximum of 500 mg) was dissolved in 10 mL of water and infused over 1 to 2 minutes. On day 1 of the study, the nasoenteric tube was inserted into the stomach. By day 3 of the study the tube had passed into the proximal small intestine (SI) just distal to the ligament of Treitz (confirmed fluoroscopically). The balloon was then inflated, and peristalsis propelled the tube distally. Tube position within the cecum

was confirmed fluoroscopically on day 5 (day 7 in 4 patients because of slow transit). If the tube had migrated too far, it was retracted into the desired location.

Serum gastrin, cysteamine and leukocyte cystine measurements. After an overnight fast (except for water) blood samples were taken at baseline and at varying intervals after intraluminal delivery of cysteamine. Serum gastrin levels were then measured at 30, 60, 90, and 120 minutes and 3 and 4 hours; cysteamine levels were measured at 0, 5, 10, 20, 30, 45, 60, 75, 90, 105, 120, and 150 minutes and 3, 4, 6, 8, 10, 12, and 16 hours; leukocyte cystine levels were measured at 1, 2, 3, 4, 6, and 12 hours in patients with cystinosis only. Gastrin was measured in picograms/mL with the Diagnostic Products Corporation (Los Angeles, Calif.) gastrin radioimmunoassay-assay kit. Leukocyte cystine levels were measured in nmol half-cystine per mg protein by the Cystine Determination Lab (La Jolla, Calif.).

To measure plasma cysteamine, 100-μL plasma samples were collected in heparinized vacutainers and spun in a centrifuge within 1 hour, and plasma was stored at −18° C. The concentration of cysteamine was measured by use of tandem mass spectroscopy (API 2000 LC/MS/MS; Applied Biosystems, Foster City, Calif.). Cysteamine concentrations were calculated with a calibration curve that was prepared by spiking plasma with buffered cysteamine solutions, and quality control samples were analyzed with each batch.

Statistical analysis. Mixed model restricted maximum likelihood (REML) repeated measures analysis of variance with subjects as a random effect was performed on the absolute leukocyte cystine levels, on the leukocyte cystine level changes from baseline, and on the "area over the curve" (AOC) for leukocyte cystine level changes from baseline after cysteamine administration for the subjects with cystinosis. AOC is computationally analogous to area under the curve, but it is applied when values are predominantly decreasing below baseline values. Large AOC values reflect large decreases, and a negative AOC reflects a net increase in value. Main effects for site of delivery, time after delivery, and the interaction between site and time were tested, except just the site effect was tested for AOCs. In the absence of significant interaction when a main effect was detected, Tukey's honestly significant difference test (HSD) was applied to identify where differences occurred within a 5% family wise error rate. The Tukey HSD procedure controls for overall significance level when performing all pairwise comparisons. An additional analysis was performed with plasma cysteamine C_{max} added to the AOC model.

REML repeated measures analyses of variance with subjects as a random effect were also performed as described above on AUC and the C_{max} over time for plasma cysteamine levels separately for the subjects with cystinosis and control subjects and with both subject groups combined. Differences between means for the 3 sites were tested, plus group and group x site interaction effects for the combined groups. If a site effect was detected, Tukey's HSD was applied to determine which sites differed from each other.

REML repeated measures analyses of variance were also performed as described above on gastrin levels. The analyses were performed on 2 versions of datasets: the full dataset and all data after omitting observations collected at 30 minutes (1 subject was missing a blood sample taken at 30 minutes after small intestinal cysteamine delivery). A 5% significance level was used without adjustment for all statistical testing.

Six patients with cystinosis, (3 male, 3 female) with a mean age of 15.2 years (range 13-19 years) were recruited into the study (Table I). Eight healthy adult control patients (6 male, 2 female) with a mean age of 23.2 years (range 19-28 years) were enrolled. None of the children with cystinosis had undergone kidney transplantation. All control subjects received 500 mg cysteamine base, whereas the mean dose for

subjects with cystinosis was 453 mg (range 406-500 mg). All subjects had normal liver function test results. In all subjects the nasoenteric tube passed successfully from the stomach into the upper SI; however, it did not progress any further in 2 subjects with cystinosis. In 2 of the control subjects the tube only reached the mid-ileum but did, however, progress to the cecum in 8 subjects (4 control subjects, 4 with cystinosis). There were no reported adverse effects with the insertion or removal of the nasoenteric tube (FIG. **1**).

Symptoms. Only 2 patients (1 male, 1 female) with cystinosis reported regular GI symptoms before the study, and these had responded to acid-suppression therapy. The male subject had severe retching and emesis about 15 minutes after receiving intragastric cysteamine but did not have any symptoms when the drug was infused into the proximal small intestine. The female child with cystinosis had mild transient nausea after SI drug delivery only. No other symptoms were reported after any other cysteamine delivery in the children with cystinosis. There were no associated adverse events with tube placement or removal.

Plasma cysteamine. Among the subjects with cystinosis as measured by analysis of variance, the mean plasma cysteamine C_{max} and AUCs (of the concentration-time gradient) differed by site of cysteamine delivery (both P<0.03). Site (†) refers to either patients with cystinosis or control subjects. For the plasma cysteamine AUCs, the means differed between the duodenal and both gastric and cecal sites of delivery (Tukey HSD global P<0.05). Among control subjects, the mean AUC did not differ among delivery sites (P>0.4), but mean C_{max} did (P<0.05). For both cystinosis and control groups the mean C_{max} values differed only between the duodenum and cecum; mean C_{max} values after duodenal versus gastric or gastric versus cecal delivery were not statistically different (Tables II and III).

TABLE II

Mean plasma cysteamine C_{max} levels (γmol/L) and area under curve (AUC) measurements in cystinosis subjects, controls, and combined cystinosis and control subjects, after delivery of cysteamine into the stomach, small intestine, and cecum

	C_{max} Cystinosis	AUC Cystinosis	C_{max} Control	AUC Control	C_{max} Combined	AUC Combined
Stomach	35.5 (20.5)	3006 (1112)	39.5 (16.4)	3613 (1384)	37.8 (17.6)	3353 (1267)
Small Intestine	55.8 (13.0)	4299 (1056)	51.1 (20.7)	3988 (1659)	53.2 (17.4)	4047 (1376)
Cecum	21.9 (13.1)	3002 (909)	23.1 (15.3)	2804 (1323)	22.5 (13.2)	2903 (1056)

The standard deviations are in parenthesis

TABLE III

Comparisons or mean plasma cysteamine C_{max} (γmol/L) and AUC measurements for combined cystinosis subjects and control subjects among delivery sites

	AUC	C_{max}
P value *	<0.01	<0.01
Stomach vs SI	+	+
Stomach vs Cecum	−	−
SI vs Cecum	+	+

+ Significant difference using Tukey's HSD test (α = 0.05)
− No significant difference
* ANOVA test for equality of three delivery sites

When data from the control subjects were combined with cystinosis subject data, there was both a group effect (P<0.05) and a site effect (P<0.01) for AUCs, with a significant differ-

ence between mean AUC levels for the duodenum versus both the stomach and cecum. C_{max} values differed among sites (P<0.01) but not between groups (P>0.4). Group (*)refers to site of intestinal delivery. C_{max} differed between duodenum versus both stomach and cecum (FIG. **2**).

Leukocyte cystine. There were significant differences among the 3 sites of delivery for cystine levels (P<0.04), changes from baseline values (P<0.0001), and AOCs for changes from baseline (P<0.02). A Tukey HSD test, which controls for multiple comparisons, showed that mean leukocyte cystine levels differed between the cecum and stomach sites, but that cecum versus duodenum and stomach versus duodenum produced similar mean values. When the absolute cystine levels or AOCs for changes from baseline levels were evaluated, the significant differences in sites were found between the duodenum and both the stomach and cecum, but not between stomach and cecum (Tukey HSD global P<0.05) (FIG. **3**). Plasma cysteamine C_{max} and AUC contributed a statistical effect on AOC (P<0.001 and <0.02, respectively), even after controlling for delivery site (FIG. **4**).

Blood gastrin. For the full gastrin dataset, there was a significant difference among the means for the different delivery sites (P<0.1), with the cecum resulting in a lower mean from that of the stomach and small intestine. Both group * and site + significant effects were detected after omitting observations from 30 minutes after delivery (P<0.05 and P<0.01, respectively). The 30-minute observations were omitted because of a missing data set. For these observations, mean levels of gastrin after delivery in the cecum were different from those from both the duodenum and stomach, although the latter did not differ from each other. The 1 boy (14 years) who had severe GI symptoms after intragastric, but not enteric or cecal, cysteamine delivery had a rise in baseline gastrin from 70 pg/mL to 121 pg/mL at 30 minutes after gastric cysteamine. Within the control group, more than half of the baseline and post-cysteamine gastrin levels remained undetectable (<25 pg/mL), and none of the control subjects had a significant rise in gastrin after cysteamine delivery into any site.

Patients with cystinosis are required to ingest oral cysteamine (Cystagon®) every 6 hours, day and night. When taken regularly, cysteamine can deplete intracellular cystine by up to 90% (as measured in circulating white blood cells), and this has been shown to reduce the rate of progression to kidney failure/transplantation and also to obviate the need for thyroid replacement therapy. Unfortunately, because of the strict treatment regimen and the associated symptoms, nonadherence with cysteamine therapy remains a problem, particularly among adolescent and young adult patients. Certainly, by reducing the frequency of required cysteamine dosing adherence can be improved. The disclosure shows a strong statistical association between the maximum plasma concentration (C_{max}) of cysteamine and AOC measurements for leukocyte cystine (P<0.001). A higher C_{max} is achieved after delivery of cysteamine into the small intestine than when infused into the stomach or colon; this may be due to improved absorption rate from the SI, greater surface area of the SI, or less cysteamine undergoing hepatic first pass elimination when absorbed rapidly through the small intestine. When data were combined for patients with cystinosis and control subjects, there was a statistical difference between duodenal versus both gastric and colonic delivery for plasma cysteamine C_{max} and AUC levels (both P<0.05). The lack of similar statistical significance for the cystinosis group alone may simply reflect the small number of patients studied. Changes from baseline leukocyte cystine levels were statistically significant for absolute cystine levels and for AOC when cysteamine was infused into the duodenum compared with both stomach and colon. As shown in FIG. **3**, the leukocyte cystine levels remained below pre-delivery levels for up to 12 hours after a single dose of cysteamine into the small intestine. This would suggest that effective absorption of cysteamine through the SI, by causing a higher C_{max} and AUC on the cysteamine concentration-time gradient, could lead to prolonged depletion of leukocyte cystine and possibly less frequent daily dosing. Another explanation would be that by achieving a high enough plasma cysteamine concentration, more drug reaches the lysosome (where cystine accumulates). In the lysosome the cysteamine reacts with cystine forming the mixed disulfide of cysteamine and cysteine. The mixed disulfide exits the lysosome presumably via the lysine carrier. In the cytosol the mixed disulfide can be reduced by its reaction with glutathione. The cysteine released can be used for protein or glutathione synthesis. The cysteamine released from the mixed disulfide reenters the lysosome where it can react with another cystine molecule. Thus 1 molecule of cysteamine may release many molecules of cystine from the lysosome. This study showed a dramatic decrease in leukocyte cystine within an hour of cysteamine delivery. In retrospect, the finding from this study was that the leukocyte cystine levels remained at the 1-hour level for 24 hours, and even at 48 hours after delivery the levels had not returned to the pre-cysteamine level.

Cysteamine is a potent gastric acid-secretagogue that has been used in laboratory animals to induce duodenal ulceration; studies in humans and animals have shown that cysteamine-induced gastric acid hypersecretion is most likely mediated through hypergastrinemia. In previous studies performed in children with cystinosis who suffered regular upper gastrointestinal symptoms, a single oral dose of cysteamine (11-23 mg/kg) was shown to cause hypergastrinemia and a 2-to 3-fold rise in gastric acid-hypersecretion. Symptoms suffered by these individuals included abdominal pain, heartburn, nausea, vomiting, and anorexia. Interestingly, only 2 of 6 subjects with cystinosis (who were known to suffer regular cysteamine-induced GI symptoms) had increased gastrin levels and symptoms, including nausea, retching, and discomfort after intragastric cysteamine. Gastrin levels were only available after small intestinal administration in 1 of the 2 children and the levels remained the same as baseline. Neither child had symptoms after enteric cysteamine delivery. None of the other patients with cystinosis or control subjects had an increase in gastrin levels with cysteamine infused into any site. This would suggest that cysteamine-induced hypergastrinemia may arise as a local effect on the gastric antral-predominant G-cells only in susceptible individuals. In addition, plasma gastrin levels usually peaks after intragastric delivery within 30 minutes, whereas the plasma cysteamine levels peaked later. 8, 10 In 2 previous studies, children with cystinosis were shown to have a significant rise in plasma gastrin levels after receiving intragastric cysteamine; as part of these study's entry criteria all subjects did, however, suffer with regular GI symptoms. Data from this study would suggest that cysteamine does not cause hypergastrinemia, and therefore acid-hypersecretion, in all patients with cystinosis. Thus acid suppression therapy would not be recommended in patients with cystinosis without upper GI symptoms.

The data suggest that direct administration of cysteamine into the jejunum may result in prolonged leukocyte cystine depletion. In a previous study, a child who had a gastrojejunal feeding tube for oral feeding aversion and severe UGI symptoms, responded to intrajejunal cysteamine with a 3-fold rise in serum gastrin as compared with drug administration into the stomach. The leukocyte cystine response was not mea-

sured in this child. Therefore patients with jejunal feeding tubes will have to be further evaluated.

FIGS. **5** and **6** shows results from a patient that remained on the twice daily EC-cysteamine for an extended period of time. Over this period the patient's leukocyte cystine levels have been measured regularly. The dose of twice daily EC-cysteamine is titrated against the patients symptoms and cystine levels. The patient's cystine levels have been 0.4, 1.0, 0.36.

This study provides data that may be used to improve the quality of life for patients with cystinosis The present formulation of Cystagon®) comprises cysteamine in a capsule that will dissolve rapidly on contact with water, most likely within the stomach.

Although a number of embodiments and features have been described above, it will be understood by those skilled in the art that modifications and variations of the described embodiments and features may be made without departing from the teachings of the disclosure or the scope of the invention as defined by the appended claims.

What is claimed is:

1. A method of administering cysteamine or cystamine, or pharmaceutically acceptable salts thereof, to a patient in need thereof comprising administering to said patient a pharmaceutical composition comprising cysteamine or cystamine, or pharmaceutically acceptable salts thereof, twice per day, wherein the composition increases delivery of cysteamine or cystamine, or pharmaceutically acceptable salts thereof, to the small intestine.

2. The method of claim **1**, wherein each dose of cysteamine or cystamine is about 0.5-1.0 g/m^2.

3. The method of claim **2**, wherein each dose of cysteamine or cystamine is about 0.7-0.8 g/m^2.

4. A method of administering cysteamine or cystamine, or pharmaceutically acceptable salts thereof, to a patient in need thereof comprising administering to said patient a pharmaceutical composition comprising an enteric coating that provides increased delivery of cysteamine or cystamine, or pharmaceutically acceptable salts thereof, to the small intestine.

5. The method of claim **4**, wherein the composition comprises a coating selected from the group consisting of polymerized gelatin, shellac, methacrylic acid copolymer type CNF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers formed from methyl acrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters.

6. The method of claim **4**, wherein the enteric coating releases the cysteamine or cystamine derivative when the composition reaches the small intestine or a region of the gastrointestinal tract of a subject in which the pH is greater than pH 4.5.

7. A method of treating a subject suffering from cystinosis, comprising administering to said subject a composition comprising an enterically coated cysteamine or cystamine, or cysteamine or cystamine derivative.

8. A method of treating a subject suffering from a neurodegenerative disease, comprising administering to said subject a composition comprising an enterically coated cysteamine or cystamine, or cysteamine or cystamine derivative.

9. A method of treating a subject in need of a hepatoprotectant agent, comprising administering to said subject a composition comprising an enterically coated cysteamine or cystamine, or cysteamine or cystamine derivative.

10. A method of administering cysteamine or cystamine, or pharmaceutically acceptable salts thereof, to a patient in need thereof comprising administering to said patient a pharmaceutical composition comprising cysteamine or cystamine, or pharmaceutically acceptable salts thereof, wherein the composition increases delivery of cysteamine or cystamine, or pharmaceutically acceptable salts thereof, to the small intestine and wherein the frequency of administering is less than four times daily.

11. A method of administering cysteamine or cystamine, or pharmaceutically acceptable salts thereof, to a patient in need thereof comprising administering to said patient a pharmaceutical composition comprising an enteric coating, wherein said pharmaceutical composition provides an increased time to C_{max} compared to a cysteamine or cystamine, or pharmaceutically acceptable salts thereof, that is not enterically formulated.

12. The method of any one of claims **1** or **10**, wherein the total daily dose of cysteamine or cystamine is about 1.35 $g/m^2/day$.

13. The method of claim **1** or **10**, wherein the composition increases delivery to the proximal or mid-small intestine or both.

14. The method of claim **1** or **10**, wherein the composition increases delivery to one or more of the duodenum, jejunum or mid-ileum.

15. The method of claim **1** or **10**, wherein the composition increases delivery to a region of the gastrointestinal tract of a subject in which the pH is greater than pH 4.5.

16. The method of any one of claims **1** or **10**, wherein the patient is suffering from cystinosis.

17. The method of any one of claims **1** or **10**, wherein the patient is suffering from a neurodegenerative disease.

18. The method of claim **17**, wherein the neurodegenerative disease is Huntington's disease or Parkinson's disease.

19. The method of claim **16**, further comprising treating the patient with a second therapeutic agent.

20. The method of any one of claims **1** or **10**, wherein the patient is suffering from a metabolic disorder.

21. The method of any one of claims **1** or **10**, wherein the patient is suffering from free radical damage.

22. The method of claim **17**, further comprising treating the patient with a second therapeutic agent.

23. The method of claim **18**, further comprising treating the patient with a second therapeutic agent.

24. The method of any of claims **1-3**, or **10**, wherein the pharmaceutical composition comprises an enteric coating.

25. The method of claim **24**, wherein the enteric coating comprises a coating selected from the group consisting of polymerized gelatin, shellac, methacrylic acid copolymer type CNF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers formed from methyl acrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters.

26. The method of claim **24**, wherein gastric acid levels are decreased in the subject as compared to treatment with a non-coated cysteamine.

27. The method of claim **24**, wherein the cysteamine induces a significant reduction in leukocyte cystine levels compared to non-coated cysteamine.

* * * * *

EXHIBIT B

US009192590B2

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

(10) **Patent No.: US 9,192,590 B2**
(45) **Date of Patent: *Nov. 24, 2015**

(54) **ENTERICALLY COATED CYSTEAMINE, CYSTAMINE AND DERIVATIVES THEREOF**

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(*) 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.

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Related U.S. Application Data

(60) Division of application No. 14/555,993, filed on Nov. 28, 2014, which is a continuation of application No. 13/399,900, filed on Feb. 17, 2012, now abandoned, which is a continuation of application No. 13/190,396, filed on Jul. 25, 2011, now Pat. No. 8,129,433, which is a division of application No. 11/990,869, filed as application No. PCT/US2007/002325 on Jan. 26, 2007, now Pat. No. 8,026,284.

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CPC ***A61K 31/145*** (2013.01); ***A61K 9/0053*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

The disclosure provides oral cysteamine and cystamine formulations useful for treating cystinosis and neurodegenerative diseases and disorders. The formulations provide controlled release compositions that improve quality of life and reduced side-effects.

24 Claims, 4 Drawing Sheets

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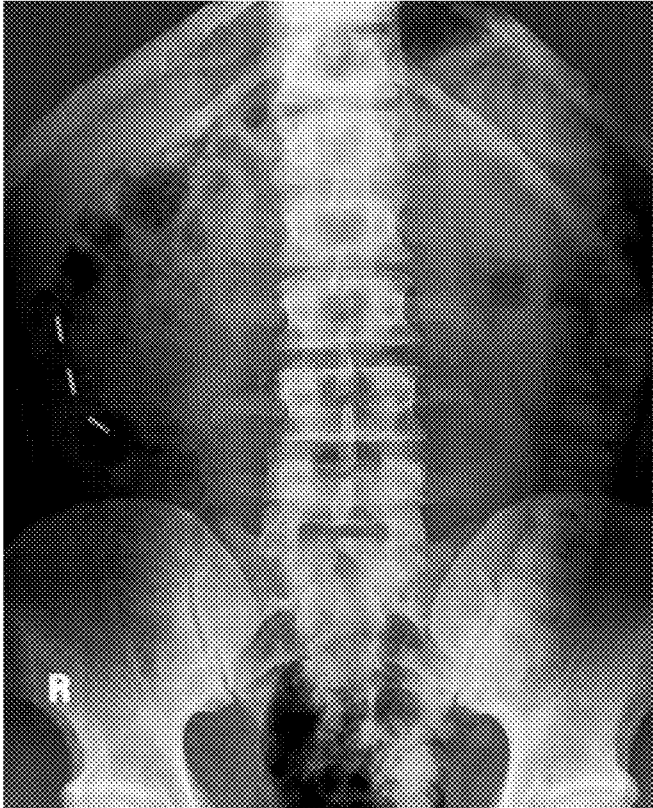

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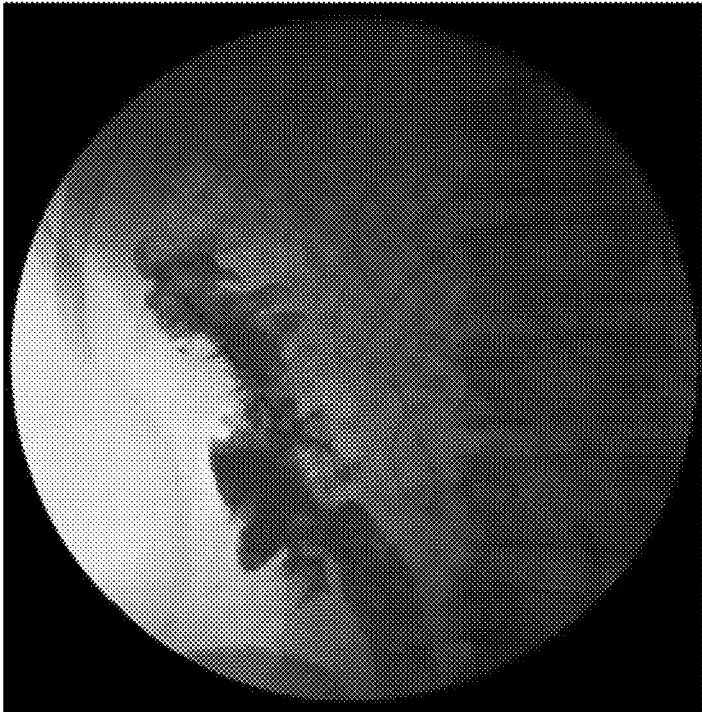

B

FIG. 1

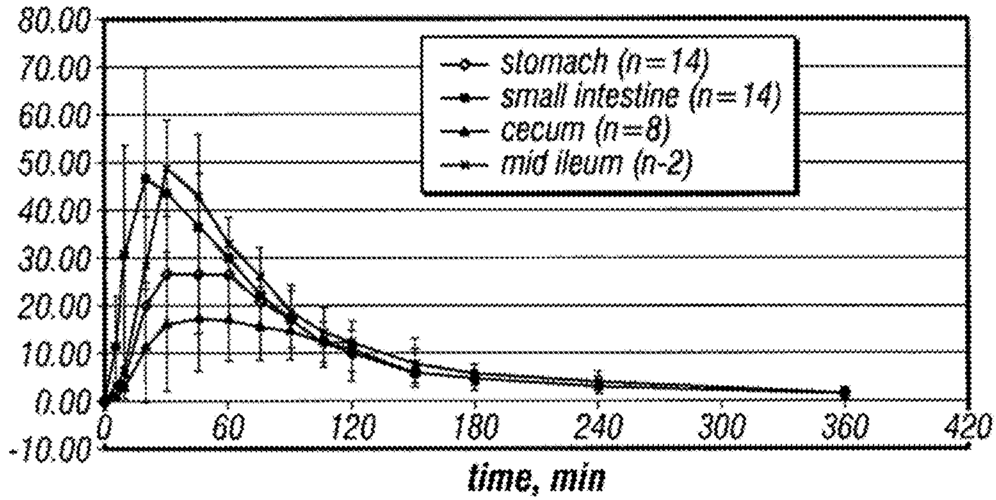

FIG. 2

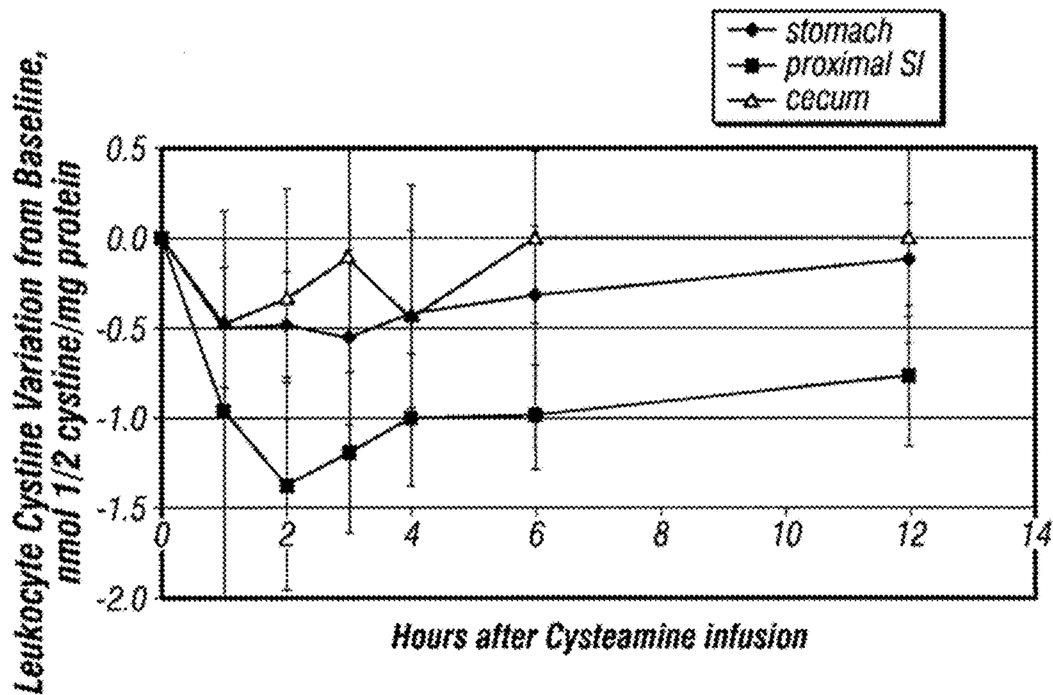

FIG. 3

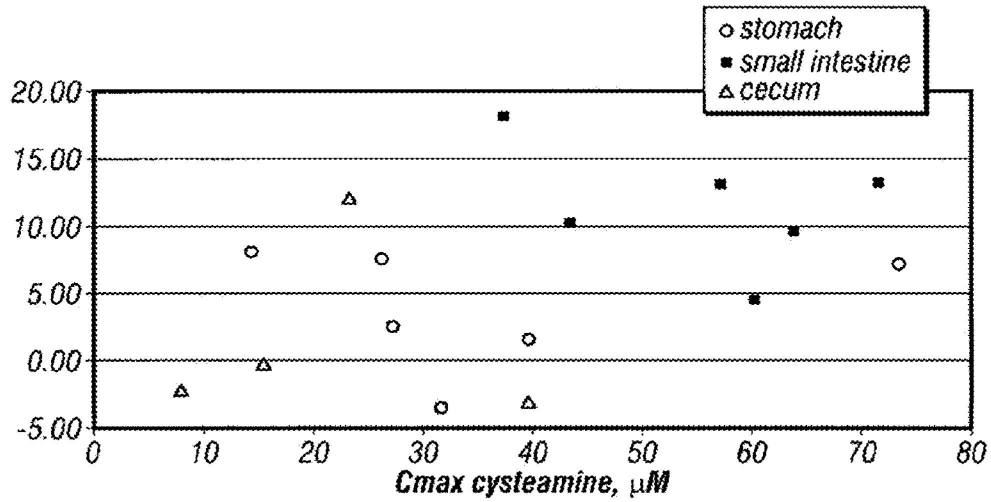

FIG. 4

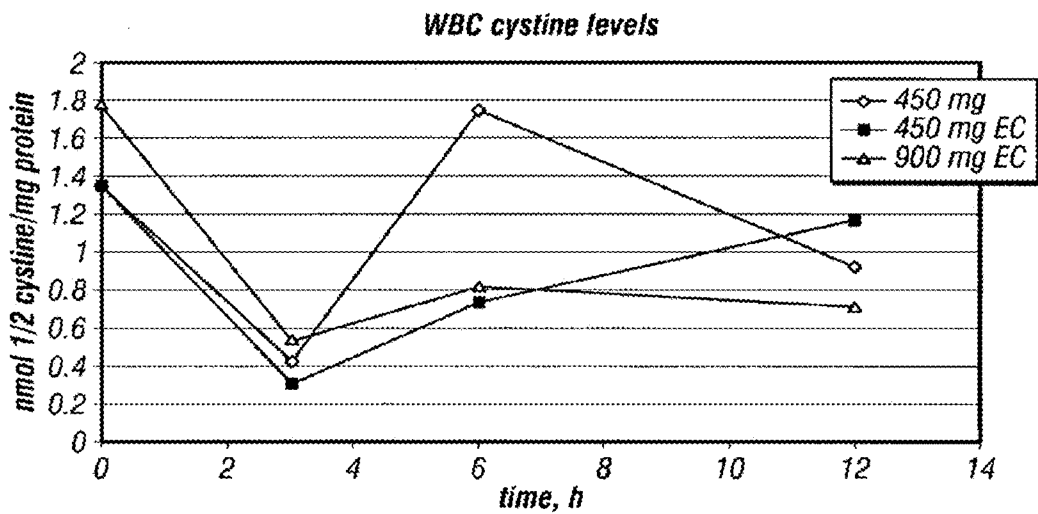

FIG. 5

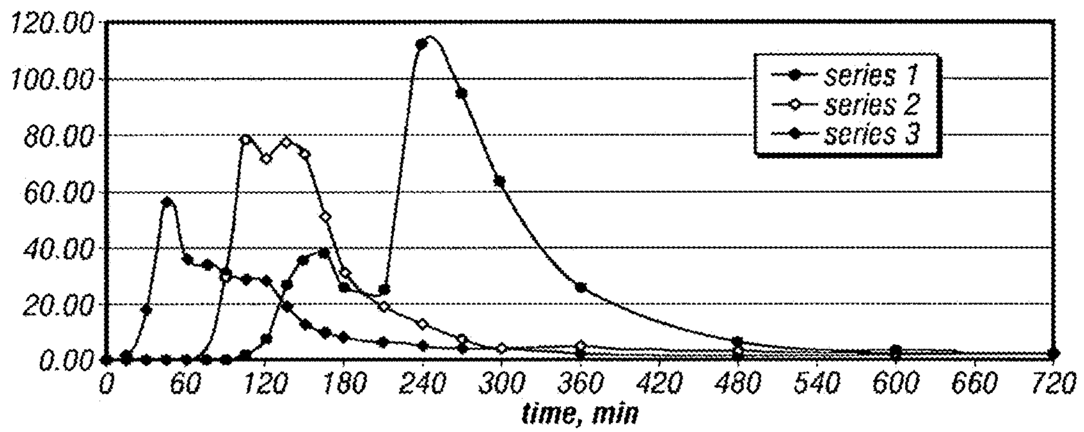

FIG. 6

ENTERICALLY COATED CYSTEAMINE, CYSTAMINE AND DERIVATIVES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 14/555,993, filed Nov. 28, 2014, which is a continuation of U.S. application Ser. No. 13/399,900, filed Feb. 17, 2012, which is a continuation of U.S. application Ser. No. 13/190,396, filed Jul. 25, 2011, which is a divisional of U.S. application Ser. No. 11/990,869, filed Nov. 13, 2008, which is a U.S. National Stage Application filed under 35 U.S.C. §371 and claims priority to International Application No. PCT/US07/02325, filed Jan. 26, 2007, which application claims priority under 35 U.S.C. §119 to U.S. Provisional Application Ser. No. 60/762,714, filed Jan. 27, 2006, the disclosures of which are incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to methods, compositions and treatments for metabolic conditions and free radical damage. More specifically, the invention relates to methods and composition useful for treating Cystinosis and neurodegenerative diseases such as Huntington's, Alzheimer's and Parkinson's disease, as free radical and radioprotectants, and as heptoprotectant agents.

BACKGROUND

Cystinosis is a rare, autosomal recessive disease caused by intra-lysosomal accumulation of the amino acid cystine within various tissues, including the spleen, liver, lymph nodes, kidney, bone marrow, and eyes. Nephropathic cystinosis is associated with kidney failure that necessitates kidney transplantation. To date, the only specific treatment for nephropathic cystinosis is the sulfhydryl agent, cysteamine. Cysteamine has been shown to lower intracellular cystine levels, thereby reducing the rate of progression of kidney failure in children.

Cysteamine, through a mechanism of increased gastrin and gastric acid production, is ulcerogenic. When administered orally to children with cystinosis, cysteamine has also been shown to cause a 3-fold increase in gastric acid production and a 50% rise of serum gastrin levels. As a consequence, subjects that use cysteamine suffer gastrointestinal (GI) symptoms and are often unable to take cysteamine regularly or at full dose.

To achieve sustained reduction of leukocyte cystine levels, patients are normally required to take oral cysteamine every 6 hours, which invariably means having to awaken from sleep. However, when a single dose of cysteamine was administered intravenously the leukocyte cystine level remained suppressed for more than 24 hours, possibly because plasma cysteamine concentrations were higher and achieved more rapidly than when the drug is administered orally. Regular intravenous administration of cysteamine would not be practical. Accordingly, there is a need for formulations and delivery methods that would result in higher plasma, and thus intracellular, concentration as well as decrease the number of daily doses and therefore improve the quality of life for patients.

SUMMARY

The invention provides a composition comprising an enterically coated cystamine or cystamine derivative.

The invention also provides a composition comprising an enterically coated cysteamine or cysteamine derivative.

The invention further provides a composition comprising a coated cystinosis therapeutic agent that has increased uptake in the small intestine compared to a non-coated cystinosis therapeutic agent when administered orally. In one aspect, the coated cystinosis therapeutic agent comprises a cysteamine or cysteamine derivative.

The invention also provides a method of treating a subject with cystinosis, comprising administering to the subject a composition of the invention.

The invention also contemplates a method of treating a subject with a neurodegenerative disease or disorder comprising administering to the subject a composition of the invention comprising an enterically coated cystamine or cystamine derivative.

The invention provides a pharmaceutical formulation comprising a composition of the invention further including various pharmaceutically acceptable agents (e.g., flavorants, binders and the like) in a pharmaceutically acceptable carrier.

The invention provides a method of treating cystinosis or a neurodegenerative disease or disorder comprising administering a composition of the invention and a second therapeutic agent.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows enterocolonic tube. (A) Is an abdominal X-ray film showing the radiopaque weighted tip of the tube entering the ascending colon. (B) Is a contrast infused picture. The tube has passed through the small intestine and the tip is confirmed.

FIG. **2** shows mean plasma cysteamine levels taken from patients with cystinosis and control subjects after delivery of drug into various intestinal sites. Error bars are standard error of the mean. In 2 control subjects, most distal point of drug delivery was the mid-ileal region.

FIG. **3** shows the mean change in leukocyte cystine levels, compared with baseline levels, over a 12-hour period following delivery of cysteamine into varying intestinal sites. Negative levels signify increased leukocyte cystine depletion compared with baseline.

FIG. **4** shows a scatterplot of plasma cysteamine C_{max} vs. AOC of WBC Cystine changes from Baseline. Positive value means decrease from baseline. Negative value means increase from baseline. AOC change from baseline was affected by C_{max} for cysteamine ($P<0.001$).

FIG. **5** shows serial leukocyte cystine levels after drug was given as normal Cystagon® and enteric-coated (EC) cysteamine on alternate days. These serial levels were taken during the inpatient phase of the study. Desired cystine levels are below 1 mmol ½ cystine/mg protein. Higher dose enteric-coated (yellow)) drug resulted in prolonged cystine suppression with 12 hour levels still within desired range.

FIG. **6** shows the blood cysteamine levels following a single 450 mg dose of Cystagon® (series 1), 450 mg EC-cysteamine (series 2) and 900 mg EC-cysteamine (series 3). The C_{max} is higher following EC drug. In addition, the time to C_{max} is longer following EC-drug, suggesting that the drug is released from the capsule within the small intestine rather than the stomach.

DETAILED DESCRIPTION

As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, refer-

ence to "a derivative" includes a plurality of such derivatives and reference to "a subject" includes reference to one or more subjects known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein.

The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior disclosure.

Cystinosis is a metabolic disease characterized by an abnormal accumulation of the amino acid cystine in various organs of the body such as the kidney, eye, muscle, pancreas, and brain. Different organs are affected at different ages.

There are three clinical forms of cystinosis. Infantile (or nephropathic) cystinosis; late-onset cystinosis; and benign cystinosis. The latter form does not produce kidney damage. Infantile cystinosis is usually diagnosed between 6 and 18 months of age with symptoms of excessive thirst and urination, failure to thrive, rickets, and episodes of dehydration. These findings are caused by a disorder called renal tubulopathy or Fanconi syndrome. As a consequence important nutrients and minerals are lost in the urine. Children with cystinosis also have crystals in their eyes (after one year of age) which may lead to photosensitivity. They also have an increased level of cystine in their white blood cells without adverse effect but allowing the diagnosis to be ascertained. Without specific treatment, children with cystinosis develop end-stage renal failure, i.e., lose their kidney function, usually between 6 and 12 years of age. Without cysteamine treatment subjects can develop complications in other organs due to the continued accumulation of cystine throughout the body. These complications can include muscle wasting, difficulty swallowing, diabetes, and hypothyroidism.

Some symptoms include the inability of the kidneys to concentrate urine and allow important quantities of sodium, potassium, phosphorus, bicarbonate and substances like carnitine to be excreted in the urine. Treatment of symptoms compensates for these urinary losses. Subjects need to drink large quantities of water, because up to 2 to 3 liters of water are lost in the urine every day driving the feeling of being thirsty. In addition, the loss of urinary electrolytes (sodium, potassium, bicarbonate, phosphorus) must be compensated in the subject. It is often necessary to add a salt supplement in the form of sodium chloride. Children also lose bicarbonate and potassium in the urine, which can be compensated for by giving sodium bicarbonate and potassium bicarbonate.

Specific treatments of cystinosis aim to reduce cystine accumulation within the cells. Cystinosis is currently treated with cysteamine (Cystagon®). Cysteamine also improves growth of cystinosis children. Cysteamine is only active in a very short period of time not exceeding 5-6 hours, thus requiring administration of Cystagon® capsules 4 times a day, that is to say about every 6 hours. This treatment is also only effective if continued day after day, indefinitely in order to control the disease. About 1000 children require lifelong treatment to prolong their lives and prevent deterioration of kidney function. However, as mentioned above, cysteamine administration results in increased gastric secretions and is ulcerogenic. In addition, routes and timing of administration provide difficulty for subjects in need of such therapy. Recently, a similar drug called cystamine (the disulfide form of cysteamine) has been studied for neurodegenerative disorders including Huntington's and Parkinson's diseases. Cystamine has similar side-effects and dosing difficulties to that of cysteamine.

Cysteamine is a potent gastric acid-secretagogue that has been used in laboratory animals to induce duodenal ulceration; studies in humans and animals have shown that cysteamine-induced gastric acid hypersecretion is most likely mediated through hypergastrinemia. In previous studies performed in children with cystinosis who suffered regular upper gastrointestinal symptoms, a single oral dose of cysteamine (11-23 mg/kg) was shown to cause hypergastrinemia and a 2- to 3-fold rise in gastric acid-hypersecretion. Symptoms suffered by these individuals included abdominal pain, heartburn, nausea, vomiting, and anorexia. The disclosure demonstrates that cysteamine-induced hypergastrinemia arises, in part, as a local effect on the gastric antral-predominant G-cells in susceptible individuals. The data also suggest that this is also a systemic effect of gastrin release by cysteamine. Depending upon the route of administration, plasma gastrin levels usually peak after intragastric delivery within 30 minutes, whereas the plasma cysteamine levels peak later.

Subjects with cystinosis are required to ingest oral cysteamine (Cystagon®) every 6 hours, day and night. When taken regularly, cysteamine can deplete intracellular cystine by up to 90% (as measured in circulating white blood cells), and this has been shown to reduce the rate of progression to kidney failure/transplantation and also to obviate the need for thyroid replacement therapy. Unfortunately, because of the strict treatment regimen and the associated symptoms, non-adherence with cysteamine therapy remains a problem, particularly among adolescent and young adult patients. By reducing the frequency of required cysteamine dosing, adherence to a therapeutic regimen can be improved. The disclosure demonstrates that delivery of cysteamine to the small intestine reduces gastric distress and ulceration and improves bioavailability of cysteamine in the circulation. Delivery of cysteamine into the small intestine is useful due to improved absorption rate from the SI, greater surface area of the SI, and/or less cysteamine undergoing hepatic first pass elimination when absorbed through the small intestine. This disclosure shows a dramatic decrease in leukocyte cystine within an hour of cysteamine delivery.

In addition, sulfhydryl (SH) compounds such as cysteamine, cystamine, and glutathione are among the most important and active intracellular antioxidants. Cysteamine protects animals against bone marrow and gastrointestinal radiation syndromes. The rationale for the importance of SH compounds is further supported by observations in mitotic cells. These are the most sensitive to radiation injury in terms of cell reproductive death and are noted to have the lowest level of SH compounds. Conversely, S-phase cells, which are the most resistant to radiation injury using the same criteria, have demonstrated the highest levels of inherent SH compounds. In addition, when mitotic cells were treated with cysteamine, they became very resistant to radiation. It has also been noted that cysteamine may directly protect cells against induced mutations. The protection is thought to result from scavenging of free radicals, either directly or via release of protein-bound GSH. An enzyme that liberates cysteamine from coenzyme A has been reported in avian liver and hog kidney. Recently, studies have appeared demonstrating a protective effect of cysteamine against the hepatotoxic agents acetaminophen, bromobenzene, and phalloidine.

Cystamine, in addition, to its role as a radioprotectant, has been found to alleviate tremors and prolong life in mice with the gene mutation for Huntington's disease (HD). The drug

may work by increasing the activity of proteins that protect nerve cells, or neurons, from degeneration. Cystamine appears to inactivate an enzyme called transglutaminase and thus results in a reduction of huntingtin protein (Nature Medicine 8, 143-149, 2002). In addition, cystamine was found to increase the levels of certain neuroprotective proteins. However, due to the current methods and formulation of delivery of cystamine, degradation and poor uptake require excessive dosing.

The disclosure is not limited with respect to a specific cysteamine or cystamine salt or ester or derivative; the compositions of the disclosure can contain any cysteamine or cystamine, cysteamine or cystamine derivative, or combination of cysteamine or cystamines. The active agents in the composition, i.e., cysteamine or cystamine, may be administered in the form of a pharmacologically acceptable salt, ester, amide, prodrug or analog or as a combination thereof. Salts, esters, amides, prodrugs and analogs of the active agents may be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry and described, for example, by J. March, "Advanced Organic Chemistry: Reactions, Mechanisms and Structure," 4th Ed. (New York: Wiley-Interscience, 1992). For example, basic addition salts are prepared from the neutral drug using conventional means, involving reaction of one or more of the active agent's free hydroxyl groups with a suitable base. Generally, the neutral form of the drug is dissolved in a polar organic solvent such as methanol or ethanol and the base is added thereto. The resulting salt either precipitates or may be brought out of solution by addition of a less polar solvent. Suitable bases for forming basic addition salts include, but are not limited to, inorganic bases such as sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, trimethylamine, or the like. Preparation of esters involves functionalization of hydroxyl groups which may be present within the molecular structure of the drug. The esters are typically acyl-substituted derivatives of free alcohol groups, i.e., moieties which are derived from carboxylic acids of the formula R—COOH where R is alkyl, and typically is lower alkyl. Esters can be reconverted to the free acids, if desired, by using conventional hydrogenolysis or hydrolysis procedures. Preparation of amides and prodrugs can be carried out in an analogous manner. Other derivatives and analogs of the active agents may be prepared using standard techniques known to those skilled in the art of synthetic organic chemistry, or may be deduced by reference to the pertinent literature.

The disclosure provides delivery methods and compositions that overcome the problems associated with cysteamine and cystamine delivery. The methods of compositions of the disclosure provide enteric-coated compositions that result in less frequent dosing (2×/day vs. 4×/day), increased patient compliance and fewer gastrointestinal side effects (e.g., pain, heartburn, acid production, vomiting) and other side effects (e.g., patients smell like rotten eggs—a particular compliance problem as subjects reach puberty). The disclosure provides enteric-coated cysteamine compositions (sulfhydryl/Cystagon®) and cystamine compositions.

The disclosure provides methods for the treatment of cystinosis, the treatment of neurodegenerative disease such as Alzheimer Disease, Huntington's and Parkinson's disease and free radical damage using enterically coated cysteamine and cystamine, respectively.

The disclosure provides composition comprising enterically formulated cysteamine and cystamine derivatives. Examples of cysteamine derivatives include hydrochloride, bitartrate and phosphocysteamine derivatives. Cystamine and cystamine derivatives include sulfated cystamine. Enteric coatings prolong release until the cystamine, cystamine derivative, or cysteamine derivative/Cystagon® reaches the intestinal tract, typically the small intestine. Because of the enteric coatings, delivery to the small intestine is improved thereby improving uptake of active ingredient while reducing gastric side effects. This will result in a reduction in the need for frequent administration that currently is associated with Cystagon® therapy, cystamine and cysteamine therapy.

An "enterically coated" drug or tablet refers to a drug or tablet that is coated with a substance—i.e., with an "enteric coating"—that remains intact in the stomach but dissolves and releases the drug once the small intestine is reached.

As used herein "enteric coating", is a material, a polymer material or materials which encase the medicament core (e.g., cystamine, cysteamine, Cystagon®). Typically, a substantial amount or all of the enteric coating material is dissolved before the medicament or therapeutically active agent is released from the dosage form, so as to achieve delayed dissolution of the medicament core. A suitable pH-sensitive polymer is one which will dissolve in intestinal juices at a higher pH level (pH greater than 4.5), such as within the small intestine and therefore permit release of the pharmacologically active substance in the regions of the small intestine and not in the upper portion of the GI tract, such as the stomach.

The coating material is selected such that the therapeutically active agent will be released when the dosage form reaches the small intestine or a region in which the pH is greater than pH 4.5. The coating may be a pH-sensitive materials, which remain intact in the lower pH environs of the stomach, but which disintegrate or dissolve at the pH commonly found in the small intestine of the patient. For example, the enteric coating material begins to dissolve in an aqueous solution at pH between about 4.5 to about 5.5. For example, pH-sensitive materials will not undergo significant dissolution until the dosage form has emptied from the stomach. The pH of the small intestine gradually increases from about 4.5 to about 6.5 in the duodenal bulb to about 7.2 in the distal portions of the small intestine (ileum). In order to provide predictable dissolution corresponding to the small intestine transit time of about 3 hours (e.g., 2-3 hours) and permit reproducible release therein, the coating should begin to dissolve within the pH range of the duodenum, and continue to dissolve at the pH range within the small intestine. Therefore, the amount of enteric polymer coating should be sufficient to substantially dissolved during the approximate three hour transit time within the small intestine (e.g., the proximal and mid-small intestine).

Enteric coatings have been used for many years to arrest the release of the drug from orally ingestible dosage forms. Depending upon the composition and/or thickness, the enteric coatings are resistant to stomach acid for required periods of time before they begin to disintegrate and permit release of the drug in the lower stomach or upper part of the small intestines. Examples of some enteric coatings are disclosed in U.S. Pat. No. 5,225,202 which is incorporated by reference fully herein. As set forth in U.S. Pat. No. 5,225,202, some examples of coating previously employed are beeswax and glyceryl monostearate; beeswax, shellac and cellulose; and cetyl alcohol, mastic and shellac, as well as shellac and stearic acid (U.S. Pat. No. 2,809,918); polyvinyl acetate and ethyl cellulose (U.S. Pat. No. 3,835,221); and neutral copolymer of polymethacrylic acid esters (Eudragit L30D) (F. W. Goodhart et al., Pharm. Tech., pp. 64-71, April 1984); copoly-

mers of methacrylic acid and methacrylic acid methylester (Eudragits), or a neutral copolymer of polymethacrylic acid esters containing metallic stearates (Mehta et al., U.S. Pat. Nos. 4,728,512 and 4,794,001). Such coatings comprise mixtures of fats and fatty acids, shellac and shellac derivatives and the cellulose acid phthlates, e.g., those having a free carboxyl content. See, Remington's at page 1590, and Zeitova et al. (U.S. Pat. No. 4,432,966), for descriptions of suitable enteric coating compositions. Accordingly, increased adsorption in the small intestine due to enteric coatings of cystamine, cysteamine derivatives (including Cystagon®) can result in improvements in cystinosis as well as neurodegenerative diseases including, for example, Huntington's disease.

Generally, the enteric coating comprises a polymeric material that prevents cysteamine or cystamine release in the low pH environment of the stomach but that ionizes at a slightly higher pH, typically a pH of 4 or 5, and thus dissolves sufficiently in the small intestines to gradually release the active agent therein. Accordingly, among the most effective enteric coating materials are polyacids having a pK_a in the range of about 3 to 5. Suitable enteric coating materials include, but are not limited to, polymerized gelatin, shellac, methacrylic acid copolymer type C NF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers, typically formed from methyl acrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters (Eudragit NE, Eudragit RL, Eudragit RS). For example, the enterically coating can comprise Eudragit L30D, triethylcitrate, and hydroxypropylmethylcellulose (HPMC), Cystagon® (or other cysteamine derivative), wherein the coating comprises 10 to 13% of the final product.

By "pharmaceutically acceptable carrier" or "pharmaceutically acceptable vehicle" are meant materials that are suitable for oral administration and not biologically, or otherwise, undesirable, i.e., that may be administered to a subject along with an active ingredient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of a pharmaceutical composition in which it is contained.

Similarly, a "pharmaceutically acceptable" salt, ester or other derivative of an active agent comprise, for example, salts, esters or other derivatives which are not biologically or otherwise undesirable.

"Stabilizing agents" refer to compounds that lower the rate at which pharmaceutical degrades, particularly an oral pharmaceutical formulation under environmental conditions of storage.

By the terms "effective amount" or "therapeutically effective amount" of a enteric formulation of cysteamine or cystamine refers to a nontoxic but sufficient amount of the agent to provide the desired therapeutic effect. As will be pointed out below, the exact amount required will vary from subject to subject, depending on the age, weight, and general condition of the subject, the severity of the condition being treated, and the like. An appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

In one aspect of the disclosure there is provided a stabilized pharmaceutical composition for administration of an cysteamine or cystamine, wherein the cysteamine or cystamine is enterically coated.

The cysteamine or cystamine is present in the composition in a therapeutically effective amount; typically, the composition is in unit dosage form. The amount of cysteamine or cystamine administered will, of course, be dependent on the age, weight, and general condition of the subject, the severity of the condition being treated, and the judgment of the prescribing physician. Suitable therapeutic amounts will be known to those skilled in the art and/or are described in the pertinent reference texts and literature. In one aspect, the dose is administered twice per day at about 0.5-1.0 g/m^2 (e.g., 0.7-0.8 g/m^2) body surface area. Current non-enterically coated doses are about 1.35 g/m2 body surface area and are administered 4-5 times per day.

Theentericaly coated cysteamine or cystamine can comprise various excipients, as is well known in the pharmaceutical art, provided such excipients do not exhibit a destabilizing effect on any components in the composition. Thus, excipients such as binders, bulking agents, diluents, disintegrants, lubricants, fillers, carriers, and the like can be combined with the cysteamine or cystamine. For solid compositions, diluents are typically necessary to increase the bulk of a tablet so that a practical size is provided for compression. Suitable diluents include dicalcium phosphate, calcium sulfate, lactose, cellulose, kaolin, mannitol, sodium chloride, dry starch and powdered sugar. Binders are used to impart cohesive qualities to a tablet formulation, and thus ensure that a tablet remains intact after compression. Suitable binder materials include, but are not limited to, starch (including corn starch and pregelatinized starch), gelatin, sugars (including sucrose, glucose, dextrose and lactose), polyethylene glycol, waxes, and natural and synthetic gums, e.g., acacia sodium alginate, polyvinylpyrrolidone, cellulosic polymers (including hydroxypropyl cellulose, hydroxypropyl methylcellulose, methyl cellulose, hydroxyethyl cellulose, and the like), and Veegum. Lubricants are used to facilitate tablet manufacture; examples of suitable lubricants include, for example, magnesium stearate, calcium stearate, and stearic acid, and are typically present at no more than approximately 1 weight percent relative to tablet weight. Disintegrants are used to facilitate tablet disintegration or "breakup" after administration, and are generally starches, clays, celluloses, algins, gums or crosslinked polymers. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and the like. If desired, flavoring, coloring and/or sweetening agents may be added as well. Other optional components for incorporation into an oral formulation herein include, but are not limited to, preservatives, suspending agents, thickening agents, and the like. Fillers include, for example, insoluble materials such as silicon dioxide, titanium oxide, alumina, talc, kaolin, powdered cellulose, microcrystalline cellulose, and the like, as well as soluble materials such as mannitol, urea, sucrose, lactose, dextrose, sodium chloride, sorbitol, and the like.

A pharmaceutical composition may also comprise a stabilizing agent such as hydroxypropyl methylcellulose or polyvinylpyrrolidone, as disclosed in U.S. Pat. No. 4,301,146. Other stabilizing agents include, but are not limited to, cellulosic polymers such as hydroxypropyl cellulose, hydroxyethyl cellulose, methyl cellulose, ethyl cellulose, cellulose acetate, cellulose acetate phthalate, cellulose acetate trimel-

litate, hydroxypropyl methylcellulose phthalate, microcrystalline cellulose and carboxymethylcellulose sodium; and vinyl polymers and copolymers such as polyvinyl acetate, polyvinylacetate phthalate, vinylacetate crotonic acid copolymer, and ethylene-vinyl acetate copolymers. The stabilizing agent is present in an amount effective to provide the desired stabilizing effect; generally, this means that the ratio of cysteamine or cystamine to the stabilizing agent is at least about 1:500 w/w, more commonly about 1:99 w/w.

The tablets are manufactured by first enterically coating the cysteamine or cystamine. A method for forming tablets herein is by direct compression of the powders containing the enterically coated cysteamine or cystamine, optionally in combination with diluents, binders, lubricants, disintegrants, colorants, stabilizers or the like. As an alternative to direct compression, compressed tablets can be prepared using wet-granulation or dry-granulation processes. Tablets may also be molded rather than compressed, starting with a moist material containing a suitable water-soluble lubricant.

In an alternative embodiment, the enterically coated cysteamine or cystamine are granulated and the granulation is compressed into a tablet or filled into a capsule. Capsule materials may be either hard or soft, and are typically sealed, such as with gelatin bands or the like. Tablets and capsules for oral use will generally include one or more commonly used excipients as discussed herein.

For administration of the dosage form, i.e., the tablet or capsule comprising the enterically coated cysteamine or cystamine, a total weight in the range of approximately 100 mg to 1000 mg is used. The dosage form is orally administered to a patient suffering from a condition for which an cysteamine or cystamine would typically be indicated, including, but not limited to, cystinosis and neurodegenerative diseases such as Huntington's, Alzheimer's and Parkinson's disease.

The compositions of the disclosure can be used in combination with other therapies useful for treating cystinosis and neurodegenerative diseases and disorders. For example, indomethacin therapy (Indocid® or Endol®) is an anti-inflammatory used to treat rheumatoid arthritis and lumbago, but it can be used to reduce water and electrolyte urine loss. In children with cystinosis, indomethacin reduces the urine volume and therefore liquid consumption by about 30%, sometimes by half. In most cases this is associated with an appetite improvement. Indomethacin treatment is generally followed for several years.

Other therapies can be combined with the methods and compositions of the disclosure to treat diseases and disorders that are attributed or result from cystinosis. Urinary phosphorus loss, for example, entails rickets, and it may be necessary to give a phosphorus supplement. Carnitine is lost in the urine and blood levels are low. Carnitine allows fat to be used by the muscles to provide energy. Hormone supplementation is sometimes necessary. Sometimes the thyroid gland will not produce enough thyroid hormones. This is given as thyroxin (drops or tablets). Insulin treatment is sometimes necessary if diabetes appears, when the pancreas does not produce enough insulin. These treatments have become rarely necessary in children whom are treated with cysteamine, since the treatment protects the thyroid and the pancreas. Some adolescent boys require a testosterone treatment if puberty is late. Growth hormone therapy may be indicated if growth is not sufficient despite a good hydro electrolytes balance. Accordingly, such therapies can be combined with the enterically coated cysteamine and cystamine compositions and methods of the disclosure.

The effectiveness of a method or composition of the disclosure can be assessed by measuring leukocyte cystine concentrations. Dosage adjustment and therapy can be made by a medical specialist depending upon, for example, the severity of cystenosis and/or the concentration of cystine. Additional therapies including the use of omeprazole (Prilosec®) can reduce these symptoms.

In addition, various prodrugs can be "activated" by use of the enterically coated cysteamine. Prodrugs are pharmacologically inert, they themselves do not work in the body, but once they have been absorbed, the prodrug decomposes. The prodrug approach has been used successfully in a number of therapeutic areas including antibiotics, antihistamines and ulcer treatments. The advantage of using prodrugs is that the active agent is chemically camouflaged and no active agent is released until the drug has passed out of the gut and into the cells of the body. For example, a number of produgs use S—S bonds. Weak reducing agents, such as cysteamine, reduce these bonds and release the drug. Accordingly, the compositions of the disclosure are useful in combination with prodrugs for timed release of the drug. In this aspect, a pro-drug can be administered followed by administration of an enterically coated cysteamine compositions of the invention (at a desired time) to activate the pro-drug.

It is to be understood that while the invention has been described in conjunction with specific embodiments thereof, that the foregoing description as well as the examples which follow are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention

EXAMPLES

Subjects

Children with cystinosis, ≥12 years old, and taking regular cysteamine bitartrate (Cystagon®; Mylan, Morgantown, W. Va.) were recruited to the study (Table I). Adult control patients were recruited locally. Patients with cystinosis had a mean leukocyte cystine level of less than 2.0 nmol half-cystine/mg protein over the past year. Cysteamine therapy was discontinued 2 days before admission, and acid suppressants, antibiotics, nonsteroidal anti-inflammatory drugs, prokinetic agents, and antihistamines were discontinued 2 weeks before admission. None of the patients had undergone kidney transplantation. Baseline chemistry, *Helicobacter pylori* serologic study, complete blood count, and urinalysis were performed.

TABLE I

Cystinosis patient data

Patient	Age (yrs.)	Sex	Weight (kg)	Cysteamine dose (mg)*	Serum creatinine (mg/dL)
1	16	Male	61.5	500	1.0
2	14	Male	39.4	406	1.2
3	13	Female	39.1	406	1.5
4	19	Female	38.1	406	1.4
5	13	Female	50.1	500	1.0
6	16	Male	58.7	500	3.1

*Dose of cysteamine base delivered into varying delivery sites

Cysteamine Bitartrate Delivery.

Cysteamine was infused through a silicone rubber nasoenteric tube (Dentsleeve Pty Ltd, Australia), 3 mm in diameter and 4.5 meters long. The tube, specifically made for this study, had a tungsten-weighted tip, and immediately proximal to this was an inflatable balloon (5-mL capacity). Immediately proximal to the balloon was an infusion port (1 mm diameter) through which the drug was delivered. After an overnight fast (except for water), the dose of cysteamine bitartrate (10 mg/kg/dose of base, maximum of 500 mg) was dissolved in 10 mL of water and infused over 1 to 2 minutes. On day 1 of the study, the nasoenteric tube was inserted into the stomach. By day 3 of the study the tube had passed into the proximal small intestine (SI) just distal to the ligament of Treitz (confirmed fluoroscopically). The balloon was then inflated, and peristalsis propelled the tube distally. Tube position within the cecum was confirmed fluoroscopically on day 5 (day 7 in 4 patients because of slow transit). If the tube had migrated too far, it was retracted into the desired location.

Serum Gastrin, Cysteamine and Leukocyte Cystine Measurements.

After an overnight fast (except for water) blood samples were taken at baseline and at varying intervals after intraluminal delivery of cysteamine. Serum gastrin levels were then measured at 30, 60, 90, and 120 minutes and 3 and 4 hours; cysteamine levels were measured at 0, 5, 10, 20, 30, 45, 60, 75, 90, 105, 120, and 150 minutes and 3, 4, 6, 8, 10, 12, and 16 hours; leukocyte cystine levels were measured at 1, 2, 3, 4, 6, and 12 hours in patients with cystinosis only. Gastrin was measured in picograms/mL with the Diagnostic Products Corporation (Los Angeles, Calif.) gastrin radioimmunoassay-assay kit. Leukocyte cystine levels were measured in nmol half-cystine per mg protein by the Cystine Determination Lab (La Jolla, Calif.).

To measure plasma cysteamine, 100-μL plasma samples were collected in heparinized vacutainers and spun in a centrifuge within 1 hour, and plasma was stored at −18° C. The concentration of cysteamine was measured by use of tandem mass spectroscopy (API 2000 LC/MS/MS; Applied Biosystems, Foster City, Calif.). Cysteamine concentrations were calculated with a calibration curve that was prepared by spiking plasma with buffered cysteamine solutions, and quality control samples were analyzed with each batch.

Statistical Analysis.

Mixed model restricted maximum likelihood (REML) repeated measures analysis of variance with subjects as a random effect was performed on the absolute leukocyte cystine levels, on the leukocyte cystine level changes from baseline, and on the "area over the curve" (AOC) for leukocyte cystine level changes from baseline after cysteamine administration for the subjects with cystinosis. AOC is computationally analogous to area under the curve, but it is applied when values are predominantly decreasing below baseline values. Large AOC values reflect large decreases, and a negative AOC reflects a net increase in value. Main effects for site of delivery, time after delivery, and the interaction between site and time were tested, except just the site effect was tested for AOCs. In the absence of significant interaction when a main effect was detected, Tukey's honestly significant difference test (HSD) was applied to identify where differences occurred within a 5% family wise error rate. The Tukey HSD procedure controls for overall significance level when performing all pairwise comparisons. An additional analysis was performed with plasma cysteamine C_{max} added to the AOC model.

REML repeated measures analyses of variance with subjects as a random effect were also performed as described above on AUC and the C_{max} over time for plasma cysteamine levels separately for the subjects with cystinosis and control subjects and with both subject groups combined. Differences between means for the 3 sites were tested, plus group and group x site interaction effects for the combined groups. If a site effect was detected, Tukey's HSD was applied to determine which sites differed from each other.

REML repeated measures analyses of variance were also performed as described above on gastrin levels. The analyses were performed on 2 versions of datasets: the full dataset and all data after omitting observations collected at 30 minutes (1 subject was missing a blood sample taken at 30 minutes after small intestinal cysteamine delivery). A 5% significance level was used without adjustment for all statistical testing.

Six patients with cystinosis, (3 male, 3 female) with a mean age of 15.2 years (range 13-19 years) were recruited into the study (Table I). Eight healthy adult control patients (6 male, 2 female) with a mean age of 23.2 years (range 19-28 years) were enrolled. None of the children with cystinosis had undergone kidney transplantation. All control subjects received 500 mg cysteamine base, whereas the mean dose for subjects with cystinosis was 453 mg (range 406-500 mg). All subjects had normal liver function test results. In all subjects the nasoenteric tube passed successfully from the stomach into the upper SI; however, it did not progress any further in 2 subjects with cystinosis. In 2 of the control subjects the tube only reached the mid-ileum but did, however, progress to the cecum in 8 subjects (4 control subjects, 4 with cystinosis). There were no reported adverse effects with the insertion or removal of the nasoenteric tube (FIG. **1**).

Symptoms.

Only 2 patients (1 male, 1 female) with cystinosis reported regular GI symptoms before the study, and these had responded to acid-suppression therapy. The male subject had severe retching and emesis about 15 minutes after receiving intragastric cysteamine but did not have any symptoms when the drug was infused into the proximal small intestine. The female child with cystinosis had mild transient nausea after SI drug delivery only. No other symptoms were reported after any other cysteamine delivery in the children with cystinosis. There were no associated adverse events with tube placement or removal.

Plasma Cysteamine.

Among the subjects with cystinosis as measured by analysis of variance, the mean plasma cysteamine C_{max} and AUCs (of the concentration-time gradient) differed by site of cysteamine delivery (both $P<0.03$). Site (†) refers to either patients with cystinosis or control subjects. For the plasma cysteamine AUCs, the means differed between the duodenal and both gastric and cecal sites of delivery (Tukey HSD global $P<0.05$). Among control subjects, the mean AUC did not differ among delivery sites ($P>0.4$), but mean C_{max} did ($P<0.05$). For both cystinosis and control groups the mean C_{max} values differed only between the duodenum and cecum; mean C_{max} values after duodenal versus gastric or gastric versus cecal delivery were not statistically different (Tables II and III).

TABLE II

Mean plasma cysteamine C_{max} levels (γmol/L) and area under curve (AUC) measurements in cystinosis subjects, controls, and combined cystinosis and control subjects, after delivery of cysteamine into the stomach, small intestine, and cecum

	C_{max} Cystinosis	AUC Cystinosis	C_{max} Control	AUC Control	C_{max} Combined	AUC Combined
Stomach	35.5 (20.5)	3006 (1112)	39.5 (16.4)	3613 (1384)	37.8 (17.6)	3353 (1267)
Small Intestine	55.8 (13.0)	4299 (1056)	51.1 (20.7)	3988 (1659)	53.2 (17.4)	4047 (1376)
Cecum	21.9 (13.1)	3002 (909)	23.1 (15.3)	2804 (1323)	22.5 (13.2)	2903 (1056)

The standard deviations are in parenthesis

TABLE III

Comparisons of mean plasma cysteamine C_{max} (γmol/L) and AUC measurements for combined cystinosis subjects and control subjects among delivery sites

	AUC	C_{max}
P value*	<0.01	<0.01
Stomach vs SI	+	+
Stomach vs Cecum	−	−
SI vs Cecum	+	+

+ Significant difference using Tukey's HSD test (α = 0.05)
− No significant difference
*ANOVA test for equality of three delivery sites

When data from the control subjects were combined with cystinosis subject data, there was both a group effect (P<0.05) and a site effect (P<0.01) for AUCs, with a significant difference between mean AUC levels for the duodenum versus both the stomach and cecum. C_{max} values differed among sites (P<0.01) but not between groups (P>0.4). Group (*) refers to site of intestinal delivery. C_{max} differed between duodenum versus both stomach and cecum (FIG. **2**).

Leukocyte Cystine.

There were significant differences among the 3 sites of delivery for cystine levels (P<0.04), changes from baseline values (P<0.0001), and AOCs for changes from baseline (P<0.02). A Tukey HSD test, which controls for multiple comparisons, showed that mean leukocyte cystine levels differed between the cecum and stomach sites, but that cecum versus duodenum and stomach versus duodenum produced similar mean values. When the absolute cystine levels or AOCs for changes from baseline levels were evaluated, the significant differences in sites were found between the duodenum and both the stomach and cecum, but not between stomach and cecum (Tukey HSD global P<0.05) (FIG. **3**). Plasma cysteamine C_{max} and AUC contributed a statistical effect on AOC (P<0.001 and <0.02, respectively), even after controlling for delivery site (FIG. **4**).

Blood Gastrin.

For the full gastrin dataset, there was a significant difference among the means for the different delivery sites (P<0.1), with the cecum resulting in a lower mean from that of the stomach and small intestine. Both group * and site † significant effects were detected after omitting observations from 30 minutes after delivery (P<0.05 and P<0.01, respectively). The 30-minute observations were omitted because of a missing data set. For these observations, mean levels of gastrin after delivery in the cecum were different from those from both the duodenum and stomach, although the latter did not differ from each other. The 1 boy (14 years) who had severe GI symptoms after intragastric, but not enteric or cecal, cysteamine delivery had a rise in baseline gastrin from 70 pg/mL to 121 pg/mL at 30 minutes after gastric cysteamine. Within the control group, more than half of the baseline and post-cysteamine gastrin levels remained undetectable (<25 pg/mL), and none of the control subjects had a significant rise in gastrin after cysteamine delivery into any site.

Patients with cystinosis are required to ingest oral cysteamine (Cystagon®) every 6 hours, day and night. When taken regularly, cysteamine can deplete intracellular cystine by up to 90% (as measured in circulating white blood cells), and this has been shown to reduce the rate of progression to kidney failure/transplantation and also to obviate the need for thyroid replacement therapy. Unfortunately, because of the strict treatment regimen and the associated symptoms, non-adherence with cysteamine therapy remains a problem, particularly among adolescent and young adult patients. Certainly, by reducing the frequency of required cysteamine dosing adherence can be improved. The disclosure shows a strong statistical association between the maximum plasma concentration (C_{max}) of cysteamine and AOC measurements for leukocyte cystine (P<0.001). A higher C_{max} is achieved after delivery of cysteamine into the small intestine than when infused into the stomach or colon; this may be due to improved absorption rate from the SI, greater surface area of the SI, or less cysteamine undergoing hepatic first pass elimination when absorbed rapidly through the small intestine. When data were combined for patients with cystinosis and control subjects, there was a statistical difference between duodenal versus both gastric and colonic delivery for plasma cysteamine C_{max} and AUC levels (both P<0.05). The lack of similar statistical significance for the cystinosis group alone may simply reflect the small number of patients studied. Changes from baseline leukocyte cystine levels were statistically significant for absolute cystine levels and for AOC when cysteamine was infused into the duodenum compared with both stomach and colon. As shown in FIG. **3**, the leukocyte cystine levels remained below pre-delivery levels for up to 12 hours after a single dose of cysteamine into the small intestine. This would suggest that effective absorption of cysteamine through the SI, by causing a higher C_{max} and AUC on the cysteamine concentration-time gradient, could lead to prolonged depletion of leukocyte cystine and possibly less frequent daily dosing. Another explanation would be that by achieving a high enough plasma cysteamine concentration, more drug reaches the lysosome (where cystine accumulates). In the lysosome the cysteamine reacts with cystine forming the mixed disulfide of cysteamine and cysteine. The mixed disulfide exits the lysosome presumably via the lysine carrier. In the cytosol the mixed disulfide can be reduced by its reaction with glutathione. The cysteine released can be used for protein or glutathione synthesis. The cysteamine released from the mixed disulfide reenters the lysosome where it can react with another cystine molecule. Thus 1 molecule of cysteamine may release many molecules of cystine from the lysosome. This study showed a dramatic decrease in leukocyte

cystine within an hour of cysteamine delivery. In retrospect, the finding from this study was that the leukocyte cystine levels remained at the 1-hour level for 24 hours, and even at 48 hours after delivery the levels had not returned to the pre-cysteamine level.

Cysteamine is a potent gastric acid-secretagogue that has been used in laboratory animals to induce duodenal ulceration; studies in humans and animals have shown that cysteamine-induced gastric acid hypersecretion is most likely mediated through hypergastrinemia. In previous studies performed in children with cystinosis who suffered regular upper gastrointestinal symptoms, a single oral dose of cysteamine (11-23 mg/kg) was shown to cause hypergastrinemia and a 2- to 3-fold rise in gastric acid-hypersecretion. Symptoms suffered by these individuals included abdominal pain, heartburn, nausea, vomiting, and anorexia. Interestingly, only 2 of 6 subjects with cystinosis (who were known to suffer regular cysteamine-induced GI symptoms) had increased gastrin levels and symptoms, including nausea, retching, and discomfort after Intragastric cysteamine. Gastrin levels were only available after small intestinal administration in 1 of the 2 children and the levels remained the same as baseline. Neither child had symptoms after enteric cysteamine delivery. None of the other patients with cystinosis or control subjects had an increase in gastrin levels with cysteamine infused into any site. This would suggest that cysteamine-induced hypergastrinemia may arise as a local effect on the gastric antral-predominant G-cells only in susceptible individuals. In addition, plasma gastrin levels usually peaks after intragastric delivery within 30 minutes, whereas the plasma cysteamine levels peaked later. 8,10 In 2 previous studies, children with cystinosis were shown to have a significant rise in plasma gastrin levels after receiving intragastric cysteamine; as part of these study's entry criteria all subjects did, however, suffer with regular GI symptoms. Data from this study would suggest that cysteamine does not cause hypergastrinemia, and therefore acid-hypersecretion, in all patients with cystinosis. Thus acid suppression therapy would not be recommended in patients with cystinosis without upper GI symptoms.

The data suggest that direct administration of cysteamine into the jejunum may result in prolonged leukocyte cystine depletion. In a previous study, a child who had a gastrojejunal feeding tube for oral feeding aversion and severe UGI symptoms, responded to intrajejunal cysteamine with a 3-fold rise in serum gastrin as compared with drug administration into the stomach. The leukocyte cystine response was not measured in this child. Therefore patients with jejunal feeding tubes will have to be further evaluated.

FIGS. **5** and **6** shows results from a patient that remained on the twice daily EC-cysteamine for an extended period of time. Over this period the patient's leukocyte cystine levels have been measured regularly. The dose of twice daily EC-cysteamine is titrated against the patient's symptoms and cystine levels. The patient's cystine levels have been 0.4, 1.0, 0.36.

This study provides data that may be used to improve the quality of life for patients with cystinosis. The present formulation of Cystagon® comprises cysteamine in a capsule that will dissolve rapidly on contact with water, most likely within the stomach.

Although a number of embodiments and features have been described above, it will be understood by those skilled in the art that modifications and variations of the described embodiments and features may be made without departing from the teachings of the disclosure or the scope of the invention as defined by the appended claims.

What is claimed is:

1. A method of treating a patient with cystinosis comprising administering to said patient twice per day a composition comprising cysteamine, or a pharmaceutically acceptable salt thereof, and one or more materials that provides increased delivery of said cysteamine to the small intestine, wherein said composition is formulated to provide white blood cell cystine suppression with a 12 hour level below 1 nmol½ cystine/mg protein.

2. The method of claim **1**, wherein each dose of cysteamine is about 0.5-1.0 g/m^2 body surface area.

3. The method of claim **1**, wherein the total daily dose of cysteamine is about 1.35 g/m^2 body surface area or less.

4. The method of claim **1**, wherein the one or more materials increases delivery to the proximal small intestine.

5. The method of claim **1**, wherein the one or more materials increases delivery to the mid-small intestine.

6. The method of claim **1**, wherein the one or more materials increases delivery to the duodenum.

7. The method of claim **1**, wherein the one or more materials increases delivery to the jejunum.

8. The method of claim **1**, wherein the one or more materials increases delivery to the mid-ileum.

9. The method of claim **1**, wherein the composition is in the form of a tablet.

10. The method of claim **1**, wherein the composition is in the form of a capsule.

11. The method of claim **1**, wherein the cysteamine salt is cysteamine bitartrate.

12. A method of treating a patient with cystinosis comprising administering to said patient twice per day a composition comprising cysteamine, or a pharmaceutically acceptable salt thereof, and an enteric coating that provides increased delivery of cysteamine to the small intestine, wherein said composition is formulated to provide white blood cell cystine suppression with a 12 hour level below 1 nmol½ cystine/mg protein.

13. The method of claim **12**, wherein each dose of cysteamine is about 0.5-1.0 g/m^2 body surface area.

14. The method of claim **12**, wherein the total daily dose of cysteamine is about 1.35 g/m^2 body surface area or less.

15. The method of claim **12**, wherein the enteric coating begins to dissolve in an aqueous solution at pH of about 5.5.

16. The method of claim **12**, wherein the composition increases delivery to a region of the gastrointestinal tract of a subject in which the pH is greater than pH 5.5.

17. The method of claim **12**, wherein the coating is selected from the group consisting of polymerized gelatin, shellac, methacrylic acid copolymer type CNF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers formed from methyl acrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters.

18. The method of claim **12**, wherein the cysteamine salt is cysteamine bitartrate.

19. An improved method of administering cysteamine comprising administering to a patient in need thereof, twice per day, a composition comprising cysteamine, or a pharmaceutically acceptable salt thereof, and one or more materials that provides increased delivery of said cysteamine to the small intestine, wherein said composition is capable of pro-

viding white blood cell cystine suppression with a 12 hour level below 1 nmol½ cystine/mg protein.

20. The method of claim **19**, wherein each dose of cysteamine is about 0.5-1.0 g/m^2 body surface area.

21. The method of claim **19**, wherein the total daily dose of cysteamine is about 1.35 g/m^2 body surface area or less.

22. The method of claim **19**, wherein the one or more materials increases delivery to a region of the gastrointestinal tract of a subject in which the pH is greater than pH 5.5.

23. The method of claim **19**, wherein the composition comprises an enteric coating.

24. The method of claim **23**, wherein the enteric coating begins to dissolve in an aqueous solution at pH of about 5.5.

* * * * *

EXHIBIT C

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

(10) **Patent No.:** **US 9,198,882 B2**
(45) **Date of Patent:** ***Dec. 1, 2015**

(54) **ENTERICALLY COATED CYSTEAMINE, CYSTAMINE AND DERIVATIVES THEREOF**

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(*) 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.

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(60) Division of application No. 14/555,993, filed on Nov. 28, 2014, which is a continuation of application No. 13/399,900, filed on Feb. 17, 2012, now abandoned, which is a continuation of application No. 13/190,396, filed on Jul. 25, 2011, now Pat. No. 8,129,433, which is a division of application No. 11/990,869, filed as application No. PCT/US2007/002325 on Jan. 26, 2007, now Pat. No. 8,026,284.

(60) Provisional application No. 60/762,715, filed on Jan. 27, 2006.

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CPC ***A61K 31/145*** (2013.01); ***A61K 9/0053*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

The disclosure provides oral cysteamine and cystamine formulations useful for treating cystinosis and neurodegenerative diseases and disorders. The formulations provide controlled release compositions that improve quality of life and reduced side-effects.

18 Claims, 4 Drawing Sheets

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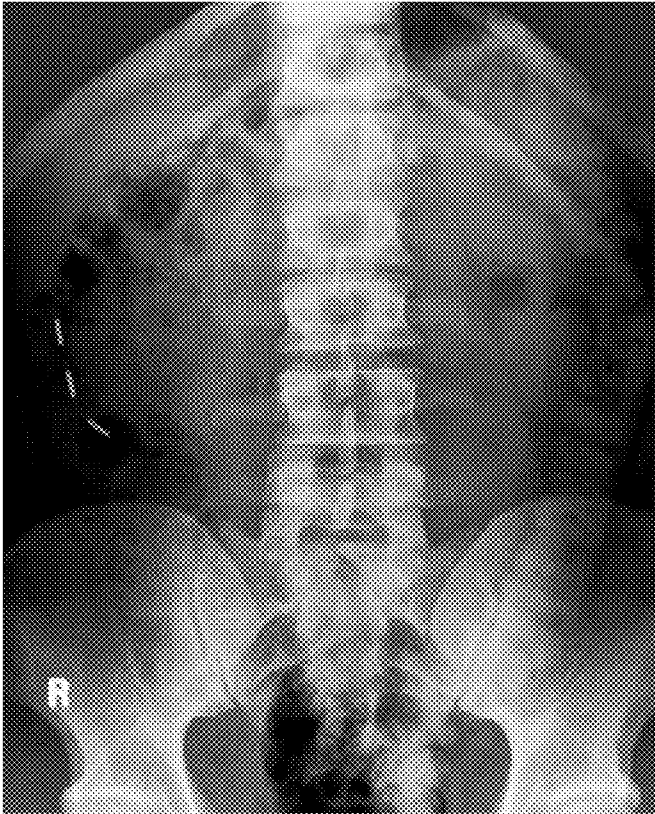

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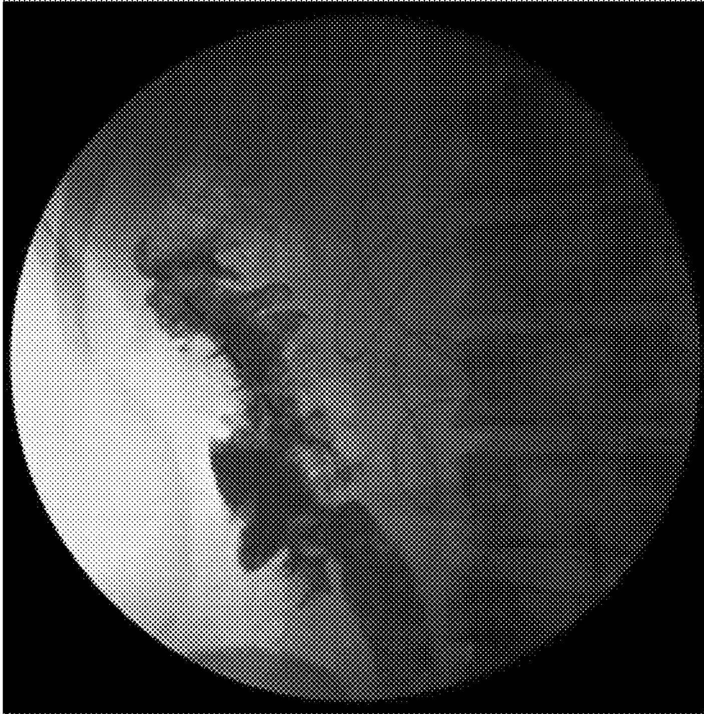

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FIG. 1

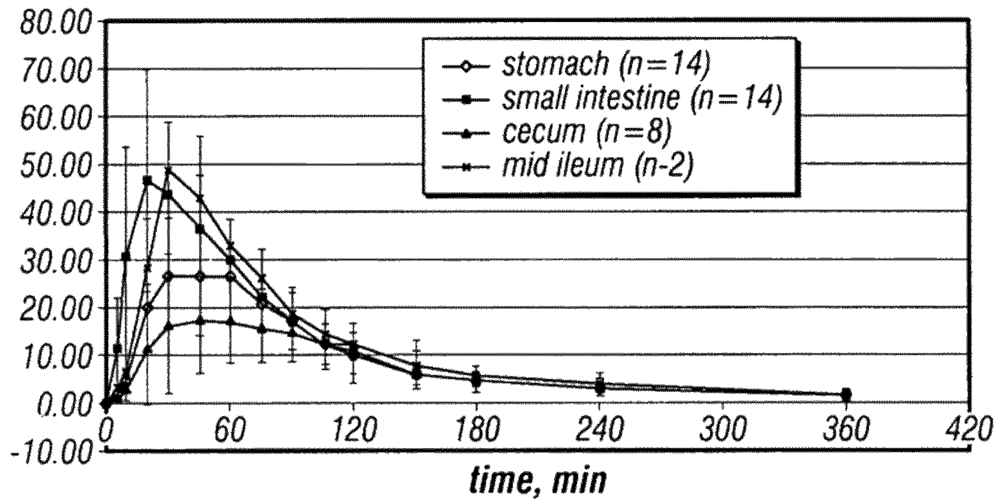

FIG. 2

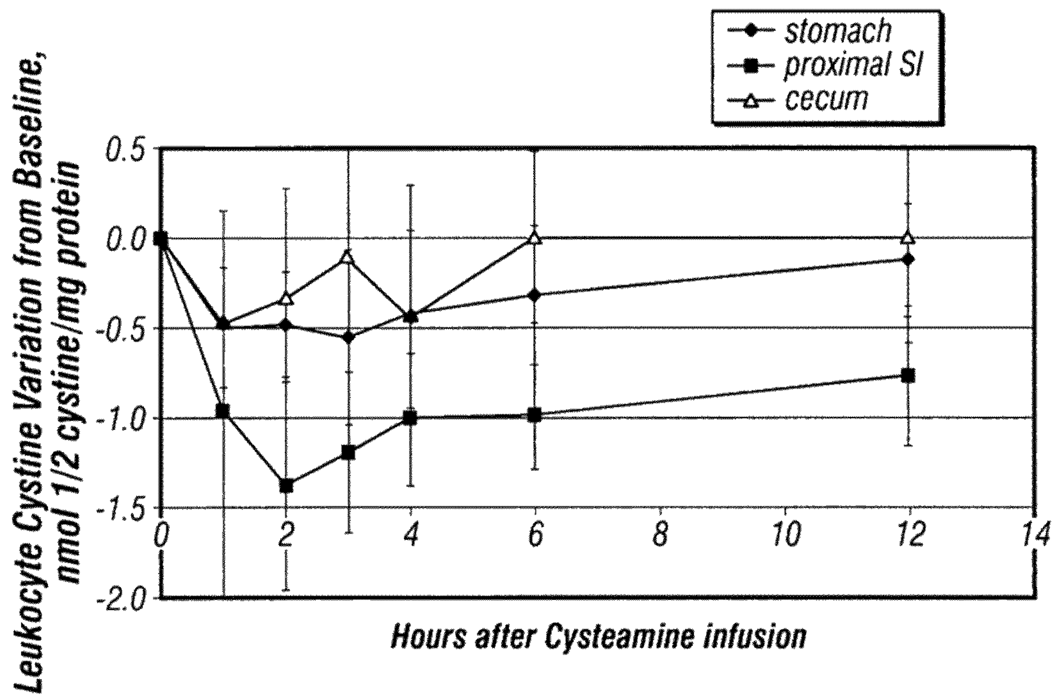

FIG. 3

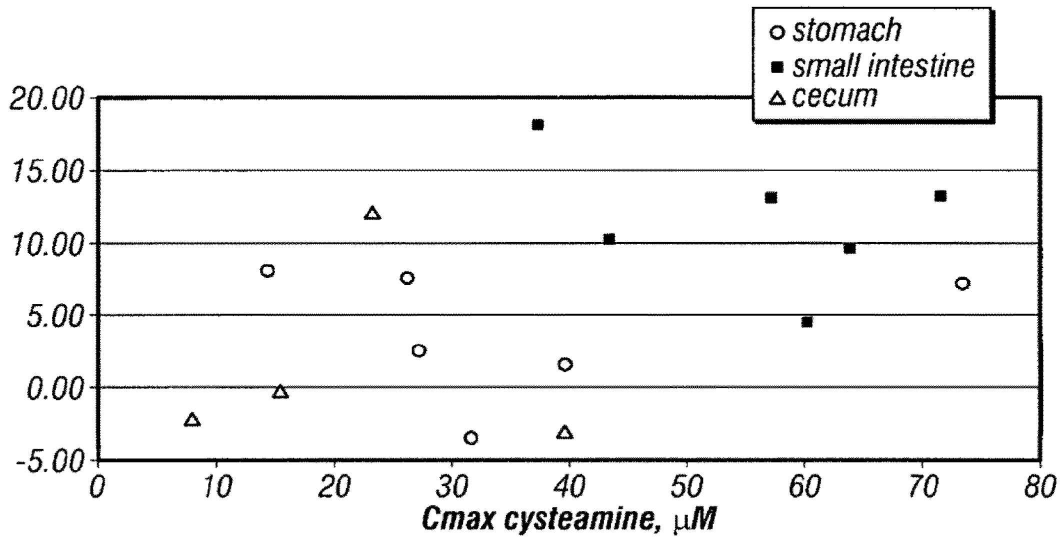

FIG. 4

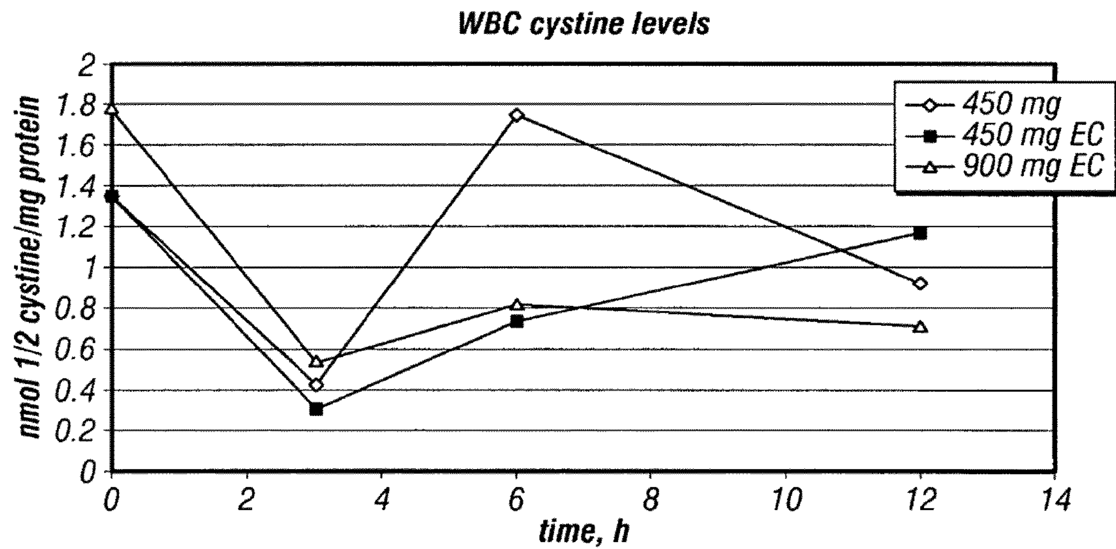

FIG. 5

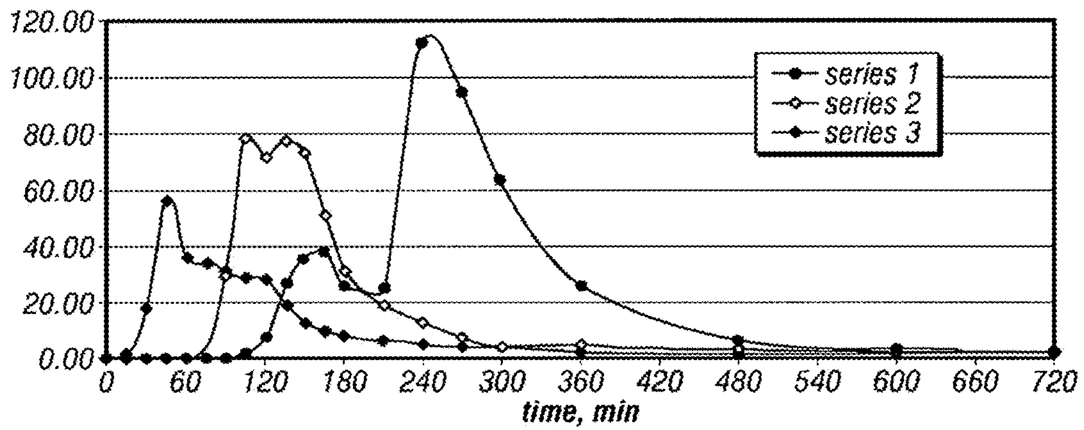

FIG. 6

ENTERICALLY COATED CYSTEAMINE, CYSTAMINE AND DERIVATIVES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 14/555,993, filed Nov. 28, 2014, which is a continuation of U.S. application Ser. No. 13/399,900, filed Feb. 17, 2012, which is a continuation of U.S. application Ser. No. 13/190,396, filed Jul. 25, 2011, which is a divisional of U.S. application Ser. No. 11/990,869, filed Nov. 13, 2008, which is a U.S. National Stage Application filed under 35 U.S.C. §371 and claims priority to International Application No. PCT/US07/02325, filed Jan. 26, 2007, which application claims priority under 35 U.S.C. §119 to U.S. Provisional Application Ser. No. 60/762,715, filed Jan. 27, 2006, the disclosures of which are incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to methods, compositions and treatments for metabolic conditions and free radical damage. More specifically, the invention relates to methods and composition useful for treating Cystinosis and neurodegenerative diseases such as Huntington's, Alzheimer's and Parkinson's disease, as free radical and radioprotectants, and as heptoprotectant agents.

BACKGROUND

Cystinosis is a rare, autosomal recessive disease caused by intra-lysosomal accumulation of the amino acid cystine within various tissues, including the spleen, liver, lymph nodes, kidney, bone marrow, and eyes. Nephropathic cystinosis is associated with kidney failure that necessitates kidney transplantation. To date, the only specific treatment for nephropathic cystinosis is the sulfhydryl agent, cysteamine. Cysteamine has been shown to lower intracellular cystine levels, thereby reducing the rate of progression of kidney failure in children.

Cysteamine, through a mechanism of increased gastrin and gastric acid production, is ulcerogenic. When administered orally to children with cystinosis, cysteamine has also been shown to cause a 3-fold increase in gastric acid production and a 50% rise of serum gastrin levels. As a consequence, subjects that use cysteamine suffer gastrointestinal (GI) symptoms and are often unable to take cysteamine regularly or at full dose.

To achieve sustained reduction of leukocyte cystine levels, patients are normally required to take oral cysteamine every 6 hours, which invariably means having to awaken from sleep. However, when a single dose of cysteamine was administered intravenously the leukocyte cystine level remained suppressed for more than 24 hours, possibly because plasma cysteamine concentrations were higher and achieved more rapidly than when the drug is administered orally. Regular intravenous administration of cysteamine would not be practical. Accordingly, there is a need for formulations and delivery methods that would result in higher plasma, and thus intracellular, concentration as well as decrease the number of daily doses and therefore improve the quality of life for patients.

SUMMARY

The invention provides a composition comprising an enterically coated cystamine or cystamine derivative.

The invention also provides a composition comprising an enterically coated cysteamine or cysteamine derivative.

The invention further provides a composition comprising a coated cystinosis therapeutic agent that has increased uptake in the small intestine compared to a non-coated cystinosis therapeutic agent when administered orally. In one aspect, the coated cystinosis therapeutic agent comprises a cysteamine or cysteamine derivative.

The invention also provides a method of treating a subject with cystinosis, comprising administering to the subject a composition of the invention.

The invention also contemplates a method of treating a subject with a neurodegenerative disease or disorder comprising administering to the subject a composition of the invention comprising an enterically coated cystamine or cystamine derivative.

The invention provides a pharmaceutical formulation comprising a composition of the invention further including various pharmaceutically acceptable agents (e.g., flavorants, binders and the like) in a pharmaceutically acceptable carrier.

The invention provides a method of treating cystinosis or a neurodegenerative disease or disorder comprising administering a composition of the invention and a second therapeutic agent.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows enterocolonic tube. (A) Is an abdominal X-ray film showing the radiopaque weighted tip of the tube entering the ascending colon. (B) Is a contrast infused picture. The tube has passed through the small intestine and the tip is confirmed.

FIG. **2** shows mean plasma cysteamine levels taken from patients with cystinosis and control subjects after delivery of drug into various intestinal sites. Error bars are standard error of the mean. In 2 control subjects, most distal point of drug delivery was the mid-ileal region.

FIG. **3** shows the mean change in leukocyte cystine levels, compared with baseline levels, over a 12-hour period following delivery of cysteamine into varying intestinal sites. Negative levels signify increased leukocyte cystine depletion compared with baseline.

FIG. **4** shows a scatterplot of plasma cysteamine C_{max} vs. AOC of WBC Cystine changes from Baseline. Positive value means decrease from baseline. Negative value means increase from baseline. AOC change from baseline was affected by C_{max} for cysteamine ($P<0.001$).

FIG. **5** shows serial leukocyte cystine levels after drug was given as normal Cystagon® and enteric-coated (EC) cysteamine on alternate days. These serial levels were taken during the inpatient phase of the study. Desired cystine levels are below 1 mmol ½ cystine/mg protein. Higher dose enteric-coated (yellow)) drug resulted in prolonged cystine suppression with 12 hour levels still within desired range.

FIG. **6** shows the blood cysteamine levels following a single 450 mg dose of Cystagon® (series 1), 450 mg EC-cysteamine (series 2) and 900 mg EC-cysteamine (series 3). The C_{max} is higher following EC drug. In addition, the time to C_{max} is longer following EC-drug, suggesting that the drug is released from the capsule within the small intestine rather than the stomach.

DETAILED DESCRIPTION

As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, refer-

ence to "a derivative" includes a plurality of such derivatives and reference to "a subject" includes reference to one or more subjects known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods, devices and materials are described herein.

The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior disclosure.

Cystinosis is a metabolic disease characterized by an abnormal accumulation of the amino acid cystine in various organs of the body such as the kidney, eye, muscle, pancreas, and brain. Different organs are affected at different ages.

There are three clinical forms of cystinosis. Infantile (or nephropathic) cystinosis; late-onset cystinosis; and benign cystinosis. The latter form does not produce kidney damage. Infantile cystinosis is usually diagnosed between 6 and 18 months of age with symptoms of excessive thirst and urination, failure to thrive, rickets, and episodes of dehydration. These findings are caused by a disorder called renal tubulopathy or Fanconi syndrome. As a consequence important nutrients and minerals are lost in the urine. Children with cystinosis also have crystals in their eyes (after one year of age) which may lead to photosensitivity. They also have an increased level of cystine in their white blood cells without adverse effect but allowing the diagnosis to be ascertained. Without specific treatment, children with cystinosis develop end-stage renal failure, i.e., lose their kidney function, usually between 6 and 12 years of age. Without cysteamine treatment subjects can develop complications in other organs due to the continued accumulation of cystine throughout the body. These complications can include muscle wasting, difficulty swallowing, diabetes, and hypothyroidism.

Some symptoms include the inability of the kidneys to concentrate urine and allow important quantities of sodium, potassium, phosphorus, bicarbonate and substances like carnitine to be excreted in the urine. Treatment of symptoms compensates for these urinary losses. Subjects need to drink large quantities of water, because up to 2 to 3 liters of water are lost in the urine every day driving the feeling of being thirsty. In addition, the loss of urinary electrolytes (sodium, potassium, bicarbonate, phosphorus) must be compensated in the subject. It is often necessary to add a salt supplement in the form of sodium chloride. Children also lose bicarbonate and potassium in the urine, which can be compensated for by giving sodium bicarbonate and potassium bicarbonate.

Specific treatments of cystinosis aim to reduce cystine accumulation within the cells. Cystinosis is currently treated with cysteamine (Cystagon®). Cysteamine also improves growth of cystinosis children. Cysteamine is only active in a very short period of time not exceeding 5-6 hours, thus requiring administration of Cystagon® capsules 4 times a day, that is to say about every 6 hours. This treatment is also only effective if continued day after day, indefinitely in order to control the disease. About 1000 children require lifelong treatment to prolong their lives and prevent deterioration of kidney function. However, as mentioned above, cysteamine administration results in increased gastric secretions and is ulcerogenic. In addition, routes and timing of administration provide difficulty for subjects in need of such therapy. Recently, a similar drug called cystamine (the disulfide form

of cysteamine) has been studied for neurodegenerative disorders including Huntington's and Parkinson's diseases. Cystamine has similar side-effects and dosing difficulties to that of cysteamine.

Cysteamine is a potent gastric acid-secretagogue that has been used in laboratory animals to induce duodenal ulceration; studies in humans and animals have shown that cysteamine-induced gastric acid hypersecretion is most likely mediated through hypergastrinemia. In previous studies performed in children with cystinosis who suffered regular upper gastrointestinal symptoms, a single oral dose of cysteamine (11-23 mg/kg) was shown to cause hypergastrinemia and a 2- to 3-fold rise in gastric acid-hypersecretion. Symptoms suffered by these individuals included abdominal pain, heartburn, nausea, vomiting, and anorexia. The disclosure demonstrates that cysteamine-induced hypergastrinemia arises, in part, as a local effect on the gastric antral-predominant G-cells in susceptible individuals. The data also suggest that this is also a systemic effect of gastrin release by cysteamine. Depending upon the route of administration, plasma gastrin levels usually peak after intragastric delivery within 30 minutes, whereas the plasma cysteamine levels peak later.

Subjects with cystinosis are required to ingest oral cysteamine (Cystagon®) every 6 hours, day and night. When taken regularly, cysteamine can deplete intracellular cystine by up to 90% (as measured in circulating white blood cells), and this has been shown to reduce the rate of progression to kidney failure/transplantation and also to obviate the need for thyroid replacement therapy. Unfortunately, because of the strict treatment regimen and the associated symptoms, non-adherence with cysteamine therapy remains a problem, particularly among adolescent and young adult patients. By reducing the frequency of required cysteamine dosing, adherence to a therapeutic regimen can be improved. The disclosure demonstrates that delivery of cysteamine to the small intestine reduces gastric distress and ulceration and improves bioavailability of cysteamine in the circulation. Delivery of cysteamine into the small intestine is useful due to improved absorption rate from the SI, greater surface area of the SI, and/or less cysteamine undergoing hepatic first pass elimination when absorbed through the small intestine. This disclosure shows a dramatic decrease in leukocyte cystine within an hour of cysteamine delivery.

In addition, sulfhydryl (SH) compounds such as cysteamine, cystamine, and glutathione are among the most important and active intracellular antioxidants. Cysteamine protects animals against bone marrow and gastrointestinal radiation syndromes. The rationale for the importance of SH compounds is further supported by observations in mitotic cells. These are the most sensitive to radiation injury in terms of cell reproductive death and are noted to have the lowest level of SH compounds. Conversely, S-phase cells, which are the most resistant to radiation injury using the same criteria, have demonstrated the highest levels of inherent SH compounds. In addition, when mitotic cells were treated with cysteamine, they became very resistant to radiation. It has also been noted that cysteamine may directly protect cells against induced mutations. The protection is thought to result from scavenging of free radicals, either directly or via release of protein-bound GSH. An enzyme that liberates cysteamine from coenzyme A has been reported in avian liver and hog kidney. Recently, studies have appeared demonstrating a protective effect of cysteamine against the hepatotoxic agents acetaminophen, bromobenzene, and phalloidine.

Cystamine, in addition, to its role as a radioprotectant, has been found to alleviate tremors and prolong life in mice with the gene mutation for Huntington's disease (HD). The drug

may work by increasing the activity of proteins that protect nerve cells, or neurons, from degeneration. Cystamine appears to inactivate an enzyme called transglutaminase and thus results in a reduction of huntingtin protein (Nature Medicine 8, 143-149, 2002). In addition, cystamine was found to increase the levels of certain neuroprotective proteins. However, due to the current methods and formulation of delivery of cystamine, degradation and poor uptake require excessive dosing.

The disclosure is not limited with respect to a specific cysteamine or cystamine salt or ester or derivative; the compositions of the disclosure can contain any cysteamine or cystamine, cysteamine or cystamine derivative, or combination of cysteamine or cystamines. The active agents in the composition, i.e., cysteamine or cystamine, may be administered in the form of a pharmacologically acceptable salt, ester, amide, prodrug or analog or as a combination thereof. Salts, esters, amides, prodrugs and analogs of the active agents may be prepared using standard procedures known to those skilled in the art of synthetic organic chemistry and described, for example, by J. March, "Advanced Organic Chemistry: Reactions, Mechanisms and Structure," 4th Ed. (New York: Wiley-Interscience, 1992). For example, basic addition salts are prepared from the neutral drug using conventional means, involving reaction of one or more of the active agent's free hydroxyl groups with a suitable base. Generally, the neutral form of the drug is dissolved in a polar organic solvent such as methanol or ethanol and the base is added thereto. The resulting salt either precipitates or may be brought out of solution by addition of a less polar solvent. Suitable bases for forming basic addition salts include, but are not limited to, inorganic bases such as sodium hydroxide, potassium hydroxide, ammonium hydroxide, calcium hydroxide, trimethylamine, or the like. Preparation of esters involves functionalization of hydroxyl groups which may be present within the molecular structure of the drug. The esters are typically acyl-substituted derivatives of free alcohol groups, i.e., moieties which are derived from carboxylic acids of the formula R—COOH where R is alkyl, and typically is lower alkyl. Esters can be reconverted to the free acids, if desired, by using conventional hydrogenolysis or hydrolysis procedures. Preparation of amides and prodrugs can be carried out in an analogous manner. Other derivatives and analogs of the active agents may be prepared using standard techniques known to those skilled in the art of synthetic organic chemistry, or may be deduced by reference to the pertinent literature.

The disclosure provides delivery methods and compositions that overcome the problems associated with cysteamine and cystamine delivery. The methods of compositions of the disclosure provide enteric-coated compositions that result in less frequent dosing (2×/day vs. 4×/day), increased patient compliance and fewer gastrointestinal side effects (e.g., pain, heartburn, acid production, vomiting) and other side effects (e.g., patients smell like rotten eggs—a particular compliance problem as subjects reach puberty). The disclosure provides enteric-coated cysteamine compositions (sulfhydryl/Cystagon®) and cystamine compositions.

The disclosure provides methods for the treatment of cystinosis, the treatment of neurodegenerative disease such as Alzheimer Disease, Huntington's and Parkinson's disease and free radical damage using enterically coated cysteamine and cystamine, respectively.

The disclosure provides composition comprising enterically formulated cysteamine and cystamine derivatives. Examples of cysteamine derivatives include hydrochloride, bitartrate and phosphocysteamine derivatives. Cystamine and cystamine derivatives include sulfated cystamine. Enteric coatings prolong release until the cystamine, cystamine derivative, or cysteamine derivative/Cystagon® reaches the intestinal tract, typically the small intestine. Because of the enteric coatings, delivery to the small intestine is improved thereby improving uptake of active ingredient while reducing gastric side effects. This will result in a reduction in the need for frequent administration that currently is associated with Cystagon® therapy, cystamine and cysteamine therapy.

An "enterically coated" drug or tablet refers to a drug or tablet that is coated with a substance—i.e., with an "enteric coating"—that remains intact in the stomach but dissolves and releases the drug once the small intestine is reached.

As used herein "enteric coating", is a material, a polymer material or materials which encase the medicament core (e.g., cystamine, cysteamine, Cystagon®). Typically, a substantial amount or all of the enteric coating material is dissolved before the medicament or therapeutically active agent is released from the dosage form, so as to achieve delayed dissolution of the medicament core. A suitable pH-sensitive polymer is one which will dissolve in intestinal juices at a higher pH level (pH greater than 4.5), such as within the small intestine and therefore permit release of the pharmacologically active substance in the regions of the small intestine and not in the upper portion of the GI tract, such as the stomach.

The coating material is selected such that the therapeutically active agent will be released when the dosage form reaches the small intestine or a region in which the pH is greater than pH 4.5. The coating may be a pH-sensitive materials, which remain intact in the lower pH environs of the stomach, but which disintegrate or dissolve at the pH commonly found in the small intestine of the patient. For example, the enteric coating material begins to dissolve in an aqueous solution at pH between about 4.5 to about 5.5. For example, pH-sensitive materials will not undergo significant dissolution until the dosage form has emptied from the stomach. The pH of the small intestine gradually increases from about 4.5 to about 6.5 in the duodenal bulb to about 7.2 in the distal portions of the small intestine (ileum). In order to provide predictable dissolution corresponding to the small intestine transit time of about 3 hours (e.g., 2-3 hours) and permit reproducible release therein, the coating should begin to dissolve within the pH range of the duodenum, and continue to dissolve at the pH range within the small intestine. Therefore, the amount of enteric polymer coating should be sufficient to substantially dissolved during the approximate three hour transit time within the small intestine (e.g., the proximal and mid-small intestine).

Enteric coatings have been used for many years to arrest the release of the drug from orally ingestible dosage forms. Depending upon the composition and/or thickness, the enteric coatings are resistant to stomach acid for required periods of time before they begin to disintegrate and permit release of the drug in the lower stomach or upper part of the small intestines. Examples of some enteric coatings are disclosed in U.S. Pat. No. 5,225,202 which is incorporated by reference fully herein. As set forth in U.S. Pat. No. 5,225,202, some examples of coating previously employed are beeswax and glyceryl monostearate; beeswax, shellac and cellulose; and cetyl alcohol, mastic and shellac, as well as shellac and stearic acid (U.S. Pat. No. 2,809,918); polyvinyl acetate and ethyl cellulose (U.S. Pat. No. 3,835,221); and neutral copolymer of polymethacrylic acid esters (Eudragit L30D) (F. W. Goodhart et al., Pharm. Tech., pp. 64-71, April 1984); copolymers of methacrylic acid and methacrylic acid methylester (Eudragits), or a neutral copolymer of polymethacrylic acid esters containing metallic stearates (Mehta et al., U.S. Pat. Nos. 4,728,512 and 4,794,001). Such coatings comprise mix-

tures of fats and fatty acids, shellac and shellac derivatives and the cellulose acid phthlates, e.g., those having a free carboxyl content. See, Remington's at page 1590, and Zeitova et al. (U.S. Pat. No. 4,432,966), for descriptions of suitable enteric coating compositions. Accordingly, increased adsorption in the small intestine due to enteric coatings of cystamine, cysteamine derivatives (including Cystagon®) can result in improvements in cystinosis as well as neurodegenerative diseases including, for example, Huntington's disease.

Generally, the enteric coating comprises a polymeric material that prevents cysteamine or cystamine release in the low pH environment of the stomach but that ionizes at a slightly higher pH, typically a pH of 4 or 5, and thus dissolves sufficiently in the small intestines to gradually release the active agent therein. Accordingly, among the most effective enteric coating materials are polyacids having a pK_a in the range of about 3 to 5. Suitable enteric coating materials include, but are not limited to, polymerized gelatin, shellac, methacrylic acid copolymer type C NF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers, typically formed from methyl acrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters (Eudragit NE, Eudragit RL, Eudragit RS). For example, the enterically coating can comprise Eudragit L30D, triethylcitrate, and hydroxypropylmethylcellulose (HPMC), Cystagon® (or other cysteamine derivative), wherein the coating comprises 10 to 13% of the final product.

By "pharmaceutically acceptable carrier" or "pharmaceutically acceptable vehicle" are meant materials that are suitable for oral administration and not biologically, or otherwise, undesirable, i.e., that may be administered to a subject along with an active ingredient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of a pharmaceutical composition in which it is contained.

Similarly, a "pharmaceutically acceptable" salt, ester or other derivative of an active agent comprise, for example, salts, esters or other derivatives which are not biologically or otherwise undesirable.

"Stabilizing agents" refer to compounds that lower the rate at which pharmaceutical degrades, particularly an oral pharmaceutical formulation under environmental conditions of storage.

By the terms "effective amount" or "therapeutically effective amount" of a enteric formulation of cysteamine or cystamine refers to a nontoxic but sufficient amount of the agent to provide the desired therapeutic effect. As will be pointed out below, the exact amount required will vary from subject to subject, depending on the age, weight, and general condition of the subject, the severity of the condition being treated, and the like. An appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using only routine experimentation.

In one aspect of the disclosure there is provided a stabilized pharmaceutical composition for administration of an cysteamine or cystamine, wherein the cysteamine or cystamine is enterically coated.

The cysteamine or cystamine is present in the composition in a therapeutically effective amount; typically, the composition is in unit dosage form. The amount of cysteamine or cystamine administered will, of course, be dependent on the age, weight, and general condition of the subject, the severity of the condition being treated, and the judgment of the prescribing physician. Suitable therapeutic amounts will be known to those skilled in the art and/or are described in the pertinent reference texts and literature. In one aspect, the dose is administered twice per day at about 0.5-1.0 g/m^2 (e.g., 0.7-0.8 g/m^2) body surface area. Current non-enterically coated doses are about 1.35 g/m2 body surface area and are administered 4-5 times per day.

The entericaly coated cysteamine or cystamine can comprise various excipients, as is well known in the pharmaceutical art, provided such excipients do not exhibit a destabilizing effect on any components in the composition. Thus, excipients such as binders, bulking agents, diluents, disintegrants, lubricants, fillers, carriers, and the like can be combined with the cysteamine or cystamine. For solid compositions, diluents are typically necessary to increase the bulk of a tablet so that a practical size is provided for compression. Suitable diluents include dicalcium phosphate, calcium sulfate, lactose, cellulose, kaolin, mannitol, sodium chloride, dry starch and powdered sugar. Binders are used to impart cohesive qualities to a tablet formulation, and thus ensure that a tablet remains intact after compression. Suitable binder materials include, but are not limited to, starch (including corn starch and pregelatinized starch), gelatin, sugars (including sucrose, glucose, dextrose and lactose), polyethylene glycol, waxes, and natural and synthetic gums, e.g., acacia sodium alginate, polyvinylpyrrolidone, cellulosic polymers (including hydroxypropyl cellulose, hydroxypropyl methylcellulose, methyl cellulose, hydroxyethyl cellulose, and the like), and Veegum. Lubricants are used to facilitate tablet manufacture; examples of suitable lubricants include, for example, magnesium stearate, calcium stearate, and stearic acid, and are typically present at no more than approximately 1 weight percent relative to tablet weight. Disintegrants are used to facilitate tablet disintegration or "breakup" after administration, and are generally starches, clays, celluloses, algins, gums or crosslinked polymers. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and the like. If desired, flavoring, coloring and/or sweetening agents may be added as well. Other optional components for incorporation into an oral formulation herein include, but are not limited to, preservatives, suspending agents, thickening agents, and the like. Fillers include, for example, insoluble materials such as silicon dioxide, titanium oxide, alumina, talc, kaolin, powdered cellulose, microcrystalline cellulose, and the like, as well as soluble materials such as mannitol, urea, sucrose, lactose, dextrose, sodium chloride, sorbitol, and the like.

A pharmaceutical composition may also comprise a stabilizing agent such as hydroxypropyl methylcellulose or polyvinylpyrrolidone, as disclosed in U.S. Pat. No. 4,301,146. Other stabilizing agents include, but are not limited to, cellulosic polymers such as hydroxypropyl cellulose, hydroxyethyl cellulose, methyl cellulose, ethyl cellulose, cellulose acetate, cellulose acetate phthalate, cellulose acetate trimellitate, hydroxypropyl methylcellulose phthalate, microcrystalline cellulose and carboxymethylcellulose sodium; and vinyl polymers and copolymers such as polyvinyl acetate, polyvinylacetate phthalate, vinylacetate crotonic acid copolymer, and ethylene-vinyl acetate copolymers. The stabilizing agent is present in an amount effective to provide the

desired stabilizing effect; generally, this means that the ratio of cysteamine or cystamine to the stabilizing agent is at least about 1:500 w/w, more commonly about 1:99 w/w.

The tablets are manufactured by first enterically coating the cysteamine or cystamine. A method for forming tablets herein is by direct compression of the powders containing the enterically coated cysteamine or cystamine, optionally in combination with diluents, binders, lubricants, disintegrants, colorants, stabilizers or the like. As an alternative to direct compression, compressed tablets can be prepared using wet-granulation or dry-granulation processes. Tablets may also be molded rather than compressed, starting with a moist material containing a suitable water-soluble lubricant.

In an alternative embodiment, the enterically coated cysteamine or cystamine are granulated and the granulation is compressed into a tablet or filled into a capsule. Capsule materials may be either hard or soft, and are typically sealed, such as with gelatin bands or the like. Tablets and capsules for oral use will generally include one or more commonly used excipients as discussed herein.

For administration of the dosage form, i.e., the tablet or capsule comprising the enterically coated cysteamine or cystamine, a total weight in the range of approximately 100 mg to 1000 mg is used. The dosage form is orally administered to a patient suffering from a condition for which an cysteamine or cystamine would typically be indicated, including, but not limited to, cystinosis and neurodegenerative diseases such as Huntington's, Alzheimer's and Parkinson's disease.

The compositions of the disclosure can be used in combination with other therapies useful for treating cystinosis and neurodegenerative diseases and disorders. For example, indomethacin therapy (Indocid® or Endol®) is an anti-inflammatory used to treat rheumatoid arthritis and lumbago, but it can be used to reduce water and electrolyte urine loss. In children with cystinosis, indomethacin reduces the urine volume and therefore liquid consumption by about 30%, sometimes by half. In most cases this is associated with an appetite improvement. Indomethacin treatment is generally followed for several years.

Other therapies can be combined with the methods and compositions of the disclosure to treat diseases and disorders that are attributed or result from cystinosis. Urinary phosphorus loss, for example, entails rickets, and it may be necessary to give a phosphorus supplement. Carnitine is lost in the urine and blood levels are low. Carnitine allows fat to be used by the muscles to provide energy. Hormone supplementation is sometimes necessary. Sometimes the thyroid gland will not produce enough thyroid hormones. This is given as thyroxin (drops or tablets). Insulin treatment is sometimes necessary if diabetes appears, when the pancreas does not produce enough insulin. These treatments have become rarely necessary in children whom are treated with cysteamine, since the treatment protects the thyroid and the pancreas. Some adolescent boys require a testosterone treatment if puberty is late. Growth hormone therapy may be indicated if growth is not sufficient despite a good hydro electrolytes balance. Accordingly, such therapies can be combined with the enterically coated cysteamine and cystamine compositions and methods of the disclosure.

The effectiveness of a method or composition of the disclosure can be assessed by measuring leukocyte cystine concentrations. Dosage adjustment and therapy can be made by a medical specialist depending upon, for example, the severity of cystenosis and/or the concentration of cystine. Additional therapies including the use of omeprazole (Prilosec®) can reduce these symptoms.

In addition, various prodrugs can be "activated" by use of the enterically coated cysteamine. Prodrugs are pharmacologically inert, they themselves do not work in the body, but once they have been absorbed, the prodrug decomposes. The prodrug approach has been used successfully in a number of therapeutic areas including antibiotics, antihistamines and ulcer treatments. The advantage of using prodrugs is that the active agent is chemically camouflaged and no active agent is released until the drug has passed out of the gut and into the cells of the body. For example, a number of produgs use S—S bonds. Weak reducing agents, such as cysteamine, reduce these bonds and release the drug. Accordingly, the compositions of the disclosure are useful in combination with prodrugs for timed release of the drug. In this aspect, a pro-drug can be administered followed by administration of an enterically coated cysteamine compositions of the invention (at a desired time) to activate the pro-drug.

It is to be understood that while the invention has been described in conjunction with specific embodiments thereof, that the foregoing description as well as the examples which follow are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention

EXAMPLES

Subjects

Children with cystinosis, ≥12 years old, and taking regular cysteamine bitartrate (Cystagon®; Mylan, Morgantown, W. Va.) were recruited to the study (Table I). Adult control patients were recruited locally. Patients with cystinosis had a mean leukocyte cystine level of less than 2.0 nmol half-cystine/mg protein over the past year. Cysteamine therapy was discontinued 2 days before admission, and acid suppressants, antibiotics, nonsteroidal anti-inflammatory drugs, prokinetic agents, and antihistamines were discontinued 2 weeks before admission. None of the patients had undergone kidney transplantation. Baseline chemistry, *Helicobacter pylori* serologic study, complete blood count, and urinalysis were performed.

TABLE I

Cystinosis patient data

Patient	Age (yrs.)	Sex	Weight (kg)	Cysteamine dose (mg)*	Serum creatinine (mg/dL)
1	16	Male	61.5	500	1.0
2	14	Male	39.4	406	1.2
3	13	Female	39.1	406	1.5
4	19	Female	38.1	406	1.4
5	13	Female	50.1	500	1.0
6	16	Male	58.7	500	3.1

*Dose of cysteamine base delivered into varying delivery sites

Cysteamine Bitartrate Delivery.

Cysteamine was infused through a silicone rubber nasoenteric tube (Dentsleeve Pty Ltd, Australia), 3 mm in diameter and 4.5 meters long. The tube, specifically made for this study, had a tungsten-weighted tip, and immediately proximal to this was an inflatable balloon (5-mL capacity). Immediately proximal to the balloon was an infusion port (1 mm diameter) through which the drug was delivered. After an overnight fast (except for water), the dose of cysteamine bitartrate (10 mg/kg/dose of base, maximum of 500 mg) was dissolved in 10 mL of water and infused over 1 to 2 minutes. On day 1 of the study, the nasoenteric tube was inserted into the stomach. By day 3 of the study the tube had passed into the proximal small intestine (SI) just distal to the ligament of Treitz (confirmed fluoroscopically). The balloon was then inflated, and peristalsis propelled the tube distally. Tube posi-

tion within the cecum was confirmed fluoroscopically on day 5 (day 7 in 4 patients because of slow transit). If the tube had migrated too far, it was retracted into the desired location.

Serum Gastrin, Cysteamine and Leukocyte Cystine Measurements.

After an overnight fast (except for water) blood samples were taken at baseline and at varying intervals after intraluminal delivery of cysteamine. Serum gastrin levels were then measured at 30, 60, 90, and 120 minutes and 3 and 4 hours; cysteamine levels were measured at 0, 5, 10, 20, 30, 45, 60, 75, 90, 105, 120, and 150 minutes and 3, 4, 6, 8, 10, 12, and 16 hours; leukocyte cystine levels were measured at 1, 2, 3, 4, 6, and 12 hours in patients with cystinosis only. Gastrin was measured in picograms/mL with the Diagnostic Products Corporation (Los Angeles, Calif.) gastrin radioimmunoassay-assay kit. Leukocyte cystine levels were measured in nmol half-cystine per mg protein by the Cystine Determination Lab (La Jolla, Calif.).

To measure plasma cysteamine, 100-μL plasma samples were collected in heparinized vacutainers and spun in a centrifuge within 1 hour, and plasma was stored at −18° C. The concentration of cysteamine was measured by use of tandem mass spectroscopy (API 2000 LC/MS/MS; Applied Biosystems, Foster City, Calif.). Cysteamine concentrations were calculated with a calibration curve that was prepared by spiking plasma with buffered cysteamine solutions, and quality control samples were analyzed with each batch.

Statistical Analysis.

Mixed model restricted maximum likelihood (REML) repeated measures analysis of variance with subjects as a random effect was performed on the absolute leukocyte cystine levels, on the leukocyte cystine level changes from baseline, and on the "area over the curve" (AOC) for leukocyte cystine level changes from baseline after cysteamine administration for the subjects with cystinosis. AOC is computationally analogous to area under the curve, but it is applied when values are predominantly decreasing below baseline values. Large AOC values reflect large decreases, and a negative AOC reflects a net increase in value. Main effects for site of delivery, time after delivery, and the interaction between site and time were tested, except just the site effect was tested for AOCs. In the absence of significant interaction when a main effect was detected, Tukey's honestly significant difference test (HSD) was applied to identify where differences occurred within a 5% family wise error rate. The Tukey HSD procedure controls for overall significance level when performing all pairwise comparisons. An additional analysis was performed with plasma cysteamine C_{max} added to the AOC model.

REML repeated measures analyses of variance with subjects as a random effect were also performed as described above on AUC and the C_{max} over time for plasma cysteamine levels separately for the subjects with cystinosis and control subjects and with both subject groups combined. Differences between means for the 3 sites were tested, plus group and group x site interaction effects for the combined groups. If a site effect was detected, Tukey's HSD was applied to determine which sites differed from each other.

REML repeated measures analyses of variance were also performed as described above on gastrin levels. The analyses were performed on 2 versions of datasets: the full dataset and all data after omitting observations collected at 30 minutes (1 subject was missing a blood sample taken at 30 minutes after small intestinal cysteamine delivery). A 5% significance level was used without adjustment for all statistical testing.

Six patients with cystinosis, (3 male, 3 female) with a mean age of 15.2 years (range 13-19 years) were recruited into the study (Table I). Eight healthy adult control patients (6 male, 2 female) with a mean age of 23.2 years (range 19-28 years) were enrolled. None of the children with cystinosis had undergone kidney transplantation. All control subjects received 500 mg cysteamine base, whereas the mean dose for subjects with cystinosis was 453 mg (range 406-500 mg). All subjects had normal liver function test results. In all subjects the nasoenteric tube passed successfully from the stomach into the upper SI; however, it did not progress any further in 2 subjects with cystinosis. In 2 of the control subjects the tube only reached the mid-ileum but did, however, progress to the cecum in 8 subjects (4 control subjects, 4 with cystinosis). There were no reported adverse effects with the insertion or removal of the nasoenteric tube (FIG. **1**).

Symptoms.

Only 2 patients (1 male, 1 female) with cystinosis reported regular GI symptoms before the study, and these had responded to acid-suppression therapy. The male subject had severe retching and emesis about 15 minutes after receiving intragastric cysteamine but did not have any symptoms when the drug was infused into the proximal small intestine. The female child with cystinosis had mild transient nausea after SI drug delivery only. No other symptoms were reported after any other cysteamine delivery in the children with cystinosis. There were no associated adverse events with tube placement or removal.

Plasma Cysteamine.

Among the subjects with cystinosis as measured by analysis of variance, the mean plasma cysteamine C_{max} and AUCs (of the concentration-time gradient) differed by site of cysteamine delivery (both $P<0.03$). Site (†) refers to either patients with cystinosis or control subjects. For the plasma cysteamine AUCs, the means differed between the duodenal and both gastric and cecal sites of delivery (Tukey HSD global $P<0.05$). Among control subjects, the mean AUC did not differ among delivery sites ($P>0.4$), but mean C_{max} did ($P<0.05$). For both cystinosis and control groups the mean C_{max} values differed only between the duodenum and cecum; mean C_{max} values after duodenal versus gastric or gastric versus cecal delivery were not statistically different (Tables II and III).

TABLE II

Mean plasma cysteamine C_{max} levels (γmol/L) and area under curve (AUC) measurements in cystinosis subjects, controls, and combined cystinosis and control subjects, after delivery of cysteamine into the stomach, small intestine, and cecum

	C_{max} Cystinosis	AUC Cystinosis	C_{max} Control	AUC Control	C_{max} Combined	AUC Combined
Stomach	35.5 (20.5)	3006 (1112)	39.5 (16.4)	3613 (1384)	37.8 (17.6)	3353 (1267)
Small Intestine	55.8 (13.0)	4299 (1056)	51.1 (20.7)	3988 (1659)	53.2 (17.4)	4047 (1376)
Cecum	21.9 (13.1)	3002 (909)	23.1 (15.3)	2804 (1323)	22.5 (13.2)	2903 (1056)

The standard deviations are in parenthesis

TABLE III

Comparisons of mean plasma cysteamine C_{max} (γmol/L) and AUC measurements for combined cystinosis subjects and control subjects among delivery sites

	AUC	C_{max}
P value*	<0.01	<0.01
Stomach vs SI	+	+
Stomach vs Cecum	–	–
SI vs Cecum	+	+

+ Significant difference using Tukey's HSD test (α = 0.05)
– No significant difference
*ANOVA test for equality of three delivery sites

When data from the control subjects were combined with cystinosis subject data, there was both a group effect (P<0.05) and a site effect (P<0.01) for AUCs, with a significant difference between mean AUC levels for the duodenum versus both the stomach and cecum. C_{max} values differed among sites (P<0.01) but not between groups (P>0.4). Group (*) refers to site of intestinal delivery. C_{max} differed between duodenum versus both stomach and cecum (FIG. **2**).

Leukocyte Cystine.

There were significant differences among the 3 sites of delivery for cystine levels (P<0.04), changes from baseline values (P<0.0001), and AOCs for changes from baseline (P<0.02). A Tukey HSD test, which controls for multiple comparisons, showed that mean leukocyte cystine levels differed between the cecum and stomach sites, but that cecum versus duodenum and stomach versus duodenum produced similar mean values. When the absolute cystine levels or AOCs for changes from baseline levels were evaluated, the significant differences in sites were found between the duodenum and both the stomach and cecum, but not between stomach and cecum (Tukey HSD global P<0.05) (FIG. **3**). Plasma cysteamine C_{max} and AUC contributed a statistical effect on AOC (P<0.001 and <0.02, respectively), even after controlling for delivery site (FIG. **4**).

Blood Gastrin.

For the full gastrin dataset, there was a significant difference among the means for the different delivery sites (P<0.1), with the cecum resulting in a lower mean from that of the stomach and small intestine. Both group * and site † significant effects were detected after omitting observations from 30 minutes after delivery (P<0.05 and P<0.01, respectively). The 30-minute observations were omitted because of a missing data set. For these observations, mean levels of gastrin after delivery in the cecum were different from those from both the duodenum and stomach, although the latter did not differ from each other. The 1 boy (14 years) who had severe GI symptoms after intragastric, but not enteric or cecal, cysteamine delivery had a rise in baseline gastrin from 70 pg/mL to 121 pg/mL at 30 minutes after gastric cysteamine. Within the control group, more than half of the baseline and post-cysteamine gastrin levels remained undetectable (<25 pg/mL), and none of the control subjects had a significant rise in gastrin after cysteamine delivery into any site.

Patients with cystinosis are required to ingest oral cysteamine (Cystagon®) every 6 hours, day and night. When taken regularly, cysteamine can deplete intracellular cystine by up to 90% (as measured in circulating white blood cells), and this has been shown to reduce the rate of progression to kidney failure/transplantation and also to obviate the need for thyroid replacement therapy. Unfortunately, because of the strict treatment regimen and the associated symptoms, non-adherence with cysteamine therapy remains a problem, particularly among adolescent and young adult patients. Certainly, by reducing the frequency of required cysteamine dosing adherence can be improved. The disclosure shows a strong statistical association between the maximum plasma concentration (C_{max}) of cysteamine and AOC measurements for leukocyte cystine (P<0.001). A higher C_{max} is achieved after delivery of cysteamine into the small intestine than when infused into the stomach or colon; this may be due to improved absorption rate from the SI, greater surface area of the SI, or less cysteamine undergoing hepatic first pass elimination when absorbed rapidly through the small intestine. When data were combined for patients with cystinosis and control subjects, there was a statistical difference between duodenal versus both gastric and colonic delivery for plasma cysteamine C_{max} and AUC levels (both P<0.05). The lack of similar statistical significance for the cystinosis group alone may simply reflect the small number of patients studied. Changes from baseline leukocyte cystine levels were statistically significant for absolute cystine levels and for AOC when cysteamine was infused into the duodenum compared with both stomach and colon. As shown in FIG. **3**, the leukocyte cystine levels remained below pre-delivery levels for up to 12 hours after a single dose of cysteamine into the small intestine. This would suggest that effective absorption of cysteamine through the SI, by causing a higher C_{max} and AUC on the cysteamine concentration-time gradient, could lead to prolonged depletion of leukocyte cystine and possibly less frequent daily dosing. Another explanation would be that by achieving a high enough plasma cysteamine concentration, more drug reaches the lysosome (where cystine accumulates). In the lysosome the cysteamine reacts with cystine forming the mixed disulfide of cysteamine and cysteine. The mixed disulfide exits the lysosome presumably via the lysine carrier. In the cytosol the mixed disulfide can be reduced by its reaction with glutathione. The cysteine released can be used for protein or glutathione synthesis. The cysteamine released from the mixed disulfide reenters the lysosome where it can react with another cystine molecule. Thus 1 molecule of cysteamine may release many molecules of cystine from the lysosome. This study showed a dramatic decrease in leukocyte cystine within an hour of cysteamine delivery. In retrospect, the finding from this study was that the leukocyte cystine levels remained at the 1-hour level for 24 hours, and even at 48 hours after delivery the levels had not returned to the pre-cysteamine level.

Cysteamine is a potent gastric acid-secretagogue that has been used in laboratory animals to induce duodenal ulceration; studies in humans and animals have shown that cysteamine-induced gastric acid hypersecretion is most likely mediated through hypergastrinemia. In previous studies performed in children with cystinosis who suffered regular upper gastrointestinal symptoms, a single oral dose of cysteamine (11-23 mg/kg) was shown to cause hypergastrinemia and a 2- to 3-fold rise in gastric acid-hypersecretion. Symptoms suffered by these individuals included abdominal pain, heartburn, nausea, vomiting, and anorexia. Interestingly, only 2 of 6 subjects with cystinosis (who were known to suffer regular cysteamine-induced GI symptoms) had increased gastrin levels and symptoms, including nausea, retching, and discomfort after intragastric cysteamine. Gastrin levels were only available after small intestinal administration in 1 of the 2 children and the levels remained the same as baseline. Neither child had symptoms after enteric cysteamine delivery. None of the other patients with cystinosis or control subjects had an increase in gastrin levels with cysteamine infused into any site. This would suggest that cysteamine-induced hypergastrinemia may arise as a local effect on the gastric antral-predominant G-cells only in susceptible individuals. In addi-

tion, plasma gastrin levels usually peaks after intragastric delivery within 30 minutes, whereas the plasma cysteamine levels peaked later.8,10 In 2 previous studies, children with cystinosis were shown to have a significant rise in plasma gastrin levels after receiving intragastric cysteamine; as part of these study's entry criteria all subjects did, however, suffer with regular GI symptoms. Data from this study would suggest that cysteamine does not cause hypergastrinemia, and therefore acid-hypersecretion, in all patients with cystinosis. Thus acid suppression therapy would not be recommended in patients with cystinosis without upper GI symptoms.

The data suggest that direct administration of cysteamine into the jejunum may result in prolonged leukocyte cystine depletion. In a previous study, a child who had a gastrojejunal feeding tube for oral feeding aversion and severe UGI symptoms, responded to intrajejunal cysteamine with a 3-fold rise in serum gastrin as compared with drug administration into the stomach. The leukocyte cystine response was not measured in this child. Therefore patients with jejunal feeding tubes will have to be further evaluated.

FIGS. **5** and **6** shows results from a patient that remained on the twice daily EC-cysteamine for an extended period of time. Over this period the patient's leukocyte cystine levels have been measured regularly. The dose of twice daily EC-cysteamine is titrated against the patient's symptoms and cystine levels. The patient's cystine levels have been 0.4, 1.0, 0.36.

This study provides data that may be used to improve the quality of life for patients with cystinosis. The present formulation of Cystagon® comprises cysteamine in a capsule that will dissolve rapidly on contact with water, most likely within the stomach.

Although a number of embodiments and features have been described above, it will be understood by those skilled in the art that modifications and variations of the described embodiments and features may be made without departing from the teachings of the disclosure or the scope of the invention as defined by the appended claims.

What is claimed is:

1. A method of administering cysteamine or a pharmaceutically acceptable salt thereof to a patient with cystinosis, comprising administering to said patient a pharmaceutical composition comprising cysteamine or a pharmaceutically acceptable salt thereof, wherein the composition increases delivery of cysteamine or the pharmaceutically acceptable salt thereof to the small intestine, and wherein the frequency of administering is less than four times daily.

2. The method of claim **1**, wherein each dose of cysteamine is about 0.5-1.0 g/m^2 body surface area.

3. The method of claim **1**, wherein the total daily dose of cysteamine is about 1.35 g/m^2 body surface area or less.

4. The method of claim **1**, wherein the cysteamine or salt thereof is cysteamine bitartrate.

5. The method of claim **1**, wherein the composition comprises enterically coated cysteamine or the salt thereof.

6. The method of claim **5**, wherein each dose of cysteamine is about 0.5-1.0 g/m^2 body surface area.

7. The method of claim **5**, wherein the total daily dose of cysteamine is about 1.35 g/m^2 body surface area or less.

8. The method of claim **5**, wherein the cysteamine or salt thereof is cysteamine bitartrate.

9. The method of claim **5**, wherein the composition comprises a coating selected from the group consisting of polymerized gelatin, shellac, methacrylic acid copolymer type CNF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers formed from methyl acrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters.

10. A method of administering cysteamine or a pharmaceutically acceptable salt thereof to a patient with cystinosis, comprising administering to said patient a pharmaceutical composition comprising cysteamine or a pharmaceutically acceptable salt thereof, twice per day, wherein the composition increases delivery of cysteamine or the pharmaceutically acceptable salt thereof to the small intestine.

11. The method of claim **10**, wherein each dose of cysteamine is about 0.5-1.0 g/m^2 body surface area.

12. The method of claim **10**, wherein the total daily dose of cysteamine is about 1.35 g/m^2 body surface area or less.

13. The method of claim **10**, wherein the cysteamine or salt thereof is cysteamine bitartrate.

14. The method of claim **10**, wherein the composition comprises enterically coated cysteamine or the salt thereof.

15. The method of claim **14**, wherein each dose of cysteamine is about 0.5-1.0 g/m^2 body surface area.

16. The method of claim **14**, wherein the total daily dose of cysteamine is about 1.35 g/m^2 body surface area or less.

17. The method of claim **14**, wherein the cysteamine or salt thereof is cysteamine bitartrate.

18. The method of claim **14**, wherein the composition comprises a coating selected from the group consisting of polymerized gelatin, shellac, methacrylic acid copolymer type CNF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers formed from methyl acrylate, ethyl acrylate, methyl methacrylate and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters.

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EXHIBIT D

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Powell et al.

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(45) **Date of Patent: *Nov. 3, 2015**

(54) **DELAYED RELEASE CYSTEAMINE BEAD FORMULATION, AND METHODS OF MAKING AND USING SAME**

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(57) **ABSTRACT**

An enteric-coated bead dosage form of cysteamine, and related methods of manufacture and use, are disclosed.

8 Claims, No Drawings

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DELAYED RELEASE CYSTEAMINE BEAD FORMULATION, AND METHODS OF MAKING AND USING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation application of U.S. application Ser. No. 14/306,303, filed Jun. 17, 2014, which claims benefit under 35 U.S.C. §119(e) of U.S. Provisional Patent Application Ser. No. 61/835,965 filed Jun. 17, 2013, the disclosures of which are hereby incorporated by reference herein.

BACKGROUND

1. Field of the Disclosure

The disclosure relates generally to delayed release formulations of cysteamine and pharmaceutically acceptable salts thereof, and related methods of making and treatment, e.g. treatment of cystinosis and other metabolic and neurodegenerative diseases including non-alcoholic fatty liver disease (NAFLD), Huntingon's disease, Parkinson's disease, Rett Syndrome and others, use as free radical and radioprotectants, and as hepto-protectant agents. More particularly, the disclosure relates to enteric coated beads comprising cysteamine or a pharmaceutically acceptable salt thereof.

2. Brief Description of Related Technology

Cystinosis is a rare, autosomal recessive disease caused by intra-lysosomal accumulation of the amino acid cystine within various tissues, including the spleen, liver, lymph nodes, kidney, bone marrow, and eyes. Nephropathic cystinosis is associated with kidney failure that necessitates kidney transplantation. A specific treatment for nephropathic cystinosis is the sulfhydryl agent, cysteamine. Cysteamine has been shown to lower intracellular cystine levels, thereby reducing the rate of progression of kidney failure in children.

An enterically-coated cysteamine composition has been described, for increasing delivering of cysteamine to the small intestine and resulting in less frequent dosing compared to non enteric-coated cysteamine.

SUMMARY

One aspect of the disclosure provides a pharmaceutical dosage form including a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core, wherein the plurality of beads is characterized by a distribution of particle sizes.

Another aspect of the disclosure provides a pharmaceutical dosage form including a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core, wherein the plurality of beads is characterized by irregular bead shapes.

Yet another aspect of the disclosure provides a pharmaceutical dosage form including a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core, wherein the plurality of beads is characterized by a distribution of enteric membrane thicknesses.

Still another aspect of the disclosure provides a method of making a pharmaceutical dosage form, including any embodiment described herein, by a method including coating

a core particle including cysteamine or a pharmaceutically acceptable salt thereof and an excipient with an enteric polymer to form an enteric membrane. The method can include sorting core particles prior to enteric coating, to provide a selected core particle size distribution. The method can also include sorting enteric coated beads to provide a selected bead size distribution.

Yet another aspect of the disclosure provides a method for treating a patient in need of cysteamine comprising administering to the patient a dosage form described herein, including any embodiment described herein.

Still another aspect of the disclosure provides dosage forms and related methods according the disclosure herein wherein the primary active component is cystamine rather than cysteamine or a pharmaceutically acceptable salt thereof.

For the compositions and methods described herein, optional features, including but not limited to components, compositional ranges thereof, substituents, conditions, and steps, are contemplated to be selected from the various aspects, embodiments, and examples provided herein.

Further aspects and advantages will be apparent to those of ordinary skill in the art from a review of the following detailed description. While the dosage form, method of making, and method of treatment are susceptible of embodiments in various forms, the description hereafter includes specific embodiments with the understanding that the disclosure is illustrative, and is not intended to limit the invention to the specific embodiments described herein.

DETAILED DESCRIPTION

Described herein is pharmaceutical dosage form that includes a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core particle. The plurality of beads can be characterized by a distribution of particle sizes. The plurality of beads can be characterized by irregular bead shapes. The plurality of beads can be characterized by a distribution of enteric membrane thicknesses. Also disclosed herein are a method for the preparation of the dosage form, including coating a core particle including cysteamine or a pharmaceutically acceptable salt thereof and an excipient with an enteric polymer to form the enteric membrane. Optionally, the core particle can be formed by a wet granulation method. Optionally, granules are sorted (e.g., via sieving) to a desired particle size range prior to enteric coating, and optionally again following enteric coating. Also disclosed herein are treatment methods including administering the dosage form to a patient in need thereof.

Cysteamine-containing, enteric-coated beads characterized by a distribution of particle sizes were shown to exhibit advantageous pharmacokinetics. Without intending to be bound by any particular theory, it is contemplated that the pharmacokinetics are influenced by the plurality of enteric-coated beads having a distribution of core particle sizes.

Cysteamine-containing, enteric-coated beads characterized by a irregular bead shapes were shown to exhibit advantageous pharmacokinetics. Without intending to be bound by any particular theory, it is contemplated that the pharmacokinetics are influenced by the plurality of enteric-coated beads having irregular bead shapes.

Cysteamine-containing, enteric-coated beads characterized by a distribution of enteric membrane thicknesses were shown to exhibit advantageous pharmacokinetics. Without intending to be bound by any particular theory, it is contem-

plated that the pharmacokinetics are influenced by the plurality of enteric-coated beads having a distribution of enteric membrane thicknesses.

In one aspect the distribution of enteric membrane thicknesses can be stated in terms of weight gain of enteric membrane material based on the total weight of the coated beads. Thus, in one embodiment, the distribution of enteric membrane thicknesses will be at least 2% based on the total weight of the coated beads. In another embodiment, the distribution of enteric membrane thicknesses will be at least 3%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 4%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 5%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 6%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 7%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 8%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 9%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 10%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 11%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 12%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 13%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 14%. For example, the difference in enteric membrane thickness from bead to bead can be in a range of +/−1-7% based on the total weight of the coated beads. The distribution of enteric membrane thicknesses can be in a range of about 2% to about 14% based on the weight of the coated beads, or in a range of about 3% to about 13%, or in a range of about 4% to about 12%, or in a range of about 5% to about 11%, or in a range of about 6% to about 10%, or in a range of about 7% to 9%, or in a range of about 3% to 14%, or in a range of about 4% to 14%, or in a range of about 4% to 13%, or in a range of about 4% to about 12%, for example. In one embodiment, the absorption (AUC) of the dosage form when dosed orally is advantageously increased, compared to other dosage forms of cysteamine. Without intending to be bound by any particular theory, it is contemplated that the increase in absorption is influenced by the dosage form exhibiting a pseudo-extended release profile. The pseudo-extended release profile is contemplated to be influenced by one or more factors, including a distribution of enteric membrane thicknesses, a distribution of bead particle sizes, and the beads having irregular bead shapes. For example, in an embodiment wherein the beads have a distribution of enteric membrane thicknesses, it is contemplated that for beads which have a relatively thin coating, the coating will completely dissolve at the trigger pH relatively quickly to release the cysteamine composition, whereas for beads having a relatively thick coating the coating will take somewhat longer to completely dissolve and release the cysteamine composition. In another aspect, in an embodiment where the beads have a distribution of particle sizes and/or irregular bead shapes, it is contemplated that the gut transit time of the beads could be varied due to bead size and/or shape, such that the transit time until reaching the enteric membrane dissolution pH is varied, thus contributing to a pseudo-extended release profile. In another embodiment, the dosage form exhibits substantially equivalent (e.g., bioequivalent) Cmax and/or AUC characteristics when administered orally inside a capsule shell or without a capsule shell.

The dosage form provides a progressive and predictable absorption curve. In one type of embodiment, the Tmax of the

dosage form when dosed orally is advantageously more stable on a dose-to-dose basis, because the beads are individually enteric-coated. A predictable, consistent Tmax is highly advantageous for accomplishing a more consistent, sustained reduction of leukocyte cystine levels by use of cysteamine. For example, process-related variations in enteric membrane thickness or other influences on enteric membrane dissolution will affect only a fraction of the cysteamine in the dosage form and will tend to lead to the pseudo-extended release behavior described above. In contrast, enteric-coated capsules comprising cysteamine microspheres exhibited significant variability in absorption time from capsule to capsule.

In another embodiment, the dosage form exhibits advantageous storage stability, e.g. as measured by the amount of cystamine present following storage and/or by the total amount of related substances. The storage stability can be assessed following storage at typical ambient conditions (e.g. 25° C. and 40% relative humidity) or at accelerated stability conditions involving increased temperature and/or humidity.

The dosage form and methods are contemplated to include embodiments including any combination of one or more of the additional optional elements, features, and steps further described below (including those shown in the figures and Examples), unless stated otherwise.

In jurisdictions that forbid the patenting of methods that are practiced on the human body, the meaning of "administering" of a composition to a human subject shall be restricted to prescribing a controlled substance that a human subject will self-administer by any technique (e.g., orally, inhalation, topical application, injection, insertion, etc.). The broadest reasonable interpretation that is consistent with laws or regulations defining patentable subject matter is intended. In jurisdictions that do not forbid the patenting of methods that are practiced on the human body, the "administering" of compositions includes both methods practiced on the human body and also the foregoing activities.

As used herein, the term "comprising" indicates the potential inclusion of other agents, elements, steps, or features, in addition to those specified.

As used herein, the term wt. % is the weight percent based on the total weight, e.g. of the core particle, or enteric membrane, or total bead, as described in context. Unless stated otherwise, the wt. % is intended to describe the weight percent based on dry weight (e.g., for a core particle following drying).

All ranges set forth herein include all possible subsets of ranges and any combinations of such subset ranges. By default, ranges are inclusive of the stated endpoints, unless stated otherwise Where a range of values is provided, it is understood that each intervening value between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also contemplated to be part of the disclosure.

Unless expressly stated otherwise, all references to cysteamine herein are intended to encompass pharmaceutically-acceptable salts thereof, and for every reference to cysteamine herein the use of cysteamine bitartrate is specifically contemplated as an embodiment. As described in the Summary above, embodiments of the dosage forms and methods

described herein can employ cystamine as the primary active component, rather than cysteamine or a pharmaceutically acceptable salt thereof.

Unless expressly stated otherwise, reference herein to a bead and properties thereof is intended to be interpreted as applying equally to a collection of beads (e.g., a plurality of such beads). Likewise, unless expressly stated otherwise, reference herein to a core particle and properties thereof is intended to be interpreted as applying equally to a collection of core particles (e.g., a plurality of such core particles).

As described above, a pharmaceutical dosage form is contemplated that includes a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core particle, wherein the plurality of beads is characterized by a distribution of particle sizes.

In one embodiment, the particle sizes of the beads are in a range of about 0.7 mm to about 2.5 mm, or about 0.7 mm to about 2.8 mm, or about 0.8 mm to about 1.7 mm. For example, the target bead size can be up to 2.5 mm with no more than 10 percent variation over this size, to a maximum size of 2.8 mm.

As the particle size of the beads becomes too small, the variability in cysteamine content increases. As the particle size becomes too large, the beads are too large for use in drug products that are labeled to be administered via sprinkling (e.g., on applesauce or other soft foods, such as jellies) and swallowed without chewing, or administered via an enteral feeding tube. Also as the particle size increases, it was found that the larger particles get coated more than the smaller particles, resulting in lower relative assay when compared to use of smaller particles. To compensate, relatively more such beads would be needed in order to meet the label strength per capsule, but because salts such as cysteamine bitartrate already have a high molecular weight, filling a capsule shell with sufficient large particles to meet the label strength per capsule becomes difficult or impossible (e.g. to fill a size 0 capsule to a 75 mg strength of cysteamine free base). Accordingly the bead particle size in one type of embodiment is up to 1.7 mm.

The distribution of bead particle sizes for various non-exclusive embodiments of the invention can be characterized in ways.

In one embodiment, the beads can be characterized by 5% or less of the beads by weight being retained on a #12 mesh (1.68 mm) screen and 10% or less by weight passing through a #20 mesh (0.84 mm) screen. In another embodiment, at least 80% by weight of the beads have a particle size in a range of about 850 μm to about 1180 μm, e.g. as determined by sieving.

The distribution of bead sizes can be characterized by a gradation test via analytical sieving. Thus, in another embodiment the distribution of bead sizes is characterized by 0% of the beads being retained on a 1700 μm sieve and less than 5% by weight of the beads being retained on a 1400 μm sieve. Optionally less than 30% by weight of the beads are retained on a 1180 μm sieve. Optionally less than 70% by weight of the beads are retained on a 1000 μm sieve. Optionally less than 20% by weight of the beads are retained on a 850 μm sieve. Optionally at least 15% by weight of the beads are retained on a 1180 μm sieve. Optionally at least 50% by weight of the beads are retained on a 1000 μm sieve. Optionally at least 10% by weight of the beads being retained on a 850 μm sieve.

Thus, for example, the distribution can be characterized by 0% of the beads being retained on a 1700 μm sieve and less than 5% by weight of the beads being retained on a 1400 μm sieve, and about 20% to about 30% by weight of the beads being retained on a 1180 μm sieve and then about 50% to about 70% (or about 55% to about 65%) by weight of the beads being retained on a 1000 μm sieve and then about 10% to about 20% by weight of the beads being retained on a 850 μm sieve.

In another embodiment, the distribution of bead sizes can be characterized by a median particle size in a range of about 850 μm to about 1180 μm.

The bead core particle can comprise one or more excipients. In one type of embodiment, the excipients can include one or more fillers, binders, and surfactants. Other optional ingredients can include, but are not limited to, glidants, lubricants, disintegrants, swelling agents, and antioxidants.

Fillers include, but are not limited to, lactose, saccharose, glucose, starch, microcrystalline cellulose, microfine cellulose, mannitol, sorbitol, calcium hydrogen phosphate, aluminum silicate, amorphous silica, and sodium chloride, starch, and dibasic calcium phosphate dehydrate. In one type of embodiment, the filler is not water soluble, although it may absorb water. In one type of embodiment, the filler is a spheronization aid. Spheronization aids can include one or more of crospovidone, carrageenan, chitosan, pectinic acid, glycerides, β-CD, cellulose derivatives, microcrystalline cellulose, powdered cellulose, polyplasdone crospovidone, and polyethylene oxide. In one embodiment, the filler includes microcrystalline cellulose.

Binders include, but are not limited to, cellulose ethers, methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, propyl cellulose, hydroxypropyl cellulose, lower-substituted hydroxypropyl cellulose, hydroxypropylmethyl cellulose (hypromellose, e.g. hypromellose 2910, METHOCEL E), carboxymethyl cellulose, starch, pregelatinized starch, acacia, tragacanth, gelatine, polyvinyl pyrrolidone (povidone), cross-linked polyvinyl pyrrolidone, sodium alginate, microcrystalline cellulose, and lower-substituted hydroxypropyl cellulose. In one embodiment, the binders are selected from wet binders. In one type of embodiment, the binder is selected from cellulose ethers, e.g. hypromellose.

Surfactants include, but are not limited to, anionic surfactants, including sodium lauryl sulfate, sodium deoxycholate, dioctyl sodium sulfosuccinate, and sodium stearyl fumarate, nonionic surfactants, including polyoxyethylene ethers, and polysorbate 80, and cationic surfactants, including quaternary ammonium compounds. In one embodiment the surfactant is selected from anionic surfactants, e.g. sodium lauryl sulfate.

Disintegrants include, but are not limited to, starch, sodium cross-linked carboxymethyl cellulose, carmellose sodium, carmellose calcium, cross-linked polyvinyl pyrrolidone, and sodium starch glycolate, low-substituted hydroxypropyl cellulose, hydroxypropyl starch.

Glidants include, but are not limited to, polyethylene glycols of various molecular weights, magnesium stearate, calcium stearate, calcium silicate, fumed silicon dioxide, magnesium carbonate, magnesium lauryl sulfate, aluminum stearate, stearic acid, palmitic acid, cetanol, stearol, and talc.

Lubricants include, but are not limited to, stearic acid, magnesium stearate, calcium stearate, aluminum stearate, and siliconized talc.

The amount of cysteamine free base in the core particle can be at least 10 wt. % or at least 15 wt. %, or at least 20 wt. %, or at least 25 wt. %, or at least 30 wt. %. For example, the amount of cysteamine bitartrate can be at least 50 wt. %, or at least 55 wt. %, or at least 60 wt. %, or at least 65 wt. %, or at least 70 wt. %, or at least 75 wt. %, or at least 80 wt. %, or at least 85 wt. % of the core particle, for example in a range of about 60 wt. % to about 90 wt. % or about 65 wt. % to about

85 wt. %. It is understood that any and all ranges including these values as endpoints is contemplated, for example, at least about 15 wt. % to about 90 wt. %, or at least about 20 wt. % to about 85 wt. %, or at least about 30 wt. % to about 85 wt. %, or at least about 50 wt. % to about 90 wt. %. As the dose of cysteamine free base can be up to about 2 g/m^2/day, and the amount of free base is relatively small compared to the molecular weight of salts (e.g. the bitartrate salt) it is preferred that the core particle have as much active ingredient as possible while allowing the creation and processing of core particles.

The amount of filler in the core particle is not particularly limited. In embodiments, the amount of filler (e.g. microcrystalline cellulose) can be in a range of about 10 wt. % to about 30 wt. %, or about 16 wt. % to about 23 wt. %, or at least 19 wt. % or at least 19.5 wt. %, for example about 20 wt. %.

The amount of binder in the core particle is not particularly limited. In embodiments, the amount of binder (e.g. hypromellose) can be in a range of about 1 wt. % to about 10 wt. %, or about 2 wt. % to about 8 wt. %, or about 4 wt. % to about 6 wt. %, for example about 5 wt. %.

The amount of surfactant, e.g. as a processing aid, in the core particle is not particularly limited. In embodiments, the amount of surfactant (e.g. microcrystalline cellulose) can be in a range of about 0.1 wt. % to about 1 wt. %, or about 0.2 wt. % to about 0.8 wt. %, or about 0.4 wt. % to about 0.6 wt. %, for example about 0.5 wt. %.

The enteric (gastro-resistant) membrane material, e.g. polymer, can be one that will dissolve in intestinal juices at a pH level higher than that of the stomach, e.g. a pH of greater than 4.5, such as within the small intestine, and therefore permit release of the active substance in the regions of the small intestine and substantially not in the upper portion of the GI tract. In one type of embodiment, the enteric material begins to dissolve in an aqueous solution at pH between about 4.5 to about 5.5. In another type of embodiment, the enteric material rapidly dissolves in an aqueous solution at pH between of about 5. In another type of embodiment, the enteric material rapidly dissolves in an aqueous solution at pH between of about 5.5.

For example, pH-sensitive materials will not undergo significant dissolution until the dosage form has emptied from the stomach. The pH of the small intestine gradually increases from about 4.5 to about 6.5 in the duodenal bulb to about 7.2 in the distal portions of the small intestine (ileum). In order to provide predictable dissolution corresponding to the small intestine transit time of about 3 hours (e.g., 2-3 hours) and permit reproducible release therein, the membrane should begin to dissolve within the pH range of the duodenum, and continue to dissolve at the pH range within the small intestine. Therefore, the amount (thickness) of enteric membrane should be sufficient to be substantially dissolved during the approximate three hour transit time within the small intestine (e.g., the proximal and mid-small intestine).

Enteric (gastro-resistant) materials can include, but are not limited to, one or more of the following: cross-linked polyvinyl pyrrolidone; non-cross linked polyvinylpyrrolidone; hydroxypropylmethyl cellulose phthalate, hydroxypropylmethyl cellulose acetate succinate, cellulose acetate succinate; cellulose acetate phthalate, hydroxypropylmethyl cellulose acetate succinate, cellulose acetate trimellitate; starch acetate phthalate; polyvinyl acetate phthalate; carboxymethyl cellulose; methyl cellulose phthalate; methyl cellulose succinate; methyl cellulose phthalate succinate; methyl cellulose phthalic acid half ester; ethyl cellulose succinate; carboxymethylamide; potassium methacrylatedivinylbenzene copolymer; polyvinylalcohols; polyoxyethyleneglycols; polyethylene glycol; sodium alginate; galactomannone; carboxypolymethylene; sodium carboxymethyl starch; copolymers of acrylic acid and/or methacrylic acid with a monomer selected from the following: methyl methacrylate, ethyl methacrylate, ethyl acrylate, butyl methacrylate, hexyl methacrylate, decyl methacrylate, lauryl methacrylate, phenyl methacrylate, methyl acrylate, isopropyl acrylate, isobutyl acrylate, or octadecyl acrylate, e.g. EUDRAGIT-L and -S series, including L 100-55, L 30 D-55, L 100, S 100, L 12.5, and S 12.5, available from Evonik Industries; polyvinyl acetate; fats; oils; waxes; fatty alcohols; shellac; zein; gluten; ethylacrylate-maleic acid anhydride copolymer; maleic acid anhydride-vinyl methyl ether copolymer; styrol-maleic acid copolymer; 2-ethyl-hexyl-acrylate maleic acid anhydride; crotonic acid-vinyl acetate copolymer; glutaminic acid/glutamic acid ester copolymer; carboxymethylethylcellulose glycerol monooctanoate; polyarginine; poly(ethylene); poly (propylene); poly(ethylene oxide); poly(ethylene terephthalate); poly(vinyl isobutyl ether); poly(vinyl chloride); and polyurethane. A combination of enteric materials may also be used. In one embodiment, the enteric material rapidly dissolves at pH 5.5 and higher, to provide fast dissolution in the upper bowel. For example, the enteric material can be selected from a copolymer of methacrylic acid and methyl methacrylate, and a copolymer of methacrylic acid and ethyl acrylate. For example, an enteric polymer is poly(methacrylic acid co-ethyl acrylate) 1:1 (EUDRAGIT L 30 D-55 and EUDRAGIT L100-55).

Examples of some enteric coatings are disclosed in U.S. Pat. No. 5,225,202, including beeswax and glyceryl monostearate; beeswax, shellac and cellulose; and cetyl alcohol, mastic and shellac, as well as shellac and stearic acid (U.S. Pat. No. 2,809,918); polyvinyl acetate and ethyl cellulose (U.S. Pat. No. 3,835,221); and neutral copolymer of polymethacrylic acid esters (Eudragit L30D) (F. W. Goodhart et al., Pharm. Tech., pp. 64-71, April 1984); copolymers of methacrylic acid and methacrylic acid methylester (Eudragits), or a neutral copolymer of polymethacrylic acid esters containing metallic stearates (Mehta et al., U.S. Pat. Nos. 4,728,512 and 4,794,001). Such coatings comprise mixtures of fats and fatty acids, shellac and shellac derivatives and the cellulose acid phthlates, e.g., those having a free carboxyl content. See also Remington's Pharmaceutical Sciences, A. Osol, ed., Mack Pub. Co., Easton, Pa. (16th ed. 1980) at pages 1590-1593, and Zeitova et al. (U.S. Pat. No. 4,432,966), for descriptions of suitable enteric coating compositions.

One or more plasticizers can be added to enteric polymers in order to increase their pliability and reduce brittleness, as it is known in the art. Suitable plasticizers are known in the art and include, for example, butyl citrates, triethyl citrate, diethyl phthalate, dibutyl sebacate, PEGs (e.g. PEG 6000), acetyl triethyl citrate, and triacetin. In one type of embodiment, the plasticizer is triethyl citrate. While some enteric materials are flexible and do not require addition of plasticizers, more brittle polymers (e.g., Eudragit L/S types, Eudragit RL/RS, and Eudragit FS 30 D) benefit from plasticizers, e.g. in the range of 5 wt. % to 30 wt. % based on the dry polymer mass, e.g. about 8 wt. % to about 12 wt. % triethyl citrate with poly(methacrylic acid co-ethyl acrylate) 1:1.

One or more anti-tacking agents (antiadherents) can also be added to an enteric coating mixture in order to reduce the tackiness of the film and prevent agglomeration, as it is known in the art. Anti-tacking agents include talc, and glyceryl monostearate, fumed silica (e.g., AEROSIL 200), precipitated silica (e.g., SIPERNAT PQ), and magnesium stearate, for example. Anti-tacking agents can be used in any

suitable quantity, for example in a range of about 10 wt. % to 100 wt. % based on dry polymer mass, or about 10 wt. % to about 50 wt. %, or about 10 wt. % to about 30 wt. %, or about 15 wt. % to about 30 wt. %. For example, in one embodiment the amount of talc is in a range of 15 wt. % to about 30 wt. %, based on dry polymer mass.

One or more surfactants can also be added to an enteric coating mixture in order to improve substrate wettability and/or stabilize suspensions, as it is known in the art. Surfactants include Polysorbate 80, sorbitan monooleate, and sodium dodecyl sulfate, for example.

The enteric membrane can be formed by any suitable process. Coating processes include pan coating, fluid bed coating, and dry coating (e.g., heat dry coating and electrostatic dry coating), for example. Pan coating and fluid bed coating using solvent are well established processes. In liquid coating, the enteric material and optional excipients (e.g. pigments, plasticizers, anti-tacking agents) are mixed in an organic solvent or water to form a solution or dispersion. The coating solution or dispersion is sprayed into solid dosage forms in a pan coater or a fluid bed dryer and dried by hot air. For example, in a Wurster fluid bed coating process, the coating fluid is sprayed from the bottom of the fluid bed apparatus, whereas in an alternative the coating fluid is applied by top spraying, and in another alternative tangential spray is applied.

The amount of enteric material applied is sufficient to achieve desired acid resistance and release characteristics. For example, in one embodiment the amount of enteric membrane will be sufficient to meet United States Pharmacopeia (USP) <711> requirements (USP 36-NF 31) for delayed-release dosage forms, thereby not releasing 10.0 wt. % of drug after 2 hours in 0.1N HCl. In another aspect, the formulation will be sufficient to release at least 80% of the active in 20 minutes in pH 6.8 buffer solution, e.g. using the dissolution method of USP 36-NF 31 section <711>.

In one type of embodiment, the enteric membrane is present in an amount in a range of about 20% to 40%, or 25% to about 35% as measured by the weight gain compared to the uncoated particle cores, or in a range of about 25% to about 31% weight gain, or about 27% to about 31% weight gain, or about 28.5% to about 31% weight gain, based on the weight of the uncoated particle cores.

The beads with enteric membrane can be sorted (e.g., via sieving) to a desired particle size. In embodiments, the particle size range can be any particle size range or combination thereof described above in connection with the core particles. In one type of embodiment, the particle size range will be the same as the particle size range of the uncoated core particles. For example, the beads can be sieved such that 5% or less of the bead core particles by weight are retained on a #12 mesh (1.68 mm) screen and 10% or less by weight pass through a #20 mesh (0.84 mm) screen.

Additional lubricant (glidant, anti-tack agent) can be added to the coated beads in powder form. Anti-tacking agents include talc, glyceryl monostearate, fumed silica (e.g., AEROSIL 200), and precipitated silica (e.g., SIPERNAT PQ), for example. For example talc powder can be added to the coated beads, for example in an amount of 0.1 wt. % to about 1 wt. % based on the total bead weight.

The formulation can include a capsule shell in which the beads are disposed. Soft and hard capsule shells are known. In one embodiment, the capsule shell is a hard capsule shell, e.g. a gelatin capsule shell or a vegetable-based hard capsule shell.

Thus, for example, one type of embodiment combining various of the features described above includes a pharmaceutical dosage form including a plurality of cysteamine beads, the beads including a core particle comprising cysteamine bitartrate, a filler (optionally microcrystalline cellulose), a binder (optionally hypromellose), and an enteric membrane (optionally Eudragit L30 D-55) surrounding the core, wherein the plurality of beads is characterized by a distribution of particle sizes in a range of about 0.7 mm to about 2.5 mm, wherein the enteric membrane is present in an amount in a range of about 20% to about 40% based on the weight of the bead core particles, and wherein the beads are disposed in a capsule shell.

Pharmacokinetics

As mentioned above, the dosage form can advantageously be designed have one or more pharmacokinetic characteristics, e.g. in humans.

In one embodiment, the pharmaceutical dosage form is characterized by a mean Tmax upon oral dosing, fasted, of greater than 75 minutes, or at least 110 minutes, or at least 2 hours, or at least 3 hours, or in a range of about 2.2 hours to about 3.48 hours, or about 2.22 hours to about 3.34 hours, or about 2.78 hours, or a Tmax in a range of 80% to 125%, or 80% to 120% of such reference Tmax.

In another embodiment, the pharmaceutical dosage form is characterized by a mean Cmax upon oral dosing, fasted, in a range of about 22.16 μmol/L to about 34.63 μmol/L, or about 22.16 μmol/L to about 33.24 μmol/L, or about 22.7 μmol/L, normalized to a 450 mg dose, or a Cmax in a range of 80% to 125%, or 80% to 120% of such reference Cmax. In another embodiment, the pharmaceutical dosage form is characterized by a mean Cmax_D upon oral dosing in a range of about 0.004 to about 0.006 mg/L/mg.

In another embodiment, the pharmaceutical dosage form is characterized by a mean AUC (0-6 hours) upon oral dosing, fasted, in a range of about 60.74 μmol·h/L to about 94.91 μmol·h/L, or about 60.74 μmol·h/L to about 91.12 μmol·h/L, or about 75.93 μmol·h/L, normalized to a 450 mg dose, or a bioequivalent AUC (0-6 hours) in a range of 80% to 125%, or 80% to 120% of such reference AUC (0-6 hours). In another embodiment, the pharmaceutical dosage form is characterized by a mean AUC (0-12 hours) upon oral dosing in a range of about 79.41 μmol·h/L to about 124.08 μmol·h/L, or about 79.41 μmol·h/L to about 119.11 μmol·h/L, or about 99.26 μmol·h/L, normalized to a 450 mg dose, or a bioequivalent AUC (0-12 hours) in a range of 80% to 125%, or 80% to 120% of such reference AUC (0-12 hours). In another embodiment, the pharmaceutical dosage form is characterized by a mean AUC (0-inf_D) upon oral dosing in a range of about 0.86 min·mg/L/mg to about 1.35 min·mg/L/mg, or about 0.86 min·mg/L/mg to about 1.3 min·mg/L/mg, or a bioequivalent AUC (0-inf_D) in a range of 80% to 125%, or 80% to 120% of such reference AUC (0-inf_D).

In example embodiments, any of the described pharmaceutical dosage forms can be characterized by providing mean pharmacokinetic parameters upon oral dosing, fasted, of: Tmax 183±90 minutes, Cmax 3.5±1.7 mg/L, and/or AUC (0-inf_D) 1.08±0.46 min*mg/L/mg, or a bioequivalent Tmax, Cmax or AUC in a range of 80% to 125%, or 80% to 120% of such reference parameter.

In example embodiments, any of the described pharmaceutical dosage forms can be characterized by providing mean pharmacokinetic parameters upon oral dosing of the whole capsule, fasted, of: Tmax 194±38 minutes, Cmax 2.3±0.6 mg/L, and/or AUC (0-inf_D) 0.84±0.19 min*mg/L/mg, or a bioequivalent Tmax, Cmax or AUC in a range of 80% to 125%, or 80% to 120% of such reference parameter; and/or mean pharmacokinetic parameters upon oral dosing of the beads, sprinkled on applesauce, of: Tmax 190±61 minutes,

Cmax 2.3±0.7 mg/L, and/or AUC (0-inf_D) 0.85±0.21 min*mg/L/mg, or a bioequivalent Tmax, Cmax or AUC in a range of 80% to 125%, or 80% to 120% of such reference parameter.

In another embodiment, the pharmaceutical dosage form is characterized by being bioequivalent when administered orally, fasted, in a hard capsule shell compared to the beads being administered orally, fasted, without a capsule shell. For example, the pharmaceutical dosage form can be characterized by the dosage form when administered orally in a hard capsule shell exhibiting a Cmax in a range of 80% to 125%, or 80% to 120%, of Cmax exhibited by the beads administered orally without a capsule shell. In another embodiment, the dosage form can be characterized by the dosage form when administered orally in a hard capsule shell exhibiting an AUC (0-12 h) or AUC (0-inf) in a range of 80% to 125%, or 80% to 120%, of that exhibited by the beads administered orally without a capsule shell, respectively. In one embodiment, both the Cmax and the AUC are within the tolerance ranges just described.

Purity

In one type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, as determined by reverse phase HPLC with UV detection, as described herein. In other embodiments, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 12 months storage at 25° C. and 40% relative humidity (RH), optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 18 months storage at 25° C. and 40% RH optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 24 months storage at 25° C. and 40% RH optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 30 months storage, or more, at 25° C. and 40% RH optionally as determined by reverse phase HPLC with UV detection, as described herein. Examples of suitable reverse phase HPLC assays are described herein.

In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 12 months storage at 25° C. and 60% RH, optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 18 months storage at 25° C. and 60% RH, optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 24 months storage, or more, at 25° C. and 60% RH, optionally as determined by reverse phase HPLC with UV detection, as described herein.

In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 3 months storage at 40° C. and 75% RH, optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 6 months storage at 40° C. and 75% RH, optionally as determined by reverse phase HPLC with UV detection, as described herein.

Any of the foregoing embodiments can be further characterized by having less than 8 wt. % total related substances (impurities) based on the amount of cysteamine, under the described storage conditions and times based on reverse phase HPLC with UV detection, as described herein.

Method of Making

Also contemplated is a method for the preparation of a dosage form according to the disclosure here, including coating a core particle comprising cysteamine or a pharmaceutically acceptable salt thereof and an excipient with an enteric polymer to form the enteric membrane.

The core particle including cysteamine or a pharmaceutically acceptable salt thereof can be formed by any suitable process. In one embodiment, the core particle is formed by granulating a mixture of cysteamine or a pharmaceutically acceptable salt thereof with an excipient and milling to a desired particle size range. In another embodiment, the core particle can be formed by extrusion and spheronization of a mixture of cysteamine or a pharmaceutically acceptable salt thereof with an excipient. Granulating processes can include fluid bed granulation, wet granulation, hot melt granulation, and spray congealing, for example. Other processes include slugging and roller compaction. As it is known in the art, the mixtures which are to be granulated can first be dry-blended. The dry-blended dry ingredients can be mixed with water, prior to extrusion.

It has been found that extrusion and spheronization of a mixture of cysteamine or a pharmaceutically acceptable salt thereof with an excipient can provide desirable core particles with a distribution of particle sizes as described herein and one or more other desirable properties. Cysteamine bitartrate oxidizes in air and in water, and with heat. Thus, short processing times can lead to a more stable product. For example, reducing the amount of spheronization reduces the amount of friction and related heat. For example, reducing the amount of time that the product is exposed to air (either in the moist state and/or before packaging) also reduces the amount of oxidation. On the other hand, rapid processing by extrusion and spheronization can lead to a poor quality product, for example in having a large fraction of the pellet cores falling outside a desired particle size range. The amount of moisture absorbed by spheronization aids (which does not happen immediately, but instead over time) influences the spheronization characteristics of the beads. Accordingly, it was determined that the moisture content of the wet mass, the related wet hold time for swelling of spheronization aid(s), and the spheronization time are parameters that can be optimized to achieve both good product yield, for example in a particle size range described herein, while maintaining good stability, e.g. not more than 5 wt. % cystamine based on the amount of cysteamine, as described herein.

Accordingly, in one embodiment the moisture content of the granulation mixture, prior to drying, is in a range of about 20 wt. % to about 40 wt. %, or 25 wt. % to about 35 wt. %, or about 28 wt. % to about 32 wt. %, or at least about 28 wt. %, or at least about 28.5, or at least about 20 wt. % to about 40 wt. %, or at least about 25 wt. % to about 35 wt. %, or at least about 27 wt. % to about 31 wt. % or at least about 28.5 wt. % to about 31 wt. %.

The wet mass can be held for a period of time prior to extrusion, e.g. in order to allow the sphronization aid to swell with granulating fluid. The hold time can be at least 15 minutes, at least 30 minutes, at least 45 minutes, or at least 60

minutes, for example. The hold time can be in a range of about 15 minutes to about 120 minutes, or about 30 minutes to 100 minutes, or 60 minutes to 90 minutes, for example.

As described above in connection with description of the core particles, the method can include a step of sorting (e.g., by sieving) the core particles prior to enteric coating, to retain particles in a predetermined size range, for example sizes in a range of about 0.7 mm to about 2.8 mm, or about 0.7 mm to about 2.5 mm, or about 0.8 mm to about 1.7 mm, or any range described above in connection with the core particles.

As described above in connection with description of the beads, the method can include a step of sorting (e.g., by sieving) the beads after enteric coating, to retain particles in a predetermined size range, for example sizes in a range of about 0.7 mm to about 2.8 mm, or about 0.7 mm to about 2.5 mm, or about 0.8 mm to about 1.7 mm, or any range described above in connection with the core particles.

In an extrusion and spheronization process, the following optional features can be employed, individually or in one or more combinations thereof. Water can be used as a granulation agent. Microcrystalline cellulose can be used in the core particles as a spheronization aid. Hypromellose can be included in the core particles as a binder. The extrusion screen size can be 1.0 mm. The friction plate of the spheronizer can be cross-hatched. The friction plate of the spheronizer can be cross-hatched with a square pitch of at least 3 mm, or greater than 3 mm, or at least 4 mm, or greater than 4 mm, or in a range of about 3 mm to about 7 mm, or about 5 mm. The spheronization time can be less than about 5 minutes, or less than about 4 minutes, or less than about 3 minutes, or less than about 2 minutes, or up to 1 minute. The spheronized particles can include non-spherical particles (i.e. irregular shapes), e.g. a substantial fraction thereof, e.g. at least 20 wt. % or at least 30 wt. %, or at least 40 wt. % or at least 50 wt. % or at least 60 wt. %, or at least 70 wt. % thereof.

The beads and/or filled capsules can be stored with a desiccant. The beads and/or filled capsules can be stored with an oxygen absorber.

For example, one embodiment of the method combining various of the parameters described above includes a method for the preparation of a pharmaceutical dosage form including cysteamine beads, including forming a wet mass comprising cysteamine bitartrate and an excipient, optionally microcrystalline cellulose, with a moisture content in a range of in a range of about 20 wt. % to about 40 wt. %, extruding and spheronizing the wet mass including cysteamine bitartrate and excipient to make core particles, sorting the core particles to a target particle size range, optionally 0.7 mm to 2.5 mm, coating the sorted core particles with an enteric polymer to form including beads comprising a core particle and an enteric membrane, and sorting the bead particles to a target particle size range, optionally 0.7 mm to 2.5 mm.

Use/Administration

For administration of the dosage form, a total weight in the range of approximately 100 mg to 1000 mg (based on the free base) can be used. The dosage form can be orally administered to a patient suffering from a condition for which an cysteamine is indicated, including, but not limited to, cystinosis and other metabolic and neurodegenerative diseases including non-alcoholic fatty liver disease (NAFLD), Huntingon's disease, Parkinson's disease, Rett Syndrome and others, use as free radical and radioprotectants, and as heptoprotectant agents. In any method described herein, the treatment of humans is contemplated. The compositions of the disclosure can be used in combination with other therapies useful for treating cystinosis and neurodegenerative diseases and disorders. For example, indomethacin therapy (Indocid® or Endol®) is an anti-inflammatory used to treat rheumatoid arthritis and lumbago, but it can be used to reduce water and electrolyte urine loss. In children with cystinosis, indomethacin reduces the urine volume and therefore liquid consumption by about 30%, sometimes by half. In most cases this is associated with an appetite improvement. Indomethacin treatment is generally followed for several years.

Other therapies can be combined with the methods and compositions of the disclosure to treat diseases and disorders that are attributed or result from cystinosis. Urinary phosphorus loss, for example, entails rickets, and it may be necessary to give a phosphorus supplement. Carnitine is lost in the urine and blood levels are low. Carnitine allows fat to be used by the muscles to provide energy. Hormone supplementation is sometimes necessary. Sometimes the thyroid gland will not produce enough thyroid hormones. This is given as thyroxin (drops or tablets). Insulin treatment is sometimes necessary if diabetes appears, when the pancreas does not produce enough insulin. These treatments have become rarely necessary in children whom are treated with cysteamine, since the treatment protects the thyroid and the pancreas. Some adolescent boys require a testosterone treatment if puberty is late. Growth hormone therapy may be indicated if growth is not sufficient despite a good hydro electrolytes balance. Accordingly, such therapies can be combined with the compositions and methods disclosed herein.

The effectiveness of a method or composition of the disclosure can be assessed by measuring leukocyte cystine concentrations. Dosage adjustment and therapy can be made by a medical specialist depending upon, for example, the concentration of cystine in leukocytes and the ability to tolerate the drug. Additional therapies including the use of omeprazole (Prilosec®) can reduce side effects of cysteamine administration, such as abdominal pain, heartburn, nausea, vomiting, and anorexia, which can result from cysteamine-induced gastric acid hypersecretion, for example.

In addition, various prodrugs can be "activated" by use of the enterically coated cysteamine. Prodrugs are pharmacologically inert, they themselves do not work in the body, but once they have been absorbed, the prodrug decomposes. The prodrug approach has been used successfully in a number of therapeutic areas including antibiotics, antihistamines and ulcer treatments. The advantage of using prodrugs is that the active agent is chemically camouflaged and no active agent is released until the drug has passed out of the gut and into the cells of the body. For example, a number of prodrugs use S—S bonds. Weak reducing agents, such as cysteamine, reduce these bonds and release the drug. Accordingly, the compositions of the disclosure are useful in combination with pro-drugs for timed release of the drug. In this aspect, a pro-drug can be administered followed by administration of an enterically coated cysteamine composition of the invention (at a desired time) to activate the pro-drug.

EXAMPLES

The following examples are provided for illustration and are not intended to limit the scope of the invention.

Example 1

Bead Production

Cysteamine bitartrate and excipients (microcrystalline cellulose, hypromellose, sodium lauryl sulfate) were milled through a Comil equipped with a 0.094" (2.3876 mm) screen operating at 500 RPM. The amount of each ingredient (per 75

mg cysteamine capsule) is cysteamine bitartrate 258 mg+/−37.0 mg; microcrystalline cellulose 67.1 mg+/−9.6 mg; hypromellose 17.2 mg+/−2.5 mg; and sodium lauryl sulfate 1.75 mg+/0.25 mg. Cysteamine bitartrate was passed through the Comil first followed by the excipients (hypromellose 2910-5, sodium lauryl sulfate, and microcrystalline cellulose). Cysteamine bitartrate and the excipients were dry blended for approximately 15 minutes. While mixing at a setpoint speed of 47 rpm, purified water was slowly added (addition in approximately 4 minutes) into the blended components. After the water addition, the wet blend was mixed for an additional minute for a total of 5 minutes.

A sample of the wet blend was collected and moisture content was determined by loss on drying (LOD). The wet mass was discharged in polyethylene lined fiber drums and held for 60-90 minutes prior to extrusion/spheronization.

The granulated wet mass was loaded onto a NICA extruder equipped with a 1.0 mm screen at a feeder speed of 100 RPM setpoint and extruded at a setpoint speed of 55 RPM (50-60 RPM). The extruded product was immediately spheronized using a NICA Spheronizer equipped with 5.0 mm cross-hatched friction plates. Spheronization was performed at a target speed of 625 RPM (500-700 RPM) for 40-60 seconds. The particles were collected in double polyethylene lined fiber drums and stored at room temperature for further processing.

The wet particles were dried in a Niro fluid bed dryer with an inlet air temperature setpoint of 70° C. (60-80° C.). Drying was complete when the moisture content of uncrushed particles reached ≤1.0% w/w by LOD. Sampling of the particles began when the outlet air temperature reached approximately 50° C. and continued until the acceptance criterion of ≤1.0%. The dried particles were transferred to fiber drums lined with double polyethylene bags and stored at room temperature.

The dried particles were screened through a #12 mesh screen and a #20 mesh screen. Particles passing through the #12 mesh and retained on the #20 mesh were collected as product in double polyethylene lined containers with desiccant and oxygen absorber packets in the outer liner. The collected product may be re-passed through the screens as needed. Particles greater than #12 mesh and less than #20 mesh were not retained as product for coating.

An enteric coating solution of Eudragit L30 D-55, triethyl citrate, and talc in purified water was prepared in a mixing tank equipped with a propeller mixer and placed on a balance. Eudragit L 30 D-55 was added to the portable mixing tank through a 60-mesh screen. The final solution was mixed for a minimum of 30 minutes and mixed continuously during the coating process. Based on a 75 mg cysteamine capsule, the amounts of coating ingredients were: Eudragit L30 D-55 66.2 mg+/−9.5 mg; triethyl citrate 6.65 mg+/−0.95 mg; talc 15.3 mg+/−2.2 mg.

Spray lines connecting the portable mixing tank to the Niro fluid bed dryer were primed. The floor balance was tared prior to starting the coating process. The amount of coating solution sprayed was calculated as the amount required to increase the core particle weight by 25%.

The core particles were loaded into the Niro fluid bed dryer equipped with a Precision Coater which sprays from the bottom, 1.0 mm Nozzle, 30 mm Swirl Accelerator, and 300 μm Filter Bonnet. The coating process parameters are provided in the table below.

Parameter	Setpoint	Range
Inlet Air Volume	450 scfm	300-600 scfm
Inlet Air Temperature	60° C.	45-75° C.
Product Temperature	30° C.	25-45° C.
Solution Spray Rate	0.220 kg/minute	0.200-0.240 kg/minute
Atomization Air Pressure	36 psi	32-40 psi

Once the target weight of coating solution was applied (25% of dry particle weight), the beads were weighed to confirm weight increase of ≥25.0%. If the weight was not ≥25.0% of the uncoated particle weight, the coating process was continued until ≥25.0% was achieved.

The coated beads were dried at an inlet temperature setpoint of 45° C. (35-55° C.) and inlet air volume setpoint of 350 scfm (300-400 scfm) until the LOD of the coated beads was ≤2.0% w/w. Once the LOD was reached, the inlet air heating was turned off and the beads were circulated at an air inlet volume of 300-400 scfm until the product temperature reached not more than (NMT) 30° C.

The weight gain of the dried coated beads was calculated to confirm a maximum weight gain of ≤31.0% was achieved. Visual inspection confirmed that the enteric membrane thickness was not consistent bead-to-bead, but instead there was a distribution of enteric membrane thicknesses.

The dried coated beads were screened through a #12 mesh and a #20 mesh screen in sequence. Beads passing through the #12 mesh screen and retained on the #20 mesh screen were collected as product in double polyethylene lined fiber drums with a desiccant and oxygen absorber canister in the outer liner. Mesh analysis testing can be performed as an in-process test to confirm the beads are within the limits of: NMT 5% are retained on a #12 mesh screen (1.68 mm) and NMT 10% pass through a #20 mesh screen (0.84 mm). If results are not within the limits, the product can be sorted by rescreening until the mesh analysis results meet the specified limits.

The dried coated beads were lubricated with talc prior to encapsulation. The coated beads were loaded in a V-blender; talc powder was added to the coated beads (calculated as 0.5% w/w of the total coated bead weight). The contents were mixed for a minimum of five minutes. The lubricated coated beads were transferred to double polyethylene lined fiber drums with desiccant and oxygen absorber packets in the outer liner and stored at room temperature. Lubricated coated beads were used in the manufacture of 75 mg size 0 capsules and 25 mg size 3 capsules. One batch of coated beads can be filled as a 75 mg strength batch or can be split to fill both 75 mg and 25 mg strengths, for example.

The 75 mg hard gelatin capsules were filled using an automated encapsulator at a speed of 80-100 spm to the target fill weight calculated to achieve 75 mg cysteamine free base per capsule. The 25 mg hard gelatin capsules were also filled with an automated encapsulator at a speed of 50-70 spm. The beads were introduced into the encapsulation process with a hopper.

Example 2

Particle Size Distribution

Several lots of cysteamine bitartrate enteric-coated beads produced via an extrusion and spheronization process as described herein were analyzed for particle size distribution via analytical sieving. The results are tabulated below.

Sieve Size (µm)	% Retained Lot A	% Retained Lot B	% Retained Lot C	% Retained Lot D
1700	0	0	0	0
1400	1.4	3.2	3.2	1.2
1180	19.5	25.7	26.7	20.3
1000	61.9	55.5	56	62
850	16.1	14.2	13.5	15.1
<850	1.2	1.4	0.6	1.4

Example 3

Pharmacokinetics

A population PK study was performed using Cystagon® and capsules of cysteamine bitartrate gastro-resistant beads (CBGB) produced according to the method of Example 1 herein.

Pharmacokinetic (PK) and pharmacodynamic (PD) relationships following a single dose of CBGB capsules was first studied in comparison to a single dose of immediate-release cysteamine bitartrate in a study with 9 patients. Following normalization to a 450 mg dose, the maximum plasma levels C max, AUC 0-6 h and AUC 0-12 h (calculated directly from the plasma level data for CBGB and from doubling the AUC 0-6 h value for immediate-release cysteamine to represent two doses) were lower for CBGB (27.70±14.99 µmol/L, 75.93±39.22 µmol*h/L and 99.26±44.21 µmol*h/L respectively) than for immediate-release cysteamine bitartrate (37.72±12.10 µmol/L, 96.00±37.81 µmol*h/L and 192.00±75.62 µmol*h/L respectively. The pharmacokinetics of CBGB are consistent with a delayed-release formulation showing a T max of 2.78±1.56 h for CBGB cysteamine was moderately bound to human plasma proteins, predominantly to albumin, with mean protein binding of about 52%. Plasma protein binding was independent of concentration over the concentration range achieved clinically with the recommended doses.

Additional studies were carried out as follows.

CBGB-A Study

Cystagon® Treatment Assignment: one (1) pre-dose PD sample was collected at time 0 (i.e., within 15 minutes prior to the morning Cystagon® dose administration), considered as the time of trough cysteamine/peak of WBC cystine after administration of immediate-release cysteamine bitartrate (Cystagon®). One (1) additional PD sample was collected at a sample timepoint that was time-matched to 1 of 3 PK sample profile times (either 2, 4 or 6 hours) post morning Cystagon® dose. There were six associated plasma PK samples collected at time 0 (within 15 minutes prior to morning Cystagon® dose); 30 minutes post morning Cystagon® dose; and 1, 2, 4 and 6 hours (immediately prior to the afternoon Cystagon® dose)

Inventive capsule Treatment Assignment: one (1) post-dose PD sample was collected at time 0.5 hour (30 minutes), considered as the time of trough cysteamine/peak of WBC cystine after administration of capsules of CBGB. Two (2) additional PD samples were collected at sample timepoints that were time-matched to PK sample profile times (either 3, 4, 8, 10 or 12 hours) post morning CBGB dose. In order to limit the impact of autocorrelation, juxtaposed times of sampling for patients treated with CBGB were not to be taken into account for the randomization. Therefore, patients were randomized to one of the following six pairs of the sampling time points: 3 and 8 hours, 3 and 10 hours, 3 and 12 hours, 4 and 8 hours, 4 and 10 hours, 4 and 12 hours. There were nine associated plasma PK samples collected at time 0 (within 15 minutes prior to morning CBGB dose), 30 minutes, 2, 3, 4, 6, 8, 10 and 12 hours post morning CBGB dose (immediately prior to the evening CBGB dose).

As recommended in the Cystagon® SmPC, food (meal or snack) was available 30 minutes prior to receiving the morning dose and (if applicable) the next Q6H of Cystagon® administration and the morning dose and Q12H CBGB administration and (if applicable) the next Q12H CBGB dose. Cystagon® was administered with water and CBGB was administered with an acidic beverage. Dairy products should have been withheld 1 hour before and after CBGB dosing.

CBGB-B Study

Administering cysteamine in fasted healthy volunteers provides very stable PK parameters such that it was possible to demonstrate bioequivalence between administrations of CBGB capsules as a whole or as their content sprinkled on food with only 20 healthy volunteers.

The PK parameters of cysteamine were determined after a single dose, first in fasted healthy volunteers, then in patients at steady state, using the model parameters obtained with healthy volunteers as starting parameters for the models in patients. Pharmacokinetic modeling of cysteamine was based on a **2**-compartment model and pharmacodynamic modeling of WBC cystine was based on an inhibitory E_{max} model. (Belldina, E. B., M. Y. Huang, et al. (2003). "Steady-state pharmacokinetics and pharmacodynamics of cysteamine bitartrate in paediatric nephropathic cystinosis patients." Br J Clin Pharmacol 56(5): 520-525.)

Since CBGB studies in healthy volunteers were not done against Cystagon®, data in fasted healthy volunteers (Gangoiti, J. A., M. Fidler, et al. (2010). "Pharmacokinetics of enteric-coated cysteamine bitartrate in healthy adults: a pilot study." Br J Clin Pharmacol 70(3): 376-382) were used to determine initial PK model parameters for Cystagon®. And data on EC-cysteamine (i.e. Eudragit L50D 55 enteric-coated capsules of Cystagon®—a different way of providing delayed-release cysteamine bitartrate) in this dataset was used for comparison purposes.

A bioequivalence designed to demonstrate bioequivalence between oral administration of intact CBGB capsules, and contents of opened CBGB capsules mixed with applesauce and taken orally. Twenty (20) healthy adults (mean age 37 years, range 19-64 years) received both presentations, 8 (75 mg) intact vs. 8 (75 mg) open capsules, in a crossover design study.

The final results are presented in the table below.

Study/ Protocol Country	Study Design	No. Subjects Entered/Completed (M/F)	HV/P[a] (Age: Mean, Range)	Treatment	Dose (mg)
UCSD	Open label,	(4M/3F)/	P (12, 8-17)	Cystagon ®	450
(USA)	Sequential	(4M/3F)		EC-Cysteamine	450
CBGB-A	Random,	(24M/19F)/	P(12, 6-26)	Cystagon ®	250-750

-continued

(USA/ EU)	Crossover	(22M/16F)		CBGB caps	425-1300
CBGB-B	Random,	(13M/7F)/	HV(37, 19-64)	CBGB caps	600
(USA)	Crossover	(13M/7F)		CBGB sprinkled	600

Study/ Protocol Country	Non-Compartmental Analysis (Pharsight, WinNonLin 6.2) T_{max} (min)	C_{max} (mg/L)	C_{max}_D (mg/L/mg)	AUC_{inf}_D (min * mg/L/mg)
UCSD	75 ± 19	3.1 ± 1.2	0.007 ± 0.003	0.88 ± 0.30
(USA)	220 ± 74	3.2 ± 1.4	0.007 ± 0.003	0.96 ± 0.40
CBGB-A	74 ± 32	2.6 ± 1.4	0.006 ± 0.003	0.84 ± 0.31
(USA/ EU)	183 ± 90	3.5 ± 1.7	0.005 ± 0.002	1.08 ± 0.46
CBGB-B	194 ± 38	2.3 ± 0.6	0.004 ± 0.001	0.84 ± 0.19
(USA)	190 ± 61	2.3 ± 0.7	0.004 ± 0.001	0.85 ± 0.21

Study/ Protocol Country	Population PK, 2-compartment Model (Pharsight, NLME 1.1) T_{lag} (min)	Ka (1/min)	V/F (L)	Cl/F (L/min)	V_2 (L)	Cl_2 (L/min)
UCSD	26	0.029	73	1.07	131	0.41
(USA)	156	0.025	98	1.17	54	0.5
CBGB-A	23	0.025	94	1.1	191	0.5
(USA/ EU)	60	0.015	87	1.2	200	0.4
CBGB-B	95	0.016	137	1.4	187	0.44
(USA)	98	0.017	151	1.4	192	0.47

[a)]HV = Healthy Volunteers, P = Patients

The conclusion of this population PK modeling on two different presentations of CBGB (open and intact), is that the only difference between administering CBGB as intact capsules and as open capsules, sprinkled on applesauce, is expressed by the difference between lag times: as expected the start of absorption from the beads is still delayed (85 min) but slightly less than when the gelatin capsule has to be dissolved first (108 min) and this has not much of an impact on T_{max} (190 min for open capsules vs. 194 min for intact capsules) since probably only a small amount of beads dissolves early.

However, comparison between the two presentations of CBGB (open and intact) and the immediate-release cysteamine bitartrate (Cystagon®) and the delayed-release EC-cysteamine, shows that the absorption of cysteamine after CBGB dosing is not only more delayed (Cystagon® T_{lag}<<CBGB T_{lag}<<EC-cysteamine T_{lag}) but also further extended due to a slower absorption (CBGB K_a<<Cystagon® K_a≈EC-cysteamine K_a) compared to EC-cysteamine. Without intending to be bound by any particular theory, it is contemplated that the difference in absorption of the CBGB formulation is related to one or more factors including the distribution of bead sizes and time-progressive dissolution of multiple beads and/or the irregularity of bead shapes in the CBGB formulation and/or the distribution of enteric membrane thicknesses in the CBGB formulation.

Example 4

Purity and Stability

Long term stability tests have been performed on the CBGB formulation made according to Example 1. The major impurity in the CBGB product is cystamine, the well known related substance (dimer).

The use of a more sensitive and less selective method has resulted in the observation of several impurities found in the CBGB formulation and the commercial product using cysteamine bitartrate, Cystagon®. Through the use of reverse phase HPLC, six peaks observed in the CBGB formulation related substances chromatograms have been identified as product degradants (specifically cysteamine bitartrate degradants). Two lots of Cystagon® were evaluated by the same test method. The impurities observed in representative CBGB chromatograms are also observed in Cystagon®.

Impurities Assay Method

Cysteamine bitartrate samples are assessed by gradient elution HPLC using an XBRIDGE C18 column (dimensions: 150 mm×4.6 mm; packing particle size: 3.5 μm) (Waters, Milford, Mass.). The autosampler temperature is 4° C. Approximately 10 μL or approximately 100 μL of sample is injected onto the column. The column temperature is 40° C. and the sample is eluted at a flow rate of 1.0 mL/min according to the following profile:

Time (min)	HPLC Gradient Mobile Phase A (%)	Mobile Phase B (%)
0.0	100	0
2.0	100	0
20.0	60	40
25.0	60	40
25.1	100	0
40.0	100	0

Mobile Phase A contains 23.6 mM 1-octanesulfonic acid sodium and 29.0 mM sodium phosphate (pH 2.6)/acetonitrile/methanol 85/3/12 (v/v/v). Mobile Phase B contains 0.20 M 1-octanesulfonic acid sodium and 0.10 M sodium phosphate (pH 2.6)/acetonitrile/methanol 10/18/72 (v/v/v). The

purity of 1-octanesulfonic acid is ≥98%. Detection is carried out using a UV detector at 210 nm.

Reference Solution Preparation.

Reference solutions of Cysteamine Bitartrate Analytical Reference Standard are prepared as follows. Working Standard and Working Check Standard solutions are prepared having a nominal concentration of 0.54 mg/mL Cysteamine Bitartrate Analytical Reference Standard in Mobile Phase A using low actinic glassware. A Working Sensitivity solution is prepared having a nominal concentration of 0.30 mg/mL Cysteamine Bitartrate Analytical Reference Standard in Mobile Phase A using low actinic glassware, which corresponds to the limit of quantification (LOQ) for cysteamine. The water content of the Cysteamine Bitartrate Analytical Reference Standard is determined no more than 7 days before use by Karl Fischer titration or thermal gravimetric analysis (TGA). The Reference Standard is stored refrigerated and blanketed under nitrogen.

Bead Prep Assay Sample Preparation.

Cysteamine Bitartrate Gastro-resistant Beads (CBGB) are prepared for analysis according to the following procedure. About 3.7 g of CBGB beads are ground to a fine powder using a ball mill for approximately 1 minute at 27 Hz. The grind is transferred to an amber bottle for storage. Stock Bead Prep Assay sample solutions are prepared in duplicate by adding 370.4 mg±5 mg of the grind to a 250 mL low actinic volumetric flask and diluting with Mobile Phase A. The mixture is stirred with a stir bar for at least 15 minutes. Approximately 15 mL of the resulting solution is filtered through a 0.45 μm nylon filter, with the first 5 mL being discarded. The cysteamine concentration of the resulting Stock Bead Prep Assay sample solution is approximately 0.300 mg/mL. Working Bead Prep sample solutions are prepared by placing 4.0 mL of Stock Bead Prep Assay sample solution in a 25 mL low actinic volumetric flask and diluting to volume with Mobile Phase A. The cysteamine concentration of the resulting Working Bead Prep sample solution is approximately 0.048 mg/mL.

Assay Sample Preparation.

CBGB capsules are prepared for analysis according to the following procedure. To reduce exposure to light and oxygen, sample preparation (from the initial weighing of the full capsules to the loading of sample vials on the HPLC) is completed in one day. Ten capsules are weighed. The capsule contents are emptied and the empty shells are weighed to determine the average capsule fill weight. The capsule contents are ground to a fine powder using a ball mill for approximately 1 minute at 27 Hz. The grind is transferred to an amber bottle for storage. Stock sample solutions are prepared in duplicate by adding the appropriate amount of the grind for 1 capsule (as determined by the average capsule fill weight) to a 25 mL low actinic volumetric flask and diluting with Mobile Phase A. The mixture is stirred with a stir bar for at least 15 minutes. The resulting solution is centrifuged at about 3400 rpm for 5 minutes. Approximately 15 mL of the centrifuged solution is filtered through a 0.45 μm nylon filter (Acrodisc, 25 mm diameter), with the first 5 mL being discarded, to obtain Stock sample solutions. Working sample solutions are prepared by placing 6.0 mL of Stock sample solution (for 25 mg capsules) or 2.0 mL of Stock sample solution (for 75 mg capsules) in a 10 mL low actinic volumetric flask and diluting to volume with Mobile Phase A.

Content Uniformity Sample Preparation.

CBGB capsules are prepared for analysis according to the following procedure. To reduce exposure to light and oxygen, sample preparation (from the initial weighing of the full capsules to the loading of sample vials on the HPLC) is completed in one day. Ten capsules are weighed. The contents of each capsule are emptied into separate mortars and the empty shells are weighed to determine the individual capsule fill weight. About 1-2 mL of Mobile Phase A is added into the mortar. The beads are immediately ground to a paste. If needed, additional Mobile Phase A is added to the paste, up to 5 mL total. The paste is transferred to a 250 mL low actinic volumetric flask. The mortar and pestle are thoroughly rinsed with Mobile Phase A and the rinse solution is collected in to the same flask. The flask is filled about three-quarters full with Mobile Phase A and stirred for at least 15 minutes. The flask is filled to volume with Mobile Phase A. Approximately 20 mL of the resulting solution is filtered through a 0.45 μm nylon filter (Acrodisc, 25 mm diameter), with the first 5 mL being discarded, to obtain Stock CU sample solutions. Working CU sample solutions are prepared by placing 12.0 mL of Stock CU sample solution (for 25 mg capsules) or 4.0 mL of Stock CU sample solution (for 75 mg capsules) in a 25 mL low actinic volumetric flask and diluting to volume with Mobile Phase A. The cysteamine concentration of the resulting Working CU sample solutions is approximately 0.048 mg/mL.

Data Analysis.

The cysteamine Working Standard solution concentration is calculated according to the following equation:

$$\text{Cysteamine Concentration } (C_{std}) = \text{mg Cysteamine Bitartrate Analytical Reference Standard} \times P_f / 25.0 \text{ mL}$$

P_f represents a purity factor for the standard material. P_f is calculated according to the following equation:

$$P_f = B \times (100 - \text{Water}) \times C / 100$$

where B=the anhydrous cysteamine free base in the Cysteamine Bitartrate Analytical Reference Standard (expressed as a decimal value on the standard bottle label),

water=the water content as determined by Karl Fischer or TGA no more than 7 days before use (expressed as a percentage), and

C=the cystamine correction (expressed as a decimal value on the standard bottle label).

The amount of cysteamine per capsule is calculated according to the following equation:

$$\text{mg cysteamine per capsule} = (A_{Sam}/A_{Std}) \times C_{Std} \times \text{DF} \times (\text{AveWt}/\text{SamWt})$$

where A_{Sam}=the peak area of cysteamine in the sample chromatogram with a 10 μL injection,

A_{Std}=the average peak area of cysteamine in all Working Standard solution chromatograms with a 10 μL injection,

C_{Std}=the concentration (mg/mL) of cysteamine in the Working Standard solution,

DF=the dilution factor (125 for 75 mg capsules; 41.6667 for 25 mg capsules),

AveWt=the average capsule fill weight (mg), and

SamWt=the sample weight (mg).

For Content Uniformity, the amount of cysteamine per capsule is calculated according to the following equation:

$$\text{mg cysteamine per capsule} = (A_{Sam}/A_{Std}) \times C_{Std} \times \text{DF}$$

where A_{Sam}=the peak area of cysteamine in the sample chromatogram with a 10 μL injection,

A_{Std}=the average peak area of cysteamine in all Working Standard solution chromatograms with a 10 μL injection,

C_{Std}=the concentration (mg/mL) of cysteamine in the Working Standard solution, and

DF=the dilution factor (1562.5 for 75 mg capsules; 520.8 for 25 mg capsules).

For the Bead Prep Assay, the amount of cysteamine per capsule is calculated according to the following equation:

$$\text{mg cysteamine per capsule}=(A_{Sam}/A_{Std})\times C_{Std}\times DF\times (AveWt/SamWt)$$

where A_{Sam}=the peak area of cysteamine in the sample chromatogram with a 10 µL injection,
A_{Std}=the average peak area of cysteamine in all Working Standard solution chromatograms with a 10 µL injection,
C_{Std}=the concentration (mg/mL) of cysteamine in the Working Standard solution,
DF=the dilution factor (use the 75 mg Dilution Factor, 1562.5),
AveWt=the average capsule fill weight (mg) (use the target fill weight, 370.4 mg), and
SamWt=the sample weight (mg) (use the actual weight used in sample preparation).

The percentage of the label claim (% LC) is calculated for the Assay, Content Uniformity, and Bead Prep Assay sample solutions according to the following equation:

$$\%\ LC=(\text{mg cysteamine})/LC\times 100\%$$

where mg cysteamine=the amount calculated by the applicable equation above, and
LC=the amount of the label claim (75 mg or 25 mg) (use 75 mg for the Bead Prep Assay).

The amount of substances related to cysteamine bitartrate (including cysteamine impurities) such as cystamine is calculated according to the following equation:

$$\text{mg related substance}=(A_{RS}/A_{Std})\times(C_{Std}/RRF)\times DF\times (AveWt/SamWt)$$

where A_{RS}=the peak area of any related substance in the Working sample solution chromatogram with a 100 µL injection (peaks before RRT 0.48 are disregarded; peaks observed in the chromatogram of the second injection of Mobile Phase A/Blank (100 µL injection) are also disregarded),
A_{Std}=the average peak area of cysteamine in all Working Standard solution chromatograms with a 10 µL injection,
C_{Std}=the concentration (mg/mL) of cysteamine in the Working Standard solution,
RRF=the relative response factor (0.98 for cystamine; 1.00 for other related substances),
DF=the dilution factor (12.5 for 75 mg capsules; 4.16667 for 25 mg capsules),
AveWt=the average capsule fill weight (mg), and
SamWt=the weight the sample grind from the Working sample solution preparation (mg).

The weight percentage of cystamine and other individual related substances is determined according to the following equation:

$$\%\ \text{individual related substance}=\text{mg related substance}/\text{mg cysteamine}\times 100\%$$

where mg related substance=the amount of related substance calculated above, and
mg cysteamine=the amount of cysteamine for the Assay sample.

The percentage of total related substances is determined by summing all related substances greater than or equal to 0.05%. Peaks after 28 minutes are disregarded. In contrast to a previous electrochemical detection method that disregarded early-eluting peaks as not relevant to the purity calculation, the foregoing method determines that early peaks are impurities and integrates early-eluting peaks as described above.

Results

Two lots of Cystagon® were dispensed in standard pharmacy containers and verified to be well within the manufacturer's expiration date. One lot was provided by a healthcare provider. It was dispensed in a standard pharmacy bottle and verified by the healthcare provider to be well within the expiration date. Upon analysis by the Test Method, it was shown to contain 9.1% cystamine by weight and 10.3% total related substances, based on the weight of cysteamine, using the assay described above. The second analyzed Cystagon® lot was identified by lot number. Upon analysis by the assay described above, it was shown to contain 5.2% cystamine by weight and 5.7% total related substances, based on the weight of cysteamine. Each Cystagon® lot was shipped and stored under specified label conditions.

Two representative lots of the CBGB capsule formulation were analyzed by the assay described above and were shown to contain 3.7% cystamine by weight and 3.6% cystamine by weight, respectively, based on the weight of cysteamine, at the time of manufacture. For both lots, the total amount of related substances was 4.2% by weight, based on the weight of cysteamine.

The CBGB product lots were put on stability testing in various packages and storage conditions, then assayed for purity using the assay described above. The results are shown in the table below

Lot	Product dose/count/ bottle size	Conditions ° C./% RH	Cystamine %/total related substances at time point (month) Initial	1	2	3	6	9	12
1	75 mg/60/100 cc	25/60	3.7/ 4.2	3.7/ NA	3.1/ NA	3.4/ 4.2	3.5/ 4.4	3.7/ 5.1	3.8/ 5.5
1	75 mg/60/100 cc	40/75	3.7/ 4.2	3.7/ NA	3.2/ NA	3.5/ 7.9	3.9/ 12.3		
1	75 mg/150/250 cc	25/60	3.7/ 4.2	3.5/ NA	3.4/ NA	3.7/ 4.5	3.6/ 4.3	3.6/ 4.9	3.7/ 5.4
1	75 mg/150/250 cc	40/75	3.7/ 4.2	3.4/ NA	3.4/ NA	3.7/ 7.9	3.8/ 11.6		
1	75 mg/300/400 cc	25/60	3.7/ 4.2	3.5/ NA	3.3/ NA	3.4/ 4.2	3.5/ 4.4	3.7/ 5.1	3.8/ 5.7
1	75 mg/300/400 cc	40/75	3.7/ 4.3	3.4/ NA	3.2/ NA	3.6/ 7.7	4.0/ 12.8		
1	75 mg/60/bulk	25/40	3.7/ 4.2	3.4/ NA	3.4/ NA	3.2/ NA	3.3/ 4.2	3.3/ 4.5	3.2/ 4.6
1	75 mg/60/bulk	40/75	3.7/ 4.2	3.4/ NA	3.2/ NA	3.3/ NA	2.9/ 9.1		
2	75 mg/150/250 cc	25/60	3.6/ 4.2	3.1/ 4.0	3.3/ 4.3	3.0/ 4.3	3.3/ 5.0		
2	75 mg/150/250 cc	40/75	3.6/ 4.2	3.1/ 7.5	3.6/ 12.1				

Additional CBGB product samples according to Example 1 were put on long term stability testing in various packages and storage conditions, then assayed for purity using the assay described above. Results are shown in the table below.

Lot	Product dose/count/ bottle size	Conditions ° C./% RH	Cystamine %/total related substances at time point (month)									
			Initial	1	2	3	6	9	12	15	18	24
3	25 mg/60/50 cc	25/60	3.2/ 4.1	3.0/ NA	3.3/ NA	3.1/ 4.2	3.3/ 4.7	3.2/ 4.9	3.6/ 5.5	NA	4.0/ 6.8	4.6/ NA
3	25 mg/60/50 cc	40/75	3.2/ 4.1	2.9/ NA	3.0/ NA	3.0/ 7.8	3.7/ 13.4					
4	75 mg/150/ 250 cc	25/60	3.2/ 4.0	3.2/ NA	3.4/ NA	3.4/ 4.7	3.7/ 5.2	3.5/ 5.3	4.1/ 6.3	NA	4.2/ 7.1	4.7/ NA
4	75 mg/150/ 250 cc	40/75	3.2/ 4.0	3.1/ NA	3.4/ NA	3.5/ 9.0	4.0/ 13.8					
5	75 mg/60/100 cc	25/60	3.4/ 4.2	3.4/ NA	3.5/ NA	3.3/ 4.4	3.7/ 5.2	3.5/ 5.2	5.0/ NA	3.9/ 6.0	3.9/ NA	5.3/ NA
5	75 mg/60/100 cc	40/75	3.4/ 4.2	3.3/ NA	3.4/ NA	3.3/ 8.5	4.1/ 16.0[1]					
5	75 mg/300/ 400 cc	25/60	3.4/ 4.2	3.5/ NA	3.5/ NA	3.5/ 4.9	4.0/ 6.0	3.3/ 5.3	5.3/ NA	4.1/ 6.5	4.1/ NA	5.3/ NA
5	75 mg/300/ 400 cc	40/75	3.4/ 4.2	3.5/ NA	3.7/ NA	3.7/ 9.6	4.2/ 15.4[1]					
5	75 mg/60/bulk	25/60	3.4/ 4.2	3.5/ NA	3.5/ NA	3.4/ NA	3.7/ 5.2	3.2/ NA	4.1/ 5.5			
5	75 mg/60/bulk	40/75	3.4/ 4.2	3.4/ NA	3.4/ NA	3.1/ NA	3.0/ 11.2					
6	25 mg/60/50 cc	25/60	3.3/ 4.1	3.3/ NA	3.2/ NA	3.2/ 4.3	3.7/ 5.5	3.3/ 5.0	4.0/ NA	3.9/ 5.9	4.4/ NA	5.1/ NA
6	25 mg/60/50 cc	40/75	3.3/ 4.1	3.2/ NA	3.1/ NA	3.0/ 7.8	3.8/ 15.7[1]					
6	25 mg/420/ 250 cc	25/60	3.3/ 4.1	3.3/ NA	3.5/ NA	3.6/ 4.9	4.0/ 6.0	3.8/ 5.9	4.6/ NA	4.8/ 7.4	4.1/ NA	5.2/ NA
6	25 mg/420/ 250 cc	40/75	3.3/ 4.1	3.4/ NA	3.5/ NA	3.5/ 9.7	4.7/ 17.1[1]					
6	25 mg/60/bulk	25/60	3.3/ 4.1	3.4/ NA	3.4/ NA	3.3/ NA	3.7/ 5.4	3.1/ NA	3.5/ 5.3			
6	25 mg/60/bulk	40/75	3.3/ 4.1	3.3/ NA	3.2/ NA	2.9/ NA	2.9/ 11.4					
7	75 mg/60/100 cc	25/60	3.2/ 3.9	3.2/ NA	3.3/ NA	3.3/ 4.5	3.5/ 5.3	3.2/ 4.9	4.6/ NA	4.1/ 6.1	3.6/ NA	4.6/ NA
7	75 mg/60/100 cc	40/75	3.2/ 3.9	3.2/ NA	3.1/ NA	3.2/ 8.0	3.7/ 13.5[1]					
7	75 mg/300/ 400 cc	25/60	3.2/ 3.9	3.4/ NA	3.4/ NA	3.4/ 4.8	3.7/ 5.5	3.4/ 5.2	4.9/ NA	4.2/ 6.4	3.8/ NA	4.7/ NA
7	75 mg/300/ 400 cc	40/75	3.2/ 3.9	3.3/ NA	3.3/ NA	3.5/ 9.1	3.9/ 13.6[1]					
7	75 mg/60/bulk	25/60	3.2/ 3.9	3.3/ NA	3.3/ NA	3.2/ NA	3.5/ 5.2	2.9/ NA	4.0/ 5.5			
7	75 mg/60/bulk	40/75	3.2/ 3.9	3.2/ NA	3.2/ NA	2.9/ NA	2.8/ 10.3					
8	25 mg/60/50 cc	25/60	3.1/ 3.9	3.1/ NA	3.1/ NA	3.0/ 4.1	3.4/ 4.9	3.1/ 4.9	3.7/ NA	3.4/ 5.3	3.4/ NA	4.3/ NA
8	25 mg/60/50 cc	40/75	3.1/ 3.9	3.0/ NA	2.9/ NA	2.7/ 7.4	3.4/ 13.4[1]					
8	25 mg/420/ 250 cc	25/60	3.1/ 3.9	3.2/ NA	3.3/ NA	3.3/ 4.6	3.6/ 5.3	3.4/ 5.0	4.1/ NA	4.5/ 6.8	3.8/ NA	4.6/ NA
8	25 mg/420/ 250 cc	40/75	3.1/ 3.9	3.3/ NA	3.3/ NA	3.2/ 9.1	4.0/ 16.2[1]					
8	25 mg/60/bulk	25/60	3.1/ 3.9	3.3/ NA	3.2/ NA	3.1/ NA	3.3/ 4.7	3.0/ NA	3.4/ 4.9			
8	25 mg/60/bulk	40/75	3.1/ 3.9	3.2/ NA	3.0/ NA	2.8/ NA	2.7/ 10.6					
9	25 mg/60/50 cc	25/60	3.6/ 4.2	3.5/ NA	2.9/ NA	3.2/ 4.0	3.4/ 4.4	3.3/ 4.6	3.4/ 5.0			
9	25 mg/60/50 cc	40/75	3.6/ 4.2	3.4/ NA	2.7/ NA	3.0/ 6.8	3.4/ 11.4					
9	25 mg/420/ 250 cc	25/60	3.6/ 4.2	3.5/ NA	3.0/ NA	3.4/ 4.3	3.4/ 4.4	3.5/ 5.0	3.8/ 5.8			
9	25 mg/420/ 250 cc	40/75	3.6/ 4.2	3.5/ NA	3.0/ NA	3.5/ 7.9	3.8/ 12.9					
9	25 mg/60/bulk	25/40	3.6/ 4.2	3.5/ NA	3.0/ NA	3.3/ NA	3.3/ 4.2	3.1/ 4.2	3.1/ 4.6			
9	25 mg/60/bulk	40/75	3.6/ 4.2	3.3/ NA	2.8/ NA	3.0/ NA	2.8/ 9.1					

-continued

Lot	Product dose/count/ bottle size	Conditions ° C./% RH	Cystamine %/total related substances at time point (month) Initial	1	2	3	6	9	12	15	18	24
10	25 mg/60/50 cc	25/60	3.4/ 4.0	NA	NA	3.1/ 3.9	3.1/ 4.1	2.9/ 4.2	3.1/ 4.7			
10	25 mg/60/50 cc	40/75	3.4/ 4.0	NA	NA	2.7/ 7.0	3.2/ 11.9					

[1]Samples pulled at 6 months but held at room temperature until new reference standard was qualified (at 8 months)

All of the foregoing CBGB samples met the acid resistance criteria (Not more than 10% (Q) of the label claim of cysteamine is dissolved after 2 hours in 0.1N HCl) and dissolution criteria (Not less than 70% (Q) of the label claim of cysteamine is dissolved after 30 minutes in 0.2M sodium phosphate buffer, pH 6.8)

The foregoing description is given for clearness of understanding only, and no unnecessary limitations should be understood therefrom, as modifications within the scope of the invention may be apparent to those having ordinary skill in the art.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise" and variations such as "comprises" and "comprising" will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Throughout the specification, where compositions are described as including components or materials, it is contemplated that the compositions can also consist essentially of, or consist of, any combination of the recited components or materials, unless described otherwise. Likewise, where methods are described as including particular steps, it is contemplated that the methods can also consist essentially of, or consist of, any combination of the recited steps, unless described otherwise. The invention illustratively disclosed herein suitably may be practiced in the absence of any element or step which is not specifically disclosed herein.

The practice of a method disclosed herein, and individual steps thereof, can be performed manually and/or with the aid of or automation provided by electronic equipment. Although processes have been described with reference to particular embodiments, a person of ordinary skill in the art will readily appreciate that other ways of performing the acts associated with the methods may be used. For example, the order of various of the steps may be changed without departing from the scope or spirit of the method, unless described otherwise. In addition, some of the individual steps can be combined, omitted, or further subdivided into additional steps.

All patents, publications and references cited herein are hereby fully incorporated by reference. In case of conflict between the present disclosure and incorporated patents, publications and references, the present disclosure should control.

What is claimed is:

1. A pharmaceutical dosage form comprising delayed-release cysteamine beads, the beads comprising:

(i) a core particle comprising a mixture of cysteamine bitartrate and a binder, and

(ii) an enteric membrane surrounding the core particle;

wherein the beads have a distribution of particle sizes in a range of about 0.7 mm to about 2.8 mm;

wherein the enteric membrane begins to dissolve within a pH range of about 4.5 to about 6.5;

wherein the enteric membrane is present in an amount in a range of about 25% to about 35% by weight, based on the weight of the core particles; and

wherein the pharmaceutical dosage form, upon administration in a capsule to fasted healthy normal subjects at 600 mg free cysteamine base, provides:

(a) a mean Cmax upon oral dosing in a range of 2.3±0.6 mg/L or in a range of 80% to 125% thereof; and

(b) a mean AUC (0-inf_D) upon oral dosing in a range of 0.84±0.19 min*mg/L/mg or in a range of 80% to 125% thereof.

2. The pharmaceutical dosage form of claim **1**, wherein the cysteamine (as free base) comprises at least 10 wt. % of the core particle.

3. The pharmaceutical dosage form of claim **1**, wherein the cysteamine or pharmaceutically acceptable salt thereof is cysteamine bitartrate.

4. The pharmaceutical dosage form of claim **3**, wherein the cysteamine bitartrate comprises at least 50 wt. % of the core particle.

5. The pharmaceutical dosage form of claim **1**, wherein the beads provide a mean Cmax and mean AUC (0-inf_D) upon oral dosing, fasted, when administered inside a capsule shell that are bioequivalent to the mean Cmax and mean AUC (0-inf_D) upon oral dosing, fasted, when administered without a capsule shell.

6. The pharmaceutical dosage form of claim **1**, wherein the enteric membrane comprises an enteric material that begins to dissolve at pH of about 5.5 in an aqueous solution.

7. The pharmaceutical dosage form of claim **1**, wherein the pharmaceutical dosage form, upon administration in a capsule to fasted healthy normal subjects at 600 mg free cysteamine base, provides:

(a) a mean Cmax upon oral dosing in a range of 2.3±0.6 mg/L; and

(b) a mean AUC (0-inf_D) upon oral dosing in a range of 0.84±0.19 min*mg/L/mg.

8. The pharmaceutical dosage form of claim **1**, wherein the pharmaceutical dosage form, upon administration in a capsule to fasted healthy normal subjects at 600 mg free cysteamine base, provides:

(a) a mean Cmax upon oral dosing of 2.3 mg/L or in a range of 80% to 125% thereof; and

(b) a mean AUC (0-inf_D) upon oral dosing of 0.84 min*mg/L/mg or in a range of 80% to 125% thereof.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

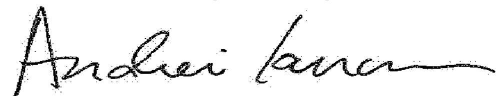
PATENT NO. : 9,173,851 B1
APPLICATION NO. : 14/751639
DATED : November 3, 2015
INVENTOR(S) : Powell et al.

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

On the Title Page

Item (72) Inventors should read as follows:
(72) Kathlene Powell, Cary, NC (US);
Ramesh Muttavarapu, Durham, NC (US);
Ranjan Dohil, San Diego, CA (US).

Signed and Sealed this
Tenth Day of July, 2018



Andrei Iancu
Director of the United States Patent and Trademark Office

EXHIBIT E

US009233077B2

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

(10) **Patent No.: US 9,233,077 B2**
(45) **Date of Patent: *Jan. 12, 2016**

(54) **DELAYED RELEASE CYSTEAMINE BEAD FORMULATION, AND METHODS OF MAKING AND USING SAME**

(71) Applicant: **RAPTOR PHARMACEUTICALS INC.**, Novato, CA (US)

(72) Inventors: **Kathlene Powell**, Cary, NC (US); **Ramesh Muttavarapu**, Durham, NC (US)

(73) Assignee: **RAPTOR PHARMACEUTICALS INC.**, Novato, CA (US)

(*) 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.: **14/306,303**

(22) Filed: **Jun. 17, 2014**

(65) **Prior Publication Data**

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A61K 31/145	(2006.01)
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A61K 9/50	(2006.01)
A61K 31/205	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 9/50*** (2013.01); ***A61K 9/5026*** (2013.01); ***A61K 31/145*** (2013.01); ***A61K 31/205*** (2013.01); *A61K 9/5084* (2013.01)

(58) **Field of Classification Search**

CPC .. A61K 9/5026; A61K 31/145; A61K 9/5084
See application file for complete search history.

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(57) **ABSTRACT**

An enteric-coated bead dosage form of cysteamine, and related methods of manufacture and use, are disclosed.

23 Claims, No Drawings

(56) **References Cited**

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DELAYED RELEASE CYSTEAMINE BEAD FORMULATION, AND METHODS OF MAKING AND USING SAME

CROSS-REFERENCE TO RELATED APPLICATION

The benefit under 35 U.S.C. §119(e) of U.S. Provisional Patent Application Ser. No. 61/835,965 filed Jun. 17, 2013, is hereby claimed, and the disclosure thereof is hereby incorporated by reference herein.

BACKGROUND

1. Field of the Disclosure

The disclosure relates generally to delayed release formulations of cysteamine and pharmaceutically acceptable salts thereof, and related methods of making and treatment, e.g. treatment of cystinosis and other metabolic and neurodegenerative diseases including non-alcoholic fatty liver disease (NAFLD), Huntingon's disease, Parkinson's disease, Rett Syndrome and others, use as free radical and radioprotectants, and as hepto-protectant agents. More particularly, the disclosure relates to enteric coated beads comprising cysteamine or a pharmaceutically acceptable salt thereof.

2. Brief Description of Related Technology

Cystinosis is a rare, autosomal recessive disease caused by intra-lysosomal accumulation of the amino acid cystine within various tissues, including the spleen, liver, lymph nodes, kidney, bone marrow, and eyes. Nephropathic cystinosis is associated with kidney failure that necessitates kidney transplantation. A specific treatment for nephropathic cystinosis is the sulfhydryl agent, cysteamine. Cysteamine has been shown to lower intracellular cystine levels, thereby reducing the rate of progression of kidney failure in children.

An enterically-coated cysteamine composition has been described, for increasing delivering of cysteamine to the small intestine and resulting in less frequent dosing compared to non enteric-coated cysteamine.

SUMMARY

One aspect of the disclosure provides a pharmaceutical dosage form including a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core, wherein the plurality of beads is characterized by a distribution of particle sizes.

Another aspect of the disclosure provides a pharmaceutical dosage form including a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core, wherein the plurality of beads is characterized by irregular bead shapes.

Yet another aspect of the disclosure provides a pharmaceutical dosage form including a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core, wherein the plurality of beads is characterized by a distribution of enteric membrane thicknesses.

Still another aspect of the disclosure provides a method of making a pharmaceutical dosage form, including any embodiment described herein, by a method including coating a core particle including cysteamine or a pharmaceutically acceptable salt thereof and an excipient with an enteric polymer to form an enteric membrane. The method can include sorting core particles prior to enteric coating, to provide a selected core particle size distribution. The method can also include sorting enteric coated beads to provide a selected bead size distribution.

Yet another aspect of the disclosure provides a method for treating a patient in need of cysteamine comprising administering to the patient a dosage form described herein, including any embodiment described herein.

Still another aspect of the disclosure provides dosage forms and related methods according the disclosure herein wherein the primary active component is cystamine rather than cysteamine or a pharmaceutically acceptable salt thereof.

For the compositions and methods described herein, optional features, including but not limited to components, compositional ranges thereof, substituents, conditions, and steps, are contemplated to be selected from the various aspects, embodiments, and examples provided herein.

Further aspects and advantages will be apparent to those of ordinary skill in the art from a review of the following detailed description. While the dosage form, method of making, and method of treatment are susceptible of embodiments in various forms, the description hereafter includes specific embodiments with the understanding that the disclosure is illustrative, and is not intended to limit the invention to the specific embodiments described herein.

DETAILED DESCRIPTION

Described herein is pharmaceutical dosage form that includes a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core particle. The plurality of beads can be characterized by a distribution of particle sizes. The plurality of beads can be characterized by irregular bead shapes. The plurality of beads can be characterized by a distribution of enteric membrane thicknesses. Also disclosed herein are a method for the preparation of the dosage form, including coating a core particle including cysteamine or a pharmaceutically acceptable salt thereof and an excipient with an enteric polymer to form the enteric membrane. Optionally, the core particle can be formed by a wet granulation method. Optionally, granules are sorted (e.g., via sieving) to a desired particle size range prior to enteric coating, and optionally again following enteric coating. Also disclosed herein are treatment methods including administering the dosage form to a patient in need thereof.

Cysteamine-containing, enteric-coated beads characterized by a distribution of particle sizes were shown to exhibit advantageous pharmacokinetics. Without intending to be bound by any particular theory, it is contemplated that the pharmacokinetics are influenced by the plurality of enteric-coated beads having a distribution of core particle sizes.

Cysteamine-containing, enteric-coated beads characterized by a irregular bead shapes were shown to exhibit advantageous pharmacokinetics. Without intending to be bound by any particular theory, it is contemplated that the pharmacokinetics are influenced by the plurality of enteric-coated beads having irregular bead shapes.

Cysteamine-containing, enteric-coated beads characterized by a distribution of enteric membrane thicknesses were shown to exhibit advantageous pharmacokinetics. Without intending to be bound by any particular theory, it is contem-

plated that the pharmacokinetics are influenced by the plurality of enteric-coated beads having a distribution of enteric membrane thicknesses.

In one aspect the distribution of enteric membrane thicknesses can be stated in terms of weight gain of enteric membrane material based on the total weight of the coated beads. Thus, in one embodiment, the distribution of enteric membrane thicknesses will be at least 2% based on the total weight of the coated beads. In another embodiment, the distribution of enteric membrane thicknesses will be at least 3%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 4%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 5%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 6%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 7%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 8%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 9%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 10%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 11%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 12%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 13%. In another embodiment, the distribution of enteric membrane thicknesses will be at least 14%. For example, the difference in enteric membrane thickness from bead to bead can be in a range of +/−1-7% based on the total weight of the coated beads. The distribution of enteric membrane thicknesses can be in a range of about 2% to about 14% based on the weight of the coated beads, or in a range of about 3% to about 13%, or in a range of about 4% to about 12%, or in a range of about 5% to about 11%, or in a range of about 6% to about 10%, or in a range of about 7% to 9%, or in a range of about 3% to 14%, or in a range of about 4% to 14%, or in a range of about 4% to 13%, or in a range of about 4% to about 12%, for example. In one embodiment, the absorption (AUC) of the dosage form when dosed orally is advantageously increased, compared to other dosage forms of cysteamine. Without intending to be bound by any particular theory, it is contemplated that the increase in absorption is influenced by the dosage form exhibiting a pseudo-extended release profile. The pseudo-extended release profile is contemplated to be influenced by one or more factors, including a distribution of enteric membrane thicknesses, a distribution of bead particle sizes, and the beads having irregular bead shapes. For example, in an embodiment wherein the beads have a distribution of enteric membrane thicknesses, it is contemplated that for beads which have a relatively thin coating, the coating will completely dissolve at the trigger pH relatively quickly to release the cysteamine composition, whereas for beads having a relatively thick coating the coating will take somewhat longer to completely dissolve and release the cysteamine composition. In another aspect, in an embodiment where the beads have a distribution of particle sizes and/or irregular bead shapes, it is contemplated that the gut transit time of the beads could be varied due to bead size and/or shape, such that the transit time until reaching the enteric membrane dissolution pH is varied, thus contributing to a pseudo-extended release profile. In another embodiment, the dosage form exhibits substantially equivalent (e.g., bioequivalent) Cmax and/or AUC characteristics when administered orally inside a capsule shell or without a capsule shell.

The dosage form provides a progressive and predictable absorption curve. In one type of embodiment, the Tmax of the dosage form when dosed orally is advantageously more stable on a dose-to-dose basis, because the beads are individually enteric-coated. A predictable, consistent Tmax is highly advantageous for accomplishing a more consistent, sustained reduction of leukocyte cystine levels by use of cysteamine. For example, process-related variations in enteric membrane thickness or other influences on enteric membrane dissolution will affect only a fraction of the cysteamine in the dosage form and will tend to lead to the pseudo-extended release behavior described above. In contrast, enteric-coated capsules comprising cysteamine microspheres exhibited significant variability in absorption time from capsule to capsule.

In another embodiment, the dosage form exhibits advantageous storage stability, e.g. as measured by the amount of cystamine present following storage and/or by the total amount of related substances. The storage stability can be assessed following storage at typical ambient conditions (e.g. 25° C. and 40% relative humidity) or at accelerated stability conditions involving increased temperature and/or humidity.

The dosage form and methods are contemplated to include embodiments including any combination of one or more of the additional optional elements, features, and steps further described below (including those shown in the figures and Examples), unless stated otherwise.

In jurisdictions that forbid the patenting of methods that are practiced on the human body, the meaning of "administering" of a composition to a human subject shall be restricted to prescribing a controlled substance that a human subject will self-administer by any technique (e.g., orally, inhalation, topical application, injection, insertion, etc.). The broadest reasonable interpretation that is consistent with laws or regulations defining patentable subject matter is intended. In jurisdictions that do not forbid the patenting of methods that are practiced on the human body, the "administering" of compositions includes both methods practiced on the human body and also the foregoing activities.

As used herein, the term "comprising" indicates the potential inclusion of other agents, elements, steps, or features, in addition to those specified.

As used herein, the term wt. % is the weight percent based on the total weight, e.g. of the core particle, or enteric membrane, or total bead, as described in context. Unless stated otherwise, the wt. % is intended to describe the weight percent based on dry weight (e.g., for a core particle following drying).

All ranges set forth herein include all possible subsets of ranges and any combinations of such subset ranges. By default, ranges are inclusive of the stated endpoints, unless stated otherwise Where a range of values is provided, it is understood that each intervening value between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also contemplated to be part of the disclosure.

Unless expressly stated otherwise, all references to cysteamine herein are intended to encompass pharmaceutically-acceptable salts thereof, and for every reference to cysteamine herein the use of cysteamine bitartrate is specifically contemplated as an embodiment. As described in the Summary above, embodiments of the dosage forms and methods

described herein can employ cystamine as the primary active component, rather than cysteamine or a pharmaceutically acceptable salt thereof.

Unless expressly stated otherwise, reference herein to a bead and properties thereof is intended to be interpreted as applying equally to a collection of beads (e.g., a plurality of such beads). Likewise, unless expressly stated otherwise, reference herein to a core particle and properties thereof is intended to be interpreted as applying equally to a collection of core particles (e.g., a plurality of such core particles).

As described above, a pharmaceutical dosage form is contemplated that includes a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core particle, wherein the plurality of beads is characterized by a distribution of particle sizes.

In one embodiment, the particle sizes of the beads are in a range of about 0.7 mm to about 2.5 mm, or about 0.7 mm to about 2.8 mm, or about 0.8 mm to about 1.7 mm. For example, the target bead size can be up to 2.5 mm with no more than 10 percent variation over this size, to a maximum size of 2.8 mm.

As the particle size of the beads becomes too small, the variability in cysteamine content increases. As the particle size becomes too large, the beads are too large for use in drug products that are labeled to be administered via sprinkling (e.g., on applesauce or other soft foods, such as jellies) and swallowed without chewing, or administered via an enteral feeding tube. Also as the particle size increases, it was found that the larger particles get coated more than the smaller particles, resulting in lower relative assay when compared to use of smaller particles. To compensate, relatively more such beads would be needed in order to meet the label strength per capsule, but because salts such as cysteamine bitartrate already have a high molecular weight, filling a capsule shell with sufficient large particles to meet the label strength per capsule becomes difficult or impossible (e.g. to fill a size 0 capsule to a 75 mg strength of cysteamine free base). Accordingly the bead particle size in one type of embodiment is up to 1.7 mm.

The distribution of bead particle sizes for various non-exclusive embodiments of the invention can be characterized in ways.

In one embodiment, the beads can be characterized by 5% or less of the beads by weight being retained on a #12 mesh (1.68 mm) screen and 10% or less by weight passing through a #20 mesh (0.84 mm) screen. In another embodiment, at least 80% by weight of the beads have a particle size in a range of about 850 μm to about 1180 μm, e.g. as determined by sieving.

The distribution of bead sizes can be characterized by a gradation test via analytical sieving. Thus, in another embodiment the distribution of bead sizes is characterized by 0% of the beads being retained on a 1700 μm sieve and less than 5% by weight of the beads being retained on a 1400 μm sieve. Optionally less than 30% by weight of the beads are retained on a 1180 μm sieve. Optionally less than 70% by weight of the beads are retained on a 1000 μm sieve. Optionally less than 20% by weight of the beads are retained on a 850 μm sieve. Optionally at least 15% by weight of the beads are retained on a 1180 μm sieve. Optionally at least 50% by weight of the beads are retained on a 1000 μm sieve. Optionally at least 10% by weight of the beads being retained on a 850 μm sieve.

Thus, for example, the distribution can be characterized by 0% of the beads being retained on a 1700 μm sieve and less than 5% by weight of the beads being retained on a 1400 μm sieve, and about 20% to about 30% by weight of the beads being retained on a 1180 μm sieve and then about 50% to about 70% (or about 55% to about 65%) by weight of the beads being retained on a 1000 μm sieve and then about 10% to about 20% by weight of the beads being retained on a 850 μm sieve.

In another embodiment, the distribution of bead sizes can be characterized by a median particle size in a range of about 850 μm to about 1180 μm.

The bead core particle can comprise one or more excipients. In one type of embodiment, the excipients can include one or more fillers, binders, and surfactants. Other optional ingredients can include, but are not limited to, glidants, lubricants, disintegrants, swelling agents, and antioxidants.

Fillers include, but are not limited to, lactose, saccharose, glucose, starch, microcrystalline cellulose, microfine cellulose, mannitol, sorbitol, calcium hydrogen phosphate, aluminum silicate, amorphous silica, and sodium chloride, starch, and dibasic calcium phosphate dehydrate. In one type of embodiment, the filler is not water soluble, although it may absorb water. In one type of embodiment, the filler is a spheronization aid. Spheronization aids can include one or more of crospovidone, carrageenan, chitosan, pectinic acid, glycerides, β-CD, cellulose derivatives, microcrystalline cellulose, powdered cellulose, polyplasdone crospovidone, and polyethylene oxide. In one embodiment, the filler includes microcrystalline cellulose.

Binders include, but are not limited to, cellulose ethers, methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, propyl cellulose, hydroxypropyl cellulose, lower-substituted hydroxypropyl cellulose, hydroxypropylmethyl cellulose (hypromellose, e.g. hypromellose 2910, METHOCEL E), carboxymethyl cellulose, starch, pregelatinized starch, acacia, tragacanth, gelatine, polyvinyl pyrrolidone (povidone), cross-linked polyvinyl pyrrolidone, sodium alginate, microcrystalline cellulose, and lower-substituted hydroxypropyl cellulose. In one embodiment, the binders are selected from wet binders. In one type of embodiment, the binder is selected from cellulose ethers, e.g. hypromellose.

Surfactants include, but are not limited to, anionic surfactants, including sodium lauryl sulfate, sodium deoxycholate, dioctyl sodium sulfosuccinate, and sodium stearyl fumarate, nonionic surfactants, including polyoxyethylene ethers, and polysorbate 80, and cationic surfactants, including quaternary ammonium compounds. In one embodiment the surfactant is selected from anionic surfactants, e.g. sodium lauryl sulfate.

Disintegrants include, but are not limited to, starch, sodium cross-linked carboxymethyl cellulose, carmellose sodium, carmellose calcium, cross-linked polyvinyl pyrrolidone, and sodium starch glycolate, low-substituted hydroxypropyl cellulose, hydroxypropyl starch.

Glidants include, but are not limited to, polyethylene glycols of various molecular weights, magnesium stearate, calcium stearate, calcium silicate, fumed silicon dioxide, magnesium carbonate, magnesium lauryl sulfate, aluminum stearate, stearic acid, palmitic acid, cetanol, stearol, and talc.

Lubricants include, but are not limited to, stearic acid, magnesium stearate, calcium stearate, aluminum stearate, and siliconized talc.

The amount of cysteamine free base in the core particle can be at least 10 wt. % or at least 15 wt. %, or at least 20 wt. %, or at least 25 wt. %, or at least 30 wt. %. For example, the amount of cysteamine bitartrate can be at least 50 wt. %, or at least 55 wt. %, or at least 60 wt. %, or at least 65 wt. %, or at least 70 wt. %, or at least 75 wt. %, or at least 80 wt. %, or at least 85 wt. % of the core particle, for example in a range of about 60 wt. % to about 90 wt. % or about 65 wt. % to about

85 wt. %. It is understood that any and all ranges including these values as endpoints is contemplated, for example, at least about 15 wt. % to about 90 wt. %, or at least about 20 wt. % to about 85 wt. %, or at least about 30 wt. % to about 85 wt. %, or at least about 50 wt. % to about 90 wt. %. As the dose of cysteamine free base can be up to about 2 g/m^2/day, and the amount of free base is relatively small compared to the molecular weight of salts (e.g. the bitartrate salt) it is preferred that the core particle have as much active ingredient as possible while allowing the creation and processing of core particles.

The amount of filler in the core particle is not particularly limited. In embodiments, the amount of filler (e.g. microcrystalline cellulose) can be in a range of about 10 wt. % to about 30 wt. %, or about 16 wt. % to about 23 wt. %, or at least 19 wt. % or at least 19.5 wt. %, for example about 20 wt. %.

The amount of binder in the core particle is not particularly limited. In embodiments, the amount of binder (e.g. hypromellose) can be in a range of about 1 wt. % to about 10 wt. %, or about 2 wt. % to about 8 wt. %, or about 4 wt. % to about 6 wt. %, for example about 5 wt. %.

The amount of surfactant, e.g. as a processing aid, in the core particle is not particularly limited. In embodiments, the amount of surfactant (e.g. microcrystalline cellulose) can be in a range of about 0.1 wt. % to about 1 wt. %, or about 0.2 wt. % to about 0.8 wt. %, or about 0.4 wt. % to about 0.6 wt. %, for example about 0.5 wt. %.

The enteric (gastro-resistant) membrane material, e.g. polymer, can be one that will dissolve in intestinal juices at a pH level higher than that of the stomach, e.g. a pH of greater than 4.5, such as within the small intestine, and therefore permit release of the active substance in the regions of the small intestine and substantially not in the upper portion of the GI tract. In one type of embodiment, the enteric material begins to dissolve in an aqueous solution at pH between about 4.5 to about 5.5. In another type of embodiment, the enteric material rapidly dissolves in an aqueous solution at pH between of about 5. In another type of embodiment, the enteric material rapidly dissolves in an aqueous solution at pH between of about 5.5.

For example, pH-sensitive materials will not undergo significant dissolution until the dosage form has emptied from the stomach. The pH of the small intestine gradually increases from about 4.5 to about 6.5 in the duodenal bulb to about 7.2 in the distal portions of the small intestine (ileum). In order to provide predictable dissolution corresponding to the small intestine transit time of about 3 hours (e.g., 2-3 hours) and permit reproducible release therein, the membrane should begin to dissolve within the pH range of the duodenum, and continue to dissolve at the pH range within the small intestine. Therefore, the amount (thickness) of enteric membrane should be sufficient to be substantially dissolved during the approximate three hour transit time within the small intestine (e.g., the proximal and mid-small intestine).

Enteric (gastro-resistant) materials can include, but are not limited to, one or more of the following: cross-linked polyvinyl pyrrolidone; non-cross linked polyvinylpyrrolidone; hydroxypropylmethyl cellulose phthalate, hydroxypropylmethyl cellulose acetate succinate, cellulose acetate succinate; cellulose acetate phthalate, hydroxypropylmethyl cellulose acetate succinate, cellulose acetate trimellitate; starch acetate phthalate; polyvinyl acetate phthalate; carboxymethyl cellulose; methyl cellulose phthalate; methyl cellulose succinate; methyl cellulose phthalate succinate; methyl cellulose phthalic acid half ester; ethyl cellulose succinate; carboxymethylamide; potassium methacrylatedivinylbenzene copolymer; polyvinylalcohols; polyoxyethyleneglycols; polyethylene glycol; sodium alginate; galactomannone; carboxypolymethylene; sodium carboxymethyl starch; copolymers of acrylic acid and/or methacrylic acid with a monomer selected from the following: methyl methacrylate, ethyl methacrylate, ethyl acrylate, butyl methacrylate, hexyl methacrylate, decyl methacrylate, lauryl methacrylate, phenyl methacrylate, methyl acrylate, isopropyl acrylate, isobutyl acrylate, or octadecyl acrylate, e.g. EUDRAGIT-L and -S series, including L 100-55, L 30 D-55, L 100, S 100, L 12.5, and S 12.5, available from Evonik Industries; polyvinyl acetate; fats; oils; waxes; fatty alcohols; shellac; zein; gluten; ethylacrylate-maleic acid anhydride copolymer; maleic acid anhydride-vinyl methyl ether copolymer; styrol-maleic acid copolymer; 2-ethyl-hexyl-acrylate maleic acid anhydride; crotonic acid-vinyl acetate copolymer; glutaminic acid/glutamic acid ester copolymer; carboxymethylethylcellulose glycerol monooctanoate; polyarginine; poly(ethylene); poly(propylene); poly(ethylene oxide); poly(ethylene terephthalate); poly(vinyl isobutyl ether); poly(vinyl chloride); and polyurethane. A combination of enteric materials may also be used. In one embodiment, the enteric material rapidly dissolves at pH 5.5 and higher, to provide fast dissolution in the upper bowel. For example, the enteric material can be selected from a copolymer of methacrylic acid and methyl methacrylate, and a copolymer of methacrylic acid and ethyl acrylate. For example, an enteric polymer is poly(methacrylic acid co-ethyl acrylate) 1:1 (EUDRAGIT L 30 D-55 and EUDRAGIT L100-55).

Examples of some enteric coatings are disclosed in U.S. Pat. No. 5,225,202, including beeswax and glyceryl monostearate; beeswax, shellac and cellulose; and cetyl alcohol, mastic and shellac, as well as shellac and stearic acid (U.S. Pat. No. 2,809,918); polyvinyl acetate and ethyl cellulose (U.S. Pat. No. 3,835,221); and neutral copolymer of polymethacrylic acid esters (Eudragit L30D) (F. W. Goodhart et al., Pharm. Tech., pp. 64-71, April 1984); copolymers of methacrylic acid and methacrylic acid methylester (Eudragits), or a neutral copolymer of polymethacrylic acid esters containing metallic stearates (Mehta et al., U.S. Pat. Nos. 4,728,512 and 4,794,001). Such coatings comprise mixtures of fats and fatty acids, shellac and shellac derivatives and the cellulose acid phthlates, e.g., those having a free carboxyl content. See also Remington's Pharmaceutical Sciences, A. Osol, ed., Mack Pub. Co., Easton, Pa. (16th ed. 1980) at pages 1590-1593, and Zeitova et al. (U.S. Pat. No. 4,432,966), for descriptions of suitable enteric coating compositions.

One or more plasticizers can be added to enteric polymers in order to increase their pliability and reduce brittleness, as it is known in the art. Suitable plasticizers are known in the art and include, for example, butyl citrates, triethyl citrate, diethyl phthalate, dibutyl sebacate, PEGs (e.g. PEG 6000), acetyl triethyl citrate, and triacetin. In one type of embodiment, the plasticizer is triethyl citrate. While some enteric materials are flexible and do not require addition of plasticizers, more brittle polymers (e.g., Eudragit L/S types, Eudragit RL/RS, and Eudragit FS 30 D) benefit from plasticizers, e.g. in the range of 5 wt. % to 30 wt. % based on the dry polymer mass, e.g. about 8 wt. % to about 12 wt. % triethyl citrate with poly(methacrylic acid co-ethyl acrylate) 1:1.

One or more anti-tacking agents (antiadherents) can also be added to an enteric coating mixture in order to reduce the tackiness of the film and prevent agglomeration, as it is known in the art. Anti-tacking agents include talc, and glyceryl monostearate, fumed silica (e.g., AEROSIL 200), precipitated silica (e.g., SIPERNAT PQ), and magnesium stearate, for example. Anti-tacking agents can be used in any

suitable quantity, for example in a range of about 10 wt. % to 100 wt. % based on dry polymer mass, or about 10 wt. % to about 50 wt. %, or about 10 wt. % to about 30 wt. %, or about 15 wt. % to about 30 wt. %. For example, in one embodiment the amount of talc is in a range of 15 wt. % to about 30 wt. %, based on dry polymer mass.

One or more surfactants can also be added to an enteric coating mixture in order to improve substrate wettability and/or stabilize suspensions, as it is known in the art. Surfactants include Polysorbate 80, sorbitan monooleate, and sodium dodecyl sulfate, for example.

The enteric membrane can be formed by any suitable process. Coating processes include pan coating, fluid bed coating, and dry coating (e.g., heat dry coating and electrostatic dry coating), for example. Pan coating and fluid bed coating using solvent are well established processes. In liquid coating, the enteric material and optional excipients (e.g. pigments, plasticizers, anti-tacking agents) are mixed in an organic solvent or water to form a solution or dispersion. The coating solution or dispersion is sprayed into solid dosage forms in a pan coater or a fluid bed dryer and dried by hot air. For example, in a Wurster fluid bed coating process, the coating fluid is sprayed from the bottom of the fluid bed apparatus, whereas in an alternative the coating fluid is applied by top spraying, and in another alternative tangential spray is applied.

The amount of enteric material applied is sufficient to achieve desired acid resistance and release characteristics. For example, in one embodiment the amount of enteric membrane will be sufficient to meet United States Pharmacopeia (USP) <711> requirements (USP 36-NF 31) for delayed-release dosage forms, thereby not releasing 10.0 wt. % of drug after 2 hours in 0.1N HCl. In another aspect, the formulation will be sufficient to release at least 80% of the active in 20 minutes in pH 6.8 buffer solution, e.g. using the dissolution method of USP 36-NF 31 section <711>.

In one type of embodiment, the enteric membrane is present in an amount in a range of about 20% to 40%, or 25% to about 35% as measured by the weight gain compared to the uncoated particle cores, or in a range of about 25% to about 31% weight gain, or about 27% to about 31% weight gain, or about 28.5% to about 31% weight gain, based on the weight of the uncoated particle cores.

The beads with enteric membrane can be sorted (e.g., via sieving) to a desired particle size. In embodiments, the particle size range can be any particle size range or combination thereof described above in connection with the core particles. In one type of embodiment, the particle size range will be the same as the particle size range of the uncoated core particles. For example, the beads can be sieved such that 5% or less of the bead core particles by weight are retained on a #12 mesh (1.68 mm) screen and 10% or less by weight pass through a #20 mesh (0.84 mm) screen.

Additional lubricant (glidant, anti-tack agent) can be added to the coated beads in powder form. Anti-tacking agents include talc, glyceryl monostearate, fumed silica (e.g., AEROSIL 200), and precipitated silica (e.g., SIPERNAT PQ), for example. For example talc powder can be added to the coated beads, for example in an amount of 0.1 wt. % to about 1 wt. % based on the total bead weight.

The formulation can include a capsule shell in which the beads are disposed. Soft and hard capsule shells are known. In one embodiment, the capsule shell is a hard capsule shell, e.g. a gelatin capsule shell or a vegetable-based hard capsule shell.

Thus, for example, one type of embodiment combining various of the features described above includes a pharmaceutical dosage form including a plurality of cysteamine beads, the beads including a core particle comprising cysteamine bitartrate, a filler (optionally microcrystalline cellulose), a binder (optionally hypromellose), and an enteric membrane (optionally Eudragit L30 D-55) surrounding the core, wherein the plurality of beads is characterized by a distribution of particle sizes in a range of about 0.7 mm to about 2.5 mm, wherein the enteric membrane is present in an amount in a range of about 20% to about 40% based on the weight of the bead core particles, and wherein the beads are disposed in a capsule shell.

Pharmacokinetics

As mentioned above, the dosage form can advantageously be designed have one or more pharmacokinetic characteristics, e.g. in humans.

In one embodiment, the pharmaceutical dosage form is characterized by a mean Tmax upon oral dosing, fasted, of greater than 75 minutes, or at least 110 minutes, or at least 2 hours, or at least 3 hours, or in a range of about 2.2 hours to about 3.48 hours, or about 2.22 hours to about 3.34 hours, or about 2.78 hours, or a Tmax in a range of 80% to 125%, or 80% to 120% of such reference Tmax.

In another embodiment, the pharmaceutical dosage form is characterized by a mean Cmax upon oral dosing, fasted, in a range of about 22.16 μmol/L to about 34.63 μmol/L, or about 22.16 μmol/L to about 33.24 μmol/L, or about 22.7 μmol/L, normalized to a 450 mg dose, or a Cmax in a range of 80% to 125%, or 80% to 120% of such reference Cmax. In another embodiment, the pharmaceutical dosage form is characterized by a mean Cmax_D upon oral dosing in a range of about 0.004 to about 0.006 mg/L/mg.

In another embodiment, the pharmaceutical dosage form is characterized by a mean AUC (0-6 hours) upon oral dosing, fasted, in a range of about 60.74 μmol·h/L to about 94.91 μmol·h/L, or about 60.74 μmol·h/L to about 91.12 μmol·h/L, or about 75.93 μmol·h/L, normalized to a 450 mg dose, or a bioequivalent AUC (0-6 hours) in a range of 80% to 125%, or 80% to 120% of such reference AUC (0-6 hours). In another embodiment, the pharmaceutical dosage form is characterized by a mean AUC (0-12 hours) upon oral dosing in a range of about 79.41 μmol·h/L to about 124.08 μmol·h/L, or about 79.41 μmol·h/L to about 119.11 μmol·h/L, or about 99.26 μmol·h/L, normalized to a 450 mg dose, or a bioequivalent AUC (0-12 hours) in a range of 80% to 125%, or 80% to 120% of such reference AUC (0-12 hours). In another embodiment, the pharmaceutical dosage form is characterized by a mean AUC (0-inf_D) upon oral dosing in a range of about 0.86 min·mg/L/mg to about 1.35 min·mg/L/mg, or about 0.86 min·mg/L/mg to about 1.3 min·mg/L/mg, or a bioequivalent AUC (0-inf_D) in a range of 80% to 125%, or 80% to 120% of such reference AUC (0-inf_D).

In example embodiments, any of the described pharmaceutical dosage forms can be characterized by providing mean pharmacokinetic parameters upon oral dosing, fasted, of: Tmax 183±90 minutes, Cmax 3.5±1.7 mg/L, and/or AUC (0-inf_D) 1.08±0.46 min*mg/L/mg, or a bioequivalent Tmax, Cmax or AUC in a range of 80% to 125%, or 80% to 120% of such reference parameter.

In example embodiments, any of the described pharmaceutical dosage forms can be characterized by providing mean pharmacokinetic parameters upon oral dosing of the whole capsule, fasted, of: Tmax 194±38 minutes, Cmax 2.3±0.6 mg/L, and/or AUC (0-inf_D) 0.84±0.19 min*mg/L/mg, or a bioequivalent Tmax, Cmax or AUC in a range of 80% to 125%, or 80% to 120% of such reference parameter; and/or mean pharmacokinetic parameters upon oral dosing of the beads, sprinkled on applesauce, of: Tmax 190±61 minutes,

Cmax 2.3±0.7 mg/L, and/or AUC (0-inf_D) 0.85±0.21 min*mg/L/mg, or a bioequivalent Tmax, Cmax or AUC in a range of 80% to 125%, or 80% to 120% of such reference parameter.

In another embodiment, the pharmaceutical dosage form is characterized by being bioequivalent when administered orally, fasted, in a hard capsule shell compared to the beads being administered orally, fasted, without a capsule shell. For example, the pharmaceutical dosage form can be characterized by the dosage form when administered orally in a hard capsule shell exhibiting a Cmax in a range of 80% to 125%, or 80% to 120%, of Cmax exhibited by the beads administered orally without a capsule shell. In another embodiment, the dosage form can be characterized by the dosage form when administered orally in a hard capsule shell exhibiting an AUC (0-12 h) or AUC (0-inf) in a range of 80% to 125%, or 80% to 120%, of that exhibited by the beads administered orally without a capsule shell, respectively. In one embodiment, both the Cmax and the AUC are within the tolerance ranges just described.

Purity

In one type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, as determined by reverse phase HPLC with UV detection, as described herein. In other embodiments, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 12 months storage at 25° C. and 40% relative humidity (RH), optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 18 months storage at 25° C. and 40% RH optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 24 months storage at 25° C. and 40% RH optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 30 months storage, or more, at 25° C. and 40% RH optionally as determined by reverse phase HPLC with UV detection, as described herein. Examples of suitable reverse phase HPLC assays are described herein.

In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 12 months storage at 25° C. and 60% RH, optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 18 months storage at 25° C. and 60% RH, optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 24 months storage, or more, at 25° C. and 60% RH, optionally as determined by reverse phase HPLC with UV detection, as described herein.

In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 3 months storage at 40° C. and 75% RH, optionally as determined by reverse phase HPLC with UV detection, as described herein. In another type of embodiment, the dosage form is characterized by having less than 5 wt. % cystamine, based on the amount of cysteamine, following 6 months storage at 40° C. and 75% RH, optionally as determined by reverse phase HPLC with UV detection, as described herein.

Any of the foregoing embodiments can be further characterized by having less than 8 wt. % total related substances (impurities) based on the amount of cysteamine, under the described storage conditions and times based on reverse phase HPLC with UV detection, as described herein.

Method of Making

Also contemplated is a method for the preparation of a dosage form according to the disclosure here, including coating a core particle comprising cysteamine or a pharmaceutically acceptable salt thereof and an excipient with an enteric polymer to form the enteric membrane.

The core particle including cysteamine or a pharmaceutically acceptable salt thereof can be formed by any suitable process. In one embodiment, the core particle is formed by granulating a mixture of cysteamine or a pharmaceutically acceptable salt thereof with an excipient and milling to a desired particle size range. In another embodiment, the core particle can be formed by extrusion and spheronization of a mixture of cysteamine or a pharmaceutically acceptable salt thereof with an excipient. Granulating processes can include fluid bed granulation, wet granulation, hot melt granulation, and spray congealing, for example. Other processes include slugging and roller compaction. As it is known in the art, the mixtures which are to be granulated can first be dry-blended. The dry-blended dry ingredients can be mixed with water, prior to extrusion.

It has been found that extrusion and spheronization of a mixture of cysteamine or a pharmaceutically acceptable salt thereof with an excipient can provide desirable core particles with a distribution of particle sizes as described herein and one or more other desirable properties. Cysteamine bitartrate oxidizes in air and in water, and with heat. Thus, short processing times can lead to a more stable product. For example, reducing the amount of spheronization reduces the amount of friction and related heat. For example, reducing the amount of time that the product is exposed to air (either in the moist state and/or before packaging) also reduces the amount of oxidation. On the other hand, rapid processing by extrusion and spheronization can lead to a poor quality product, for example in having a large fraction of the pellet cores falling outside a desired particle size range. The amount of moisture absorbed by spheronization aids (which does not happen immediately, but instead over time) influences the spheronization characteristics of the beads. Accordingly, it was determined that the moisture content of the wet mass, the related wet hold time for swelling of spheronization aid(s), and the spheronization time are parameters that can be optimized to achieve both good product yield, for example in a particle size range described herein, while maintaining good stability, e.g. not more than 5 wt. % cystamine based on the amount of cysteamine, as described herein.

Accordingly, in one embodiment the moisture content of the granulation mixture, prior to drying, is in a range of about 20 wt. % to about 40 wt. %, or 25 wt. % to about 35 wt. %, or about 28 wt. % to about 32 wt. %, or at least about 28 wt. %, or at least about 28.5, or at least about 20 wt. % to about 40 wt. %, or at least about 25 wt. % to about 35 wt. %, or at least about 27 wt. % to about 31 wt. % or at least about 28.5 wt. % to about 31 wt. %.

The wet mass can be held for a period of time prior to extrusion, e.g. in order to allow the sphronization aid to swell with granulating fluid. The hold time can be at least 15 minutes, at least 30 minutes, at least 45 minutes, or at least 60

minutes, for example. The hold time can be in a range of about 15 minutes to about 120 minutes, or about 30 minutes to 100 minutes, or 60 minutes to 90 minutes, for example.

As described above in connection with description of the core particles, the method can include a step of sorting (e.g., by sieving) the core particles prior to enteric coating, to retain particles in a predetermined size range, for example sizes in a range of about 0.7 mm to about 2.8 mm, or about 0.7 mm to about 2.5 mm, or about 0.8 mm to about 1.7 mm, or any range described above in connection with the core particles.

As described above in connection with description of the beads, the method can include a step of sorting (e.g., by sieving) the beads after enteric coating, to retain particles in a predetermined size range, for example sizes in a range of about 0.7 mm to about 2.8 mm, or about 0.7 mm to about 2.5 mm, or about 0.8 mm to about 1.7 mm, or any range described above in connection with the core particles.

In an extrusion and spheronization process, the following optional features can be employed, individually or in one or more combinations thereof. Water can be used as a granulation agent. Microcrystalline cellulose can be used in the core particles as a spheronization aid. Hypromellose can be included in the core particles as a binder. The extrusion screen size can be 1.0 mm. The friction plate of the spheronizer can be cross-hatched. The friction plate of the spheronizer can be cross-hatched with a square pitch of at least 3 mm, or greater than 3 mm, or at least 4 mm, or greater than 4 mm, or in a range of about 3 mm to about 7 mm, or about 5 mm. The spheronization time can be less than about 5 minutes, or less than about 4 minutes, or less than about 3 minutes, or less than about 2 minutes, or up to 1 minute. The spheronized particles can include non-spherical particles (i.e. irregular shapes), e.g. a substantial fraction thereof, e.g. at least 20 wt. % or at least 30 wt. %, or at least 40 wt. % or at least 50 wt. % or at least 60 wt. %, or at least 70 wt. % thereof.

The beads and/or filled capsules can be stored with a desiccant. The beads and/or filled capsules can be stored with an oxygen absorber.

For example, one embodiment of the method combining various of the parameters described above includes a method for the preparation of a pharmaceutical dosage form including cysteamine beads, including forming a wet mass comprising cysteamine bitartrate and an excipient, optionally microcrystalline cellulose, with a moisture content in a range of in a range of about 20 wt. % to about 40 wt. %, extruding and spheronizing the wet mass including cysteamine bitartrate and excipient to make core particles, sorting the core particles to a target particle size range, optionally 0.7 mm to 2.5 mm, coating the sorted core particles with an enteric polymer to form including beads comprising a core particle and an enteric membrane, and sorting the bead particles to a target particle size range, optionally 0.7 mm to 2.5 mm.

Use/Administration

For administration of the dosage form, a total weight in the range of approximately 100 mg to 1000 mg (based on the free base) can be used. The dosage form can be orally administered to a patient suffering from a condition for which an cysteamine is indicated, including, but not limited to, cystinosis and other metabolic and neurodegenerative diseases including non-alcoholic fatty liver disease (NAFLD), Huntingon's disease, Parkinson's disease, Rett Syndrome and others, use as free radical and radioprotectants, and as heptoprotectant agents. In any method described herein, the treatment of humans is contemplated. The compositions of the disclosure can be used in combination with other therapies useful for treating cystinosis and neurodegenerative diseases and disorders. For example, indomethacin therapy (Indocid® or Endol®) is an anti-inflammatory used to treat rheumatoid arthritis and lumbago, but it can be used to reduce water and electrolyte urine loss. In children with cystinosis, indomethacin reduces the urine volume and therefore liquid consumption by about 30%, sometimes by half. In most cases this is associated with an appetite improvement. Indomethacin treatment is generally followed for several years.

Other therapies can be combined with the methods and compositions of the disclosure to treat diseases and disorders that are attributed or result from cystinosis. Urinary phosphorus loss, for example, entails rickets, and it may be necessary to give a phosphorus supplement. Carnitine is lost in the urine and blood levels are low. Carnitine allows fat to be used by the muscles to provide energy. Hormone supplementation is sometimes necessary. Sometimes the thyroid gland will not produce enough thyroid hormones. This is given as thyroxin (drops or tablets). Insulin treatment is sometimes necessary if diabetes appears, when the pancreas does not produce enough insulin. These treatments have become rarely necessary in children whom are treated with cysteamine, since the treatment protects the thyroid and the pancreas. Some adolescent boys require a testosterone treatment if puberty is late. Growth hormone therapy may be indicated if growth is not sufficient despite a good hydro electrolytes balance. Accordingly, such therapies can be combined with the compositions and methods disclosed herein.

The effectiveness of a method or composition of the disclosure can be assessed by measuring leukocyte cystine concentrations. Dosage adjustment and therapy can be made by a medical specialist depending upon, for example, the concentration of cystine in leukocytes and the ability to tolerate the drug. Additional therapies including the use of omeprazole (Prilosec®) can reduce side effects of cysteamine administration, such as abdominal pain, heartburn, nausea, vomiting, and anorexia, which can result from cysteamine-induced gastric acid hypersecretion, for example.

In addition, various prodrugs can be "activated" by use of the enterically coated cysteamine. Prodrugs are pharmacologically inert, they themselves do not work in the body, but once they have been absorbed, the prodrug decomposes. The prodrug approach has been used successfully in a number of therapeutic areas including antibiotics, antihistamines and ulcer treatments. The advantage of using prodrugs is that the active agent is chemically camouflaged and no active agent is released until the drug has passed out of the gut and into the cells of the body. For example, a number of prodrugs use S—S bonds. Weak reducing agents, such as cysteamine, reduce these bonds and release the drug. Accordingly, the compositions of the disclosure are useful in combination with pro-drugs for timed release of the drug. In this aspect, a pro-drug can be administered followed by administration of an enterically coated cysteamine composition of the invention (at a desired time) to activate the pro-drug.

EXAMPLES

The following examples are provided for illustration and are not intended to limit the scope of the invention.

Example 1

Bead Production

Cysteamine bitartrate and excipients (microcrystalline cellulose, hypromellose, sodium lauryl sulfate) were milled through a Comil equipped with a 0.094" (2.3876 mm) screen operating at 500 RPM. The amount of each ingredient (per 75

mg cysteamine capsule) is cysteamine bitartrate 258 mg+/−37.0 mg; microcrystalline cellulose 67.1 mg+/−9.6 mg; hypromellose 17.2 mg+/−2.5 mg; and sodium lauryl sulfate 1.75 mg+/0.25 mg. Cysteamine bitartrate was passed through the Comil first followed by the excipients (hypromellose 2910-5, sodium lauryl sulfate, and microcrystalline cellulose). Cysteamine bitartrate and the excipients were dry blended for approximately 15 minutes. While mixing at a setpoint speed of 47 rpm, purified water was slowly added (addition in approximately 4 minutes) into the blended components. After the water addition, the wet blend was mixed for an additional minute for a total of 5 minutes.

A sample of the wet blend was collected and moisture content was determined by loss on drying (LOD). The wet mass was discharged in polyethylene lined fiber drums and held for 60-90 minutes prior to extrusion/spheronization.

The granulated wet mass was loaded onto a NICA extruder equipped with a 1.0 mm screen at a feeder speed of 100 RPM setpoint and extruded at a setpoint speed of 55 RPM (50-60 RPM). The extruded product was immediately spheronized using a NICA Spheronizer equipped with 5.0 mm cross-hatched friction plates. Spheronization was performed at a target speed of 625 RPM (500-700 RPM) for 40-60 seconds. The particles were collected in double polyethylene lined fiber drums and stored at room temperature for further processing.

The wet particles were dried in a Niro fluid bed dryer with an inlet air temperature setpoint of 70° C. (60-80° C.). Drying was complete when the moisture content of uncrushed particles reached ≤1.0% w/w by LOD. Sampling of the particles began when the outlet air temperature reached approximately 50° C. and continued until the acceptance criterion of ≤1.0%. The dried particles were transferred to fiber drums lined with double polyethylene bags and stored at room temperature.

The dried particles were screened through a #12 mesh screen and a #20 mesh screen. Particles passing through the #12 mesh and retained on the #20 mesh were collected as product in double polyethylene lined containers with desiccant and oxygen absorber packets in the outer liner. The collected product may be re-passed through the screens as needed. Particles greater than #12 mesh and less than #20 mesh were not retained as product for coating.

An enteric coating solution of Eudragit L30 D-55, triethyl citrate, and talc in purified water was prepared in a mixing tank equipped with a propeller mixer and placed on a balance. Eudragit L 30 D-55 was added to the portable mixing tank through a 60-mesh screen. The final solution was mixed for a minimum of 30 minutes and mixed continuously during the coating process. Based on a 75 mg cysteamine capsule, the amounts of coating ingredients were: Eudragit L30 D-55 66.2 mg+/−9.5 mg; triethyl citrate 6.65 mg+/−0.95 mg; talc 15.3 mg+/−2.2 mg.

Spray lines connecting the portable mixing tank to the Niro fluid bed dryer were primed. The floor balance was tared prior to starting the coating process. The amount of coating solution sprayed was calculated as the amount required to increase the core particle weight by 25%.

The core particles were loaded into the Niro fluid bed dryer equipped with a Precision Coater which sprays from the bottom, 1.0 mm Nozzle, 30 mm Swirl Accelerator, and 300 μm Filter Bonnet. The coating process parameters are provided in the table below.

Parameter	Setpoint	Range
Inlet Air Volume	450 scfm	300-600 scfm
Inlet Air Temperature	60° C.	45-75° C.
Product Temperature	30° C.	25-45° C.
Solution Spray Rate	0.220 kg/minute	0.200-0.240 kg/minute
Atomization Air Pressure	36 psi	32-40 psi

Once the target weight of coating solution was applied (25% of dry particle weight), the beads were weighed to confirm weight increase of ≥25.0%. If the weight was not ≥25.0% of the uncoated particle weight, the coating process was continued until ≥25.0% was achieved.

The coated beads were dried at an inlet temperature setpoint of 45° C. (35-55° C.) and inlet air volume setpoint of 350 scfm (300-400 scfm) until the LOD of the coated beads was ≤2.0% w/w. Once the LOD was reached, the inlet air heating was turned off and the beads were circulated at an air inlet volume of 300-400 scfm until the product temperature reached not more than (NMT) 30° C.

The weight gain of the dried coated beads was calculated to confirm a maximum weight gain of ≤31.0% was achieved. Visual inspection confirmed that the enteric membrane thickness was not consistent bead-to-bead, but instead there was a distribution of enteric membrane thicknesses.

The dried coated beads were screened through a #12 mesh and a #20 mesh screen in sequence. Beads passing through the #12 mesh screen and retained on the #20 mesh screen were collected as product in double polyethylene lined fiber drums with a desiccant and oxygen absorber canister in the outer liner. Mesh analysis testing can be performed as an in-process test to confirm the beads are within the limits of: NMT 5% are retained on a #12 mesh screen (1.68 mm) and NMT 10% pass through a #20 mesh screen (0.84 mm). If results are not within the limits, the product can be sorted by rescreening until the mesh analysis results meet the specified limits.

The dried coated beads were lubricated with talc prior to encapsulation. The coated beads were loaded in a V-blender; talc powder was added to the coated beads (calculated as 0.5% w/w of the total coated bead weight). The contents were mixed for a minimum of five minutes. The lubricated coated beads were transferred to double polyethylene lined fiber drums with desiccant and oxygen absorber packets in the outer liner and stored at room temperature. Lubricated coated beads were used in the manufacture of 75 mg size 0 capsules and 25 mg size 3 capsules. One batch of coated beads can be filled as a 75 mg strength batch or can be split to fill both 75 mg and 25 mg strengths, for example.

The 75 mg hard gelatin capsules were filled using an automated encapsulator at a speed of 80-100 spm to the target fill weight calculated to achieve 75 mg cysteamine free base per capsule. The 25 mg hard gelatin capsules were also filled with an automated encapsulator at a speed of 50-70 spm. The beads were introduced into the encapsulation process with a hopper.

Example 2

Particle Size Distribution

Several lots of cysteamine bitartrate enteric-coated beads produced via an extrusion and spheronization process as

described herein were analyzed for particle size distribution via analytical sieving. The results are tabulated below.

Sieve Size (μm)	% Retained Lot A	% Retained Lot B	% Retained Lot C	% Retained Lot D
1700	0	0	0	0
1400	1.4	3.2	3.2	1.2
1180	19.5	25.7	26.7	20.3
1000	61.9	55.5	56	62
850	16.1	14.2	13.5	15.1
<850	1.2	1.4	0.6	1.4

Example 3

Pharmacokinetics

A population PK study was performed using Cystagon® and capsules of cysteamine bitartrate gastro-resistant beads (CBGB) produced according to the method of Example 1 herein.

Pharmacokinetic (PK) and pharmacodynamic (PD) relationships following a single dose of CBGB capsules was first studied in comparison to a single dose of immediate-release cysteamine bitartrate in a study with 9 patients. Following normalization to a 450 mg dose, the maximum plasma levels C max, AUC 0-6 h and AUC 0-12 h (calculated directly from the plasma level data for CBGB and from doubling the AUC 0-6 h value for immediate-release cysteamine to represent two doses) were lower for CBGB (27.70±14.99 μmol/L, 75.93±39.22 μmol*h/L and 99.26±44.21 μmol*h/L respectively) than for immediate-release cysteamine bitartrate (37.72±12.10 μmol/L, 96.00±37.81 μmol*h/L and 192.00±75.62 μmol*h/L respectively. The pharmacokinetics of CBGB are consistent with a delayed-release formulation showing a T max of 2.78±1.56 h for CBGB cysteamine was moderately bound to human plasma proteins, predominantly to albumin, with mean protein binding of about 52%. Plasma protein binding was independent of concentration over the concentration range achieved clinically with the recommended doses.

Additional studies were carried out as follows.

CBGB-A Study

Cystagon® Treatment Assignment: one (1) pre-dose PD sample was collected at time 0 (i.e., within 15 minutes prior to the morning Cystagon® dose administration), considered as the time of trough cysteamine/peak of WBC cystine after administration of immediate-release cysteamine bitartrate (Cystagon®). One (1) additional PD sample was collected at a sample timepoint that was time-matched to 1 of 3 PK sample profile times (either 2, 4 or 6 hours) post morning Cystagon® dose. There were six associated plasma PK samples collected at time 0 (within 15 minutes prior to morning Cystagon® dose); 30 minutes post morning Cystagon® dose; and 1, 2, 4 and 6 hours (immediately prior to the afternoon Cystagon® dose)

Inventive capsule Treatment Assignment: one (1) post-dose PD sample was collected at time 0.5 hour (30 minutes), considered as the time of trough cysteamine/peak of WBC

cystine after administration of capsules of CBGB. Two (2) additional PD samples were collected at sample timepoints that were time-matched to PK sample profile times (either 3, 4, 8, 10 or 12 hours) post morning CBGB dose. In order to limit the impact of autocorrelation, juxtaposed times of sampling for patients treated with CBGB were not to be taken into account for the randomization. Therefore, patients were randomized to one of the following six pairs of the sampling time points: 3 and 8 hours, 3 and 10 hours, 3 and 12 hours, 4 and 8 hours, 4 and 10 hours, 4 and 12 hours. There were nine associated plasma PK samples collected at time 0 (within 15 minutes prior to morning CBGB dose), 30 minutes, 2, 3, 4, 6, 8, 10 and 12 hours post morning CBGB dose (immediately prior to the evening CBGB dose).

As recommended in the Cystagon® SmPC, food (meal or snack) was available 30 minutes prior to receiving the morning dose and (if applicable) the next Q6H of Cystagon® administration and the morning dose and Q12H CBGB administration and (if applicable) the next Q12H CBGB dose. Cystagon® was administered with water and CBGB was administered with an acidic beverage. Dairy products should have been withheld 1 hour before and after CBGB dosing.

CBGB-B Study

Administering cysteamine in fasted healthy volunteers provides very stable PK parameters such that it was possible to demonstrate bioequivalence between administrations of CBGB capsules as a whole or as their content sprinkled on food with only 20 healthy volunteers.

The PK parameters of cysteamine were determined after a single dose, first in fasted healthy volunteers, then in patients at steady state, using the model parameters obtained with healthy volunteers as starting parameters for the models in patients. Pharmacokinetic modeling of cysteamine was based on a 2-compartment model and pharmacodynamic modeling of WBC cystine was based on an inhibitory E_{max} model. (Belldina, E. B., M. Y. Huang, et al. (2003). “Steady-state pharmacokinetics and pharmacodynamics of cysteamine bitartrate in paediatric nephropathic cystinosis patients.” Br J Clin Pharmacol 56(5): 520-525.)

Since CBGB studies in healthy volunteers were not done against Cystagon®, data in fasted healthy volunteers (Gangoiti, J. A., M. Fidler, et al. (2010). “Pharmacokinetics of enteric-coated cysteamine bitartrate in healthy adults: a pilot study.” Br J Clin Pharmacol 70(3): 376-382) were used to determine initial PK model parameters for Cystagon®. And data on EC-cysteamine (i.e. Eudragit L50D 55 enteric-coated capsules of Cystagon®—a different way of providing delayed-release cysteamine bitartrate) in this dataset was used for comparison purposes.

A bioequivalence designed to demonstrate bioequivalence between oral administration of intact CBGB capsules, and contents of opened CBGB capsules mixed with applesauce and taken orally. Twenty (20) healthy adults (mean age 37 years, range 19-64 years) received both presentations, 8 (75 mg) intact vs. 8 (75 mg) open capsules, in a crossover design study.

The final results are presented in the table below.

Study/ Protocol Country	Study Design	No. Subjects Entered/ Completed (M/F)	HV/P[a)] (Age: Mean, Range)	Treatment	Dose (mg)	Non-Compartmental Analysis (Pharsight, WinNonLin 6.2) T_{max} (min)	C_{max} (mg/L)	C_{max}_D (mg/L/mg)	AUC_{inf}_D (min*mg/L/mg)	Population PK, 2-compartment Model (Pharsight, NLME 1.1) T_{lag} (min)	Ka (1/min)	V/F (L)	Cl/F (L/min)	V_2 (L)	Cl_2 (L/min)
UCSD (USA)	Open label, Sequential	(4M/3F)/ (4M/3F)	P (12, 8-17)	Cystagon ®	450	75 ± 19	3.1 ± 1.2	0.007 ± 0.003	0.88 ± 0.30	26	0.029	73	1.07	131	0.41
				EC-Cysteamine	450	220 ± 74	3.2 ± 1.4	0.007 ± 0.003	0.96 ± 0.40	156	0.025	98	1.17	54	0.5
CBGB-A (USA/ EU)	Random, Cross-over	(24M/ 19F)/ (22M/ 16F)	P(12, 6-26)	Cystagon ®	250-750	74 ± 32	2.6 ± 1.4	0.006 ± 0.003	0.84 ± 0.31	23	0.025	94	1.1	191	0.5
				CBGB caps	425-1300	183 ± 90	3.5 ± 1.7	0.005 ± 0.002	1.08 ± 0.46	60	0.015	87	1.2	200	0.4
CBGB-B (USA)	Random, Cross-over	(13M/ 7F)/ (13M/ 7F)	HV(37, 19-64)	CBGB caps	600	194 ± 38	2.3 ± 0.6	0.004 ± 0.001	0.84 ± 0.19	95	0.016	137	1.4	187	0.44
				CBGB sprinkled	600	190 ± 61	2.3 ± 0.7	0.004 ± 0.001	0.85 ± 0.21	98	0.017	151	1.4	192	0.47

[a)]HV = Healthy Volunteers, P = Patients

The conclusion of this population PK modeling on two different presentations of CBGB (open and intact), is that the only difference between administering CBGB as intact capsules and as open capsules, sprinkled on applesauce, is expressed by the difference between lag times: as expected the start of absorption from the beads is still delayed (85 min) but slightly less than when the gelatin capsule has to be dissolved first (108 min) and this has not much of an impact on T_{max} (190 min for open capsules vs. 194 min for intact capsules) since probably only a small amount of beads dissolves early.

However, comparison between the two presentations of CBGB (open and intact) and the immediate-release cysteamine bitartrate (Cystagon®) and the delayed-release EC-cysteamine, shows that the absorption of cysteamine after CBGB dosing is not only more delayed (Cystagon® T_{lag}<<CBGB T_{lag}<<EC-cysteamine T_{lag}) but also further extended due to a slower absorption (CBGB K_a<<Cystagon® K_a≈EC-cysteamine K_a) compared to EC-cysteamine. Without intending to be bound by any particular theory, it is contemplated that the difference in absorption of the CBGB formulation is related to one or more factors including the distribution of bead sizes and time-progressive dissolution of multiple beads and/or the irregularity of bead shapes in the CBGB formulation and/or the distribution of enteric membrane thicknesses in the CBGB formulation.

Example 4

Purity and Stability

Long term stability tests have been performed on the CBGB formulation made according to Example 1. The major impurity in the CBGB product is cystamine, the well known related substance (dimer).

The use of a more sensitive and less selective method has resulted in the observation of several impurities found in the CBGB formulation and the commercial product using cysteamine bitartrate, Cystagon®. Through the use of reverse phase HPLC, six peaks observed in the CBGB formulation related substances chromatograms have been identified as product degradants (specifically cysteamine bitartrate degradants). Two lots of Cystagon® were evaluated by the same test method. The impurities observed in representative CBGB chromatograms are also observed in Cystagon®.

Impurities Assay Method

Cysteamine bitartrate samples are assessed by gradient elution HPLC using an XBRIDGE C18 column (dimensions: 150 mm×4.6 mm; packing particle size: 3.5 μm) (Waters, Milford, Mass.). The autosampler temperature is 4° C. Approximately 10 μL or approximately 100 μL of sample is injected onto the column. The column temperature is 40° C. and the sample is eluted at a flow rate of 1.0 mL/min according to the following profile:

Time (min)	HPLC Gradient Mobile Phase A (%)	Mobile Phase B (%)
0.0	100	0
2.0	100	0
20.0	60	40
25.0	60	40
25.1	100	0
40.0	100	0

Mobile Phase A contains 23.6 mM 1-octanesulfonic acid sodium and 29.0 mM sodium phosphate (pH 2.6)/acetonitrile/methanol 85/3/12 (v/v/v). Mobile Phase B contains 0.20 M 1-octanesulfonic acid sodium and 0.10 M sodium phosphate (pH 2.6)/acetonitrile/methanol 10/18/72 (v/v/v). The purity of 1-octanesulfonic acid is ≥98%. Detection is carried out using a UV detector at 210 nm.

Reference Solution Preparation.

Reference solutions of Cysteamine Bitartrate Analytical Reference Standard are prepared as follows. Working Standard and Working Check Standard solutions are prepared having a nominal concentration of 0.54 mg/mL Cysteamine Bitartrate Analytical Reference Standard in Mobile Phase A using low actinic glassware. A Working Sensitivity solution is prepared having a nominal concentration of 0.30 mg/mL Cysteamine Bitartrate Analytical Reference Standard in Mobile Phase A using low actinic glassware, which corresponds to the limit of quantification (LOQ) for cysteamine. The water content of the Cysteamine Bitartrate Analytical Reference Standard is determined no more than 7 days before use by Karl Fischer titration or thermal gravimetric analysis (TGA). The Reference Standard is stored refrigerated and blanketed under nitrogen.

Bead Prep Assay Sample Preparation.

Cysteamine Bitartrate Gastro-resistant Beads (CBGB) are prepared for analysis according to the following procedure. About 3.7 g of CBGB beads are ground to a fine powder using a ball mill for approximately 1 minute at 27 Hz. The grind is transferred to an amber bottle for storage. Stock Bead Prep Assay sample solutions are prepared in duplicate by adding 370.4 mg±5 mg of the grind to a 250 mL low actinic volumetric flask and diluting with Mobile Phase A. The mixture is stirred with a stir bar for at least 15 minutes. Approximately 15 mL of the resulting solution is filtered through a 0.45 μm nylon filter, with the first 5 mL being discarded. The cysteamine concentration of the resulting Stock Bead Prep Assay sample solution is approximately 0.300 mg/mL. Working Bead Prep sample solutions are prepared by placing 4.0 mL of Stock Bead Prep Assay sample solution in a 25 mL low actinic volumetric flask and diluting to volume with Mobile Phase A. The cysteamine concentration of the resulting Working Bead Prep sample solution is approximately 0.048 mg/mL.

Assay Sample Preparation.

CBGB capsules are prepared for analysis according to the following procedure. To reduce exposure to light and oxygen, sample preparation (from the initial weighing of the full capsules to the loading of sample vials on the HPLC) is completed in one day. Ten capsules are weighed. The capsule contents are emptied and the empty shells are weighed to determine the average capsule fill weight. The capsule contents are ground to a fine powder using a ball mill for approximately 1 minute at 27 Hz. The grind is transferred to an amber bottle for storage. Stock sample solutions are prepared in duplicate by adding the appropriate amount of the grind for 1 capsule (as determined by the average capsule fill weight) to a 25 mL low actinic volumetric flask and diluting with Mobile Phase A. The mixture is stirred with a stir bar for at least 15 minutes. The resulting solution is centrifuged at about 3400 rpm for 5 minutes. Approximately 15 mL of the centrifuged solution is filtered through a 0.45 μm nylon filter (Acrodisc, 25 mm diameter), with the first 5 mL being discarded, to obtain Stock sample solutions. Working sample solutions are prepared by placing 6.0 mL of Stock sample solution (for 25 mg capsules) or 2.0 mL of Stock sample solution (for 75 mg capsules) in a 10 mL low actinic volumetric flask and diluting to volume with Mobile Phase A.

Content Uniformity Sample Preparation.

CBGB capsules are prepared for analysis according to the following procedure. To reduce exposure to light and oxygen, sample preparation (from the initial weighing of the full capsules to the loading of sample vials on the HPLC) is completed in one day. Ten capsules are weighed. The contents of each capsule are emptied into separate mortars and the empty shells are weighed to determine the individual capsule fill weight. About 1-2 mL of Mobile Phase A is added into the mortar. The beads are immediately ground to a paste. If needed, additional Mobile Phase A is added to the paste, up to 5 mL total. The paste is transferred to a 250 mL low actinic volumetric flask. The mortar and pestle are thoroughly rinsed with Mobile Phase A and the rinse solution is collected in to the same flask. The flask is filled about three-quarters full with Mobile Phase A and stirred for at least 15 minutes. The flask is filled to volume with Mobile Phase A. Approximately 20 mL of the resulting solution is filtered through a 0.45 μm nylon filter (Acrodisc, 25 mm diameter), with the first 5 mL being discarded, to obtain Stock CU sample solutions. Working CU sample solutions are prepared by placing 12.0 mL of Stock CU sample solution (for 25 mg capsules) or 4.0 mL of Stock CU sample solution (for 75 mg capsules) in a 25 mL low actinic volumetric flask and diluting to volume with Mobile Phase A. The cysteamine concentration of the resulting Working CU sample solutions is approximately 0.048 mg/mL.

Data Analysis.

The cysteamine Working Standard solution concentration is calculated according to the following equation: Cysteamine Concentration (C_{Std})=mg Cysteamine Bitartrate Analytical Reference Standard×P_f/25.0 mL

P_f represents a purity factor for the standard material. P_f is calculated according to the following equation:

$$P_f = B\times(100-\text{Water})\times C/100$$

where B=the anhydrous cysteamine free base in the Cysteamine Bitartrate Analytical Reference Standard (expressed as a decimal value on the standard bottle label),

water=the water content as determined by Karl Fischer or TGA no more than 7 days before use (expressed as a percentage), and

C=the cystamine correction (expressed as a decimal value on the standard bottle label).

The amount of cysteamine per capsule is calculated according to the following equation:

$$\text{mg cysteamine per capsule} = (A_{Sam}/A_{Std})\times C_{Std}\times \text{DF}\times(\text{AveWt}/\text{SamWt})$$

where A_{Sam}=the peak area of cysteamine in the sample chromatogram with a 10 μL injection,

A_{Std}=the average peak area of cysteamine in all Working Standard solution chromatograms with a 10 μL injection,

C_{Std}=the concentration (mg/mL) of cysteamine in the Working Standard solution,

DF=the dilution factor (125 for 75 mg capsules; 41.6667 for 25 mg capsules),

AveWt=the average capsule fill weight (mg), and

SamWt=the sample weight (mg).

For Content Uniformity, the amount of cysteamine per capsule is calculated according to the following equation:

$$\text{mg cysteamine per capsule} = (A_{Sam}/A_{Std})\times C_{Std}\times \text{DF}$$

where A_{Sam}=the peak area of cysteamine in the sample chromatogram with a 10 μL injection,

A_{Std}=the average peak area of cysteamine in all Working Standard solution chromatograms with a 10 μL injection,

C_{Std}=the concentration (mg/mL) of cysteamine in the Working Standard solution, and

DF=the dilution factor (1562.5 for 75 mg capsules; 520.8 for 25 mg capsules).

For the Bead Prep Assay, the amount of cysteamine per capsule is calculated according to the following equation:

$$\text{mg cysteamine per capsule} = (A_{Sam}/A_{Std})\times C_{Std}\times \text{DF}\times(\text{AveWt}/\text{SamWt})$$

where A_{Sam}=the peak area of cysteamine in the sample chromatogram with a 10 μL injection,

A_{Std}=the average peak area of cysteamine in all Working Standard solution chromatograms with a 10 μL injection,

C_{Std}=the concentration (mg/mL) of cysteamine in the Working Standard solution,

DF=the dilution factor (use the 75 mg Dilution Factor, 1562.5),

AveWt=the average capsule fill weight (mg) (use the target fill weight, 370.4 mg), and

SamWt=the sample weight (mg) (use the actual weight used in sample preparation).

The percentage of the label claim (% LC) is calculated for the Assay, Content Uniformity, and Bead Prep Assay sample solutions according to the following equation:

$$\% LC=(\text{mg cysteamine})/LC\times 100\%$$

where mg cysteamine=the amount calculated by the applicable equation above, and

LC=the amount of the label claim (75 mg or 25 mg) (use 75 mg for the Bead Prep Assay).

The amount of substances related to cysteamine bitartrate (including cysteamine impurities) such as cystamine is calculated according to the following equation:

$$\text{mg related substance}=(A_{RS}/A_{Std})\times(C_{Std}/\text{RRF})\times\text{DF}\times(\text{AveWt}/\text{SamWt})$$

where A_{RS}=the peak area of any related substance in the Working sample solution chromatogram with a 100 μL injection (peaks before RRT 0.48 are disregarded; peaks observed in the chromatogram of the second injection of Mobile Phase A/Blank (100 μL injection) are also disregarded),

A_{Std}=the average peak area of cysteamine in all Working Standard solution chromatograms with a 10 μL injection,

C_{Std}=the concentration (mg/mL) of cysteamine in the Working Standard solution,

RRF=the relative response factor (0.98 for cystamine; 1.00 for other related substances),

DF=the dilution factor (12.5 for 75 mg capsules; 4.16667 for 25 mg capsules),

AveWt=the average capsule fill weight (mg), and

SamWt=the weight the sample grind from the Working sample solution preparation (mg).

The weight percentage of cystamine and other individual related substances is determined according to the following equation:

$$\%\ \text{individual related substance}=\text{mg related substance}/\text{mg cysteamine}\times 100\%$$

where mg related substance=the amount of related substance calculated above, and

mg cysteamine=the amount of cysteamine for the Assay sample.

The percentage of total related substances is determined by summing all related substances greater than or equal to 0.05%. Peaks after 28 minutes are disregarded. In contrast to a previous electrochemical detection method that disregarded early-eluting peaks as not relevant to the purity calculation, the foregoing method determines that early peaks are impurities and integrates early-eluting peaks as described above.

Results

Two lots of Cystagon® were dispensed in standard pharmacy containers and verified to be well within the manufacturer's expiration date. One lot was provided by a healthcare provider. It was dispensed in a standard pharmacy bottle and verified by the healthcare provider to be well within the expiration date. Upon analysis by the Test Method, it was shown to contain 9.1% cystamine by weight and 10.3% total related substances, based on the weight of cysteamine, using the assay described above. The second analyzed Cystagon® lot was identified by lot number. Upon analysis by the assay described above, it was shown to contain 5.2% cystamine by weight and 5.7% total related substances, based on the weight of cysteamine. Each Cystagon® lot was shipped and stored under specified label conditions.

Two representative lots of the CBGB capsule formulation were analyzed by the assay described above and were shown to contain 3.7% cystamine by weight and 3.6% cystamine by weight, respectively, based on the weight of cysteamine, at the time of manufacture. For both lots, the total amount of related substances was 4.2% by weight, based on the weight of cysteamine.

The CBGB product lots were put on stability testing in various packages and storage conditions, then assayed for purity using the assay described above. The results are shown in the table below

Lot	Product dose/count/ bottle size	Conditions ° C./% RH	Cystamine %/total related substances at time point (month) Initial	1	2	3	6	9	12
1	75 mg/60/100 cc	25/60	3.7/4.2	3.7/NA	3.1/NA	3.4/4.2	3.5/4.4	3.7/5.1	3.8/5.5
1	75 mg/60/100 cc	40/75	3.7/4.2	3.7/NA	3.2/NA	3.5/7.9	3.9/12.3		
1	75 mg/150/250 cc	25/60	3.7/4.2	3.5/NA	3.4/NA	3.7/4.5	3.6/4.3	3.6/4.9	3.7/5.4
1	75 mg/150/250 cc	40/75	3.7/4.2	3.4/NA	3.4/NA	3.7/7.9	3.8/11.6		
1	75 mg/300/400 cc	25/60	3.7/4.2	3.5/NA	3.3/NA	3.4/4.2	3.5/4.4	3.7/5.1	3.8/5.7
1	75 mg/300/400 cc	40/75	3.7/4.3	3.4/NA	3.2/NA	3.6/7.7	4.0/12.8		
1	75 mg/60/bulk	25/40	3.7/4.2	3.4/NA	3.4/NA	3.2/NA	3.3/4.2	3.3/4.5	3.2/4.6
1	75 mg/60/bulk	40/75	3.7/4.2	3.4/NA	3.2/NA	3.3/NA	2.9/9.1		
2	75 mg/150/250 cc	25/60	3.6/4.2	3.1/4.0	3.3/4.3	3.0/4.3	3.3/5.0		
2	75 mg/150/250 cc	40/75	3.6/4.2	3.1/7.5	3.6/12.1				

Additional CBGB product samples according to Example 1 were put on long term stability testing in various packages and storage conditions, then assayed for purity using the assay described above. Results are shown in the table below.

Lot	Product dose/count/bottle size	Conditions ° C./% RH	Cystamine %/total related substances at time point (month) Initial	1	2	3	6	9	12	15	18	24
3	25 mg/60/50 cc	25/60	3.2/ 4.1	3.0/ NA	3.3/ NA	3.1/ 4.2	3.3/ 4.7	3.2/ 4.9	3.6/ 5.5	NA	4.0/ 6.8	4.6/ NA
3	25 mg/60/50 cc	40/75	3.2/ 4.1	2.9/ NA	3.0/ NA	3.0/ 7.8	3.7/ 13.4					

-continued

Lot	Product dose/count/bottle size	Conditions ° C./% RH	Cystamine %/total related substances at time point (month) Initial	1	2	3	6	9	12	15	18	24
4	75 mg/150/ 250 cc	25/60	3.2/ 4.0	3.2/ NA	3.4/ NA	3.4/ 4.7	3.7/ 5.2	3.5/ 5.3	4.1/ 6.3	NA	4.2/ 7.1	4.7/ NA
4	75 mg/150/ 250 cc	40/75	3.2/ 4.0	3.1/ NA	3.4/ NA	3.5/ 9.0	4.0/ 13.8					
5	75 mg/60/100 cc	25/60	3.4/ 4.2	3.4/ NA	3.5/ NA	3.3/ 4.4	3.7/ 5.2	3.5/ 5.2	5.0/ NA	3.9/ 6.0	3.9/ NA	5.3/ NA
5	75 mg/60/100 cc	40/75	3.4/ 4.2	3.3/ NA	3.4/ NA	3.3/ 8.5	4.1/ 16.0[1]					
5	75 mg/300/ 400 cc	25/60	3.4/ 4.2	3.5/ NA	3.5/ NA	3.5/ 4.9	4.0/ 6.0	3.3/ 5.3	5.3/ NA	4.1/ 6.5	4.1/ NA	5.3/ NA
5	75 mg/300/ 400 cc	40/75	3.4/ 4.2	3.5/ NA	3.7/ NA	3.7/ 9.6	4.2/ 15.4[1]					
5	75 mg/60/bulk	25/60	3.4/ 4.2	3.5/ NA	3.5/ NA	3.4/ NA	3.7/ 5.2	3.2/ NA	4.1/ 5.5			
5	75 mg/60/bulk	40/75	3.4/ 4.2	3.4/ NA	3.4/ NA	3.1/ NA	3.0/ 11.2					
6	25 mg/60/50 cc	25/60	3.3/ 4.1	3.3/ NA	3.2/ NA	3.2/ 4.3	3.7/ 5.5	3.3/ 5.0	4.0/ NA	3.9/ 5.9	4.4/ NA	5.1/ NA
6	25 mg/60/50 cc	40/75	3.3/ 4.1	3.2/ NA	3.1/ NA	3.0/ 7.8	3.8/ 15.7[1]					
6	25 mg/420/ 250 cc	25/60	3.3/ 4.1	3.3/ NA	3.5/ NA	3.6/ 4.9	4.0/ 6.0	3.8/ 5.9	4.6/ NA	4.8/ 7.4	4.1/ NA	5.2/ NA
6	25 mg/420/ 250 cc	40/75	3.3/ 4.1	3.4/ NA	3.5/ NA	3.5/ 9.7	4.7/ 17.1[1]					
6	25 mg/60/bulk	25/60	3.3/ 4.1	3.4/ NA	3.4/ NA	3.3/ NA	3.7/ 5.4	3.1/ NA	3.5 5.3			
6	25 mg/60/bulk	40/75	3.3/ 4.1	3.3/ NA	3.2/ NA	2.9/ NA	2.9 11.4					
7	75 mg/60/100 cc	25/60	3.2/ 3.9	3.2/ NA	3.3/ NA	3.3/ 4.5	3.5/ 5.3	3.2/ 4.9	4.6/ NA	4.1/ 6.1	3.6/ NA	4.6/ NA
7	75 mg/60/100 cc	40/75	3.2/ 3.9	3.2/ NA	3.1/ NA	3.2/ 8.0	3.7/ 13.5[1]					
7	75 mg/300/ 400 cc	25/60	3.2/ 3.9	3.4/ NA	3.4/ NA	3.4/ 4.8	3.7/ 5.5	3.4/ 5.2	4.9/ NA	4.2/ 6.4	3.8/ NA	4.7/ NA
7	75 mg/300/ 400 cc	40/75	3.2/ 3.9	3.3/ NA	3.3/ NA	3.5/ 9.1	3.9/ 13.6[1]					
7	75 mg/60/bulk	25/60	3.2/ 3.9	3.3/ NA	3.3/ NA	3.2/ NA	3.5/ 5.2	2.9/ NA	4.0/ 5.5			
7	75 mg/60/bulk	40/75	3.2/ 3.9	3.2/ NA	3.2/ NA	2.9/ NA	2.8/ 10.3					
8	25 mg/60/50 cc	25/60	3.1/ 3.9	3.1/ NA	3.1/ NA	3.0/ 4.1	3.4/ 4.9	3.1 4.9	3.7 NA	3.4/ 5.3	3.4/ NA	4.3/ NA
8	25 mg/60/50 cc	40/75	3.1/ 3.9	3.0/ NA	2.9/ NA	2.7/ 7.4	3.4/ 13.4[1]					
8	25 mg/420/ 250 cc	25/60	3.1/ 3.9	3.2/ NA	3.3/ NA	3.3/ 4.6	3.6/ 5.3	3.4/ 5.0	4.1/ NA	4.5/ 6.8	3.8/ NA	4.6/ NA
8	25 mg/420/ 250 cc	40/75	3.1/ 3.9	3.3/ NA	3.3/ NA	3.2/ 9.1	4.0/ 16.2[1]					
8	25 mg/60/bulk	25/60	3.1/ 3.9	3.3/ NA	3.2/ NA	3.1/ NA	3.3/ 4.7	3.0/ NA	3.4/ 4.9			
8	25 mg/60/bulk	40/75	3.1/ 3.9	3.2/ NA	3.0/ NA	2.8/ NA	2.7/ 10.6					
9	25 mg/60/50 cc	25/60	3.6/ 4.2	3.5/ NA	2.9/ NA	3.2/ 4.0	3.4/ 4.4	3.3/ 4.6	3.4/ 5.0			
9	25 mg/60/50 cc	40/75	3.6/ 4.2	3.4/ NA	2.7/ NA	3.0/ 6.8	3.4/ 11.4					
9	25 mg/420/ 250 cc	25/60	3.6/ 4.2	3.5/ NA	3.0/ NA	3.4/ 4.3	3.4/ 4.4	3.5/ 5.0	3.8/ 5.8			
9	25 mg/420/ 250 cc	40/75	3.6/ 4.2	3.5/ NA	3.0/ NA	3.5/ 7.9	3.8/ 12.9					
9	25 mg/60/bulk	25/40	3.6/ 4.2	3.5/ NA	3.0/ NA	3.3/ NA	3.3/ 4.2	3.1/ 4.2	3.1/ 4.6			
9	25 mg/60/bulk	40/75	3.6/ 4.2	3.3/ NA	2.8/ NA	3.0/ NA	2.8/ 9.1					
10	25 mg/60/50 cc	25/60	3.4/ 4.0	NA	NA	3.1/ 3.9	3.1/ 4.1	2.9/ 4.2	3.1/ 4.7			
10	25 mg/60/50 cc	40/75	3.4/ 4.0	NA	NA	2.7/ 7.0	3.2/ 11.9					

[1]Samples pulled at 6 months but held at room temperature until new reference standard was qualified (at 8 months)

All of the foregoing CBGB samples met the acid resistance criteria (Not more than 10% (Q) of the label claim of cysteamine is dissolved after 2 hours in 0.1N HCl) and dissolution criteria (Not less than 70% (Q) of the label claim of cysteamine is dissolved after 30 minutes in 0.2M sodium phosphate buffer, pH 6.8)

The foregoing description is given for clearness of understanding only, and no unnecessary limitations should be understood therefrom, as modifications within the scope of the invention may be apparent to those having ordinary skill in the art.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise" and variations such as "comprises" and "comprising" will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Throughout the specification, where compositions are described as including components or materials, it is contemplated that the compositions can also consist essentially of, or consist of, any combination of the recited components or materials, unless described otherwise. Likewise, where methods are described as including particular steps, it is contemplated that the methods can also consist essentially of, or consist of, any combination of the recited steps, unless described otherwise. The invention illustratively disclosed herein suitably may be practiced in the absence of any element or step which is not specifically disclosed herein.

The practice of a method disclosed herein, and individual steps thereof, can be performed manually and/or with the aid of or automation provided by electronic equipment. Although processes have been described with reference to particular embodiments, a person of ordinary skill in the art will readily appreciate that other ways of performing the acts associated with the methods may be used. For example, the order of various of the steps may be changed without departing from the scope or spirit of the method, unless described otherwise. In addition, some of the individual steps can be combined, omitted, or further subdivided into additional steps.

All patents, publications and references cited herein are hereby fully incorporated by reference. In case of conflict between the present disclosure and incorporated patents, publications and references, the present disclosure should control.

What is claimed is:

1. A pharmaceutical dosage form, comprising delayed-release cysteamine beads, the beads comprising:

(i) a core particle comprising cysteamine or a pharmaceutically acceptable salt thereof and a binder, and

(ii) an enteric membrane surrounding the core particle,

wherein the beads have a distribution of particle sizes in a range of about 0.7 mm to about 2.8 mm;

wherein the enteric membrane begins to dissolve within a pH range of about 4.5 to about 6.5;

wherein the enteric membrane is present in an amount in a range of about 25% to about 35% by weight, based on the weight of the core particles; and

wherein the pharmaceutical dosage form, upon administration in a capsule to fasted healthy normal subjects at 600 mg free cysteamine base, provides:

(a) a mean Cmax upon oral dosing in a range of 2.3±0.6 mg/L or in a range of 80% to 125% thereof; and

(b) a mean AUC (0-inf_D) upon oral dosing in a range of 0.84±0.19 min*mg/L/mg or in a range of 80% to 125% thereof.

2. The pharmaceutical composition of claim **1**, wherein the particle sizes of the beads are in a range of about 0.7 mm to about 2.5 mm.

3. The pharmaceutical dosage form of claim **1**, wherein the distribution of bead sizes is characterized by at least 80% by weight of the beads having a particle size in a range of about 850 μm to about 1180 μm.

4. The pharmaceutical composition of claim **1**, wherein 5% or less of the beads by weight are retained on a #12 mesh (1.68 mm) screen and 10% or less by weight pass through a #20 mesh (0.84 mm) screen.

5. The pharmaceutical composition of claim **1**, wherein the distribution of bead sizes is characterized by less than 5% by weight of the beads being retained on a 1400 μm sieve.

6. The pharmaceutical dosage form of claim **1**, wherein the distribution of bead sizes is characterized by less than 30% by weight of the beads being retained on a 1180 μm sieve.

7. The pharmaceutical dosage form of claim **1**, wherein the distribution of bead sizes is characterized by less than 70% by weight of the beads being retained on a 1000 μm sieve.

8. The pharmaceutical dosage form of claim **1**, wherein the distribution of bead sizes is characterized by less than 20% by weight of the beads being retained on a 850 μm sieve.

9. The pharmaceutical dosage form of claim **1**, wherein the distribution of bead sizes is characterized by at least 15% by weight of the beads being retained on a 1180 μm sieve.

10. The pharmaceutical dosage form of claim **1**, wherein the distribution of bead sizes is characterized by at least 50% by weight of the beads being retained on a 1000 μm sieve.

11. The pharmaceutical dosage form of claim **1**, wherein the distribution of bead sizes is characterized by at least 10% by weight of the beads being retained on a 850 μm sieve.

12. The pharmaceutical dosage form of claim **1**, wherein the distribution of bead sizes is characterized by a median particle size in a range of about 850 μm to about 1180 μm.

13. The pharmaceutical dosage form of claim **1**, wherein the bead core particle further comprises a filler.

14. The pharmaceutical dosage form of claim **1**, wherein the cysteamine (as free base) is present in the bead core particle in an amount of at least 10 wt. %.

15. The pharmaceutical dosage form of claim **1**, wherein the cysteamine or pharmaceutically acceptable salt thereof is a pharmaceutically acceptable salt of cysteamine.

16. The pharmaceutical dosage form of claim **1**, wherein 5% or less of the bead core particles by weight are retained on a #12 mesh (1.68 mm) screen and 10% or less by weight pass through a #20 mesh (0.84 mm) screen.

17. The pharmaceutical dosage form of claim **1**, wherein the enteric-coated beads are characterized by acid resistance such that not more than 10% of the cysteamine in the beads is dissolved after a period of two hours in a 0.1N HCl solution.

18. The pharmaceutical dosage form of claim **1**, wherein the enteric-coated beads are characterized by dissolution such that 80% of the cysteamine or pharmaceutically acceptable salt thereof is released within 20 minutes in a solution buffered at pH 6.8.

19. The pharmaceutical dosage form of claim **1**, further comprising a capsule shell enclosing the plurality of beads.

20. The pharmaceutical dosage form of claim **1**, wherein the beads provide a mean Cmax and mean AUC (0-inf_D) upon oral dosing, fasted, when administered inside a capsule shell that are bioequivalent to the mean Cmax and mean AUC (0-inf_D) upon oral dosing, fasted, when administered without a capsule shell.

21. The pharmaceutical dosage form of claim **1**, wherein the enteric membrane comprises an enteric material that begins to dissolve at pH of about 5.5 in an aqueous solution.

22. The pharmaceutical dosage form of claim **1**, wherein the pharmaceutical dosage form, upon administration in a capsule to fasted healthy normal subjects at 600 mg free cysteamine base, provides:

(a) a mean Cmax upon oral dosing in a range of 2.3±0.6 mg/L; and

(b) a mean AUC (0-inf_D) upon oral dosing in a range of 0.84±0.19 min*mg/L/mg.

23. The pharmaceutical dosage form of claim **1**, wherein the pharmaceutical dosage form, upon administration in a capsule to fasted healthy normal subjects at 600 mg free cysteamine base, provides:

(a) a mean Cmax upon oral dosing of 2.3 mg/L or in a range of 80% to 125% thereof; and

(b) a mean AUC (0-inf_D) upon oral dosing of 0.84 min*mg/L/mg or in a range of 80% to 125% thereof.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

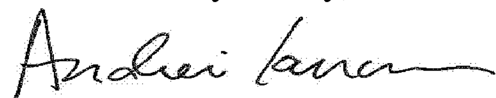
PATENT NO. : 9,233,077 B2
APPLICATION NO. : 14/306303
DATED : January 12, 2016
INVENTOR(S) : Powell et al.

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

On the Title Page

Item (72) Inventors should read as follows:
(72) Kathlene Powell, Cary, NC (US);
Ramesh Muttavarapu, Durham, NC (US);
Ranjan Dohil, San Diego, CA (US).

Signed and Sealed this
Tenth Day of July, 2018



Andrei Iancu
Director of the United States Patent and Trademark Office

EXHIBIT F

US010143665B2

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

(10) **Patent No.: US 10,143,665 B2**
(45) **Date of Patent: Dec. 4, 2018**

(54) **METHODS FOR STORING CYSTEAMINE FORMULATIONS AND RELATED METHODS OF TREATMENT**

(71) Applicant: **Horizon Orphan LLC**, Lake Forest, IL (US)

(72) Inventors: **Michael DesJardin**, Aptos, CA (US); **Mark Johnson**, Fort Collins, CO (US)

(73) Assignee: **Horizon Orphan LLC**, Lake Forest, IL (US)

(*) 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.: **15/238,037**

(22) Filed: **Aug. 16, 2016**

(65) **Prior Publication Data**

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A61K 31/095	(2006.01)
A61K 9/20	(2006.01)
A61K 9/28	(2006.01)
A61K 31/145	(2006.01)
A61K 9/50	(2006.01)

(52) **U.S. Cl.**
CPC ***A61K 31/145*** (2013.01); ***A61K 9/5026*** (2013.01)

(58) **Field of Classification Search**
None
See application file for complete search history.

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(57) **ABSTRACT**

Methods of storing and methods of stabilizing pharmaceutical compositions comprising cysteamine, or a pharmaceutically acceptable salt thereof, are provided. Methods of distributing pharmaceutical compositions comprising cysteamine, or a pharmaceutically acceptable salt thereof, and methods of treating cystinosis also are provided.

8 Claims, 19 Drawing Sheets

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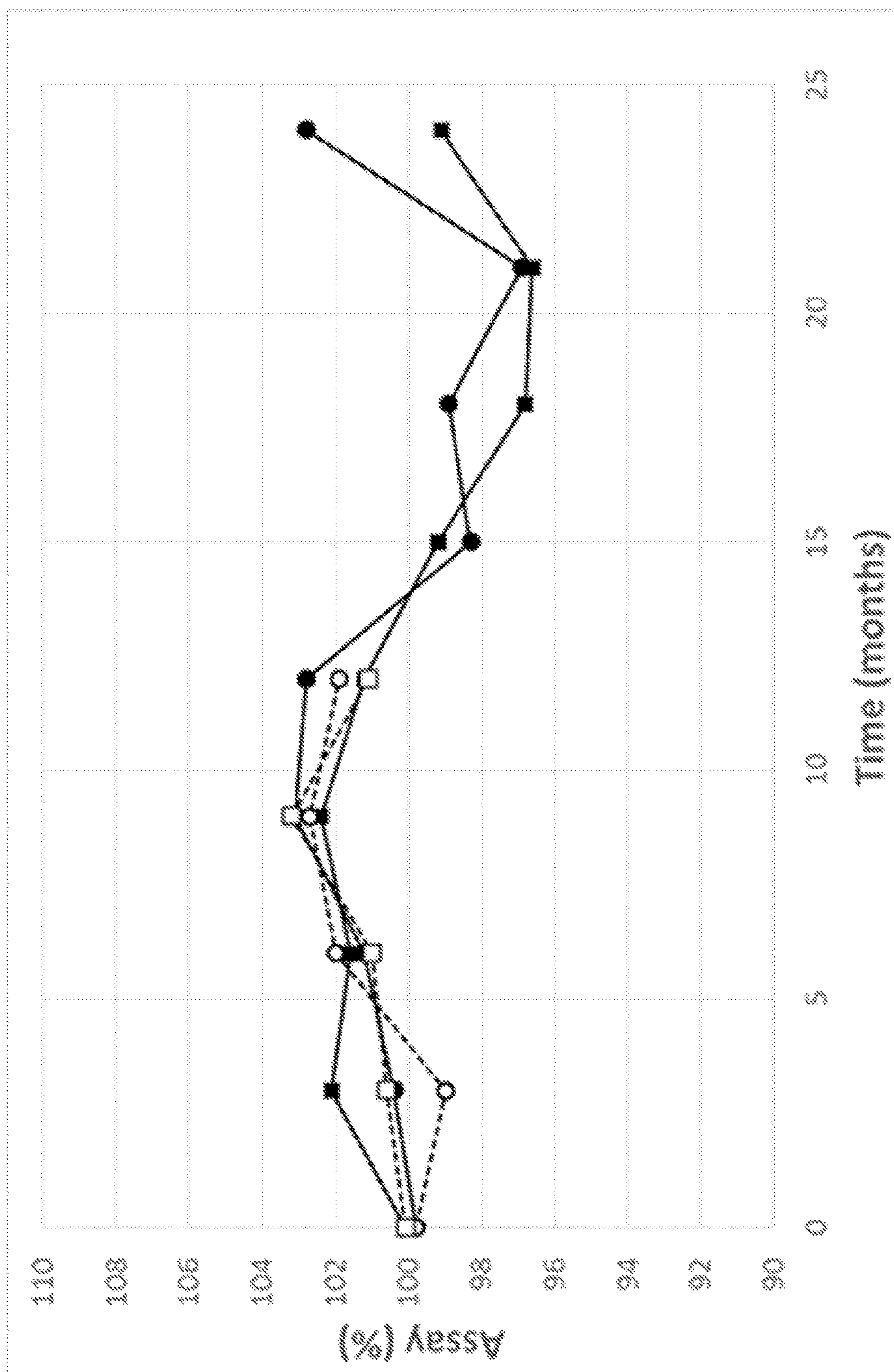

FIG. 1A

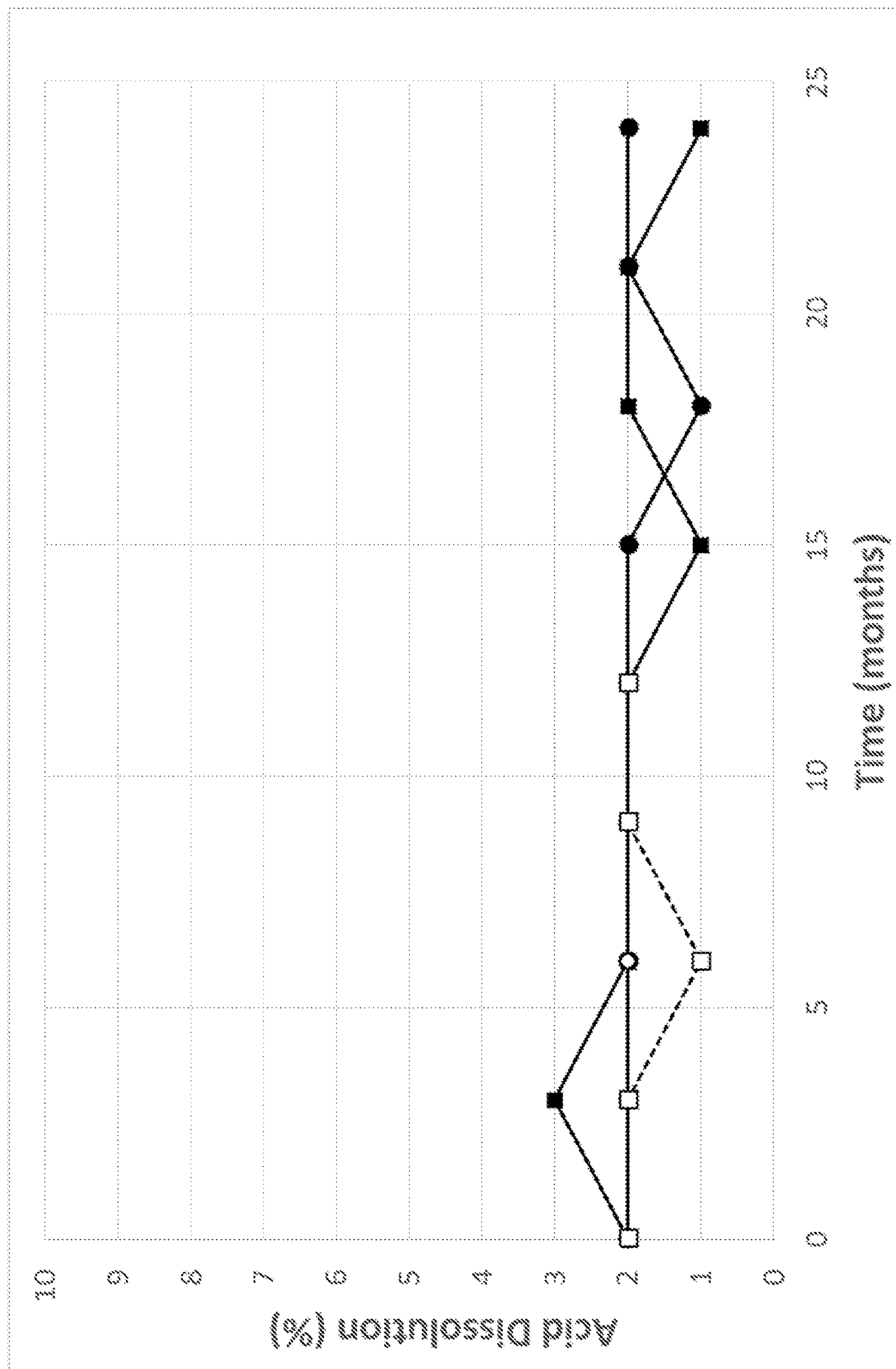

FIG. 1B

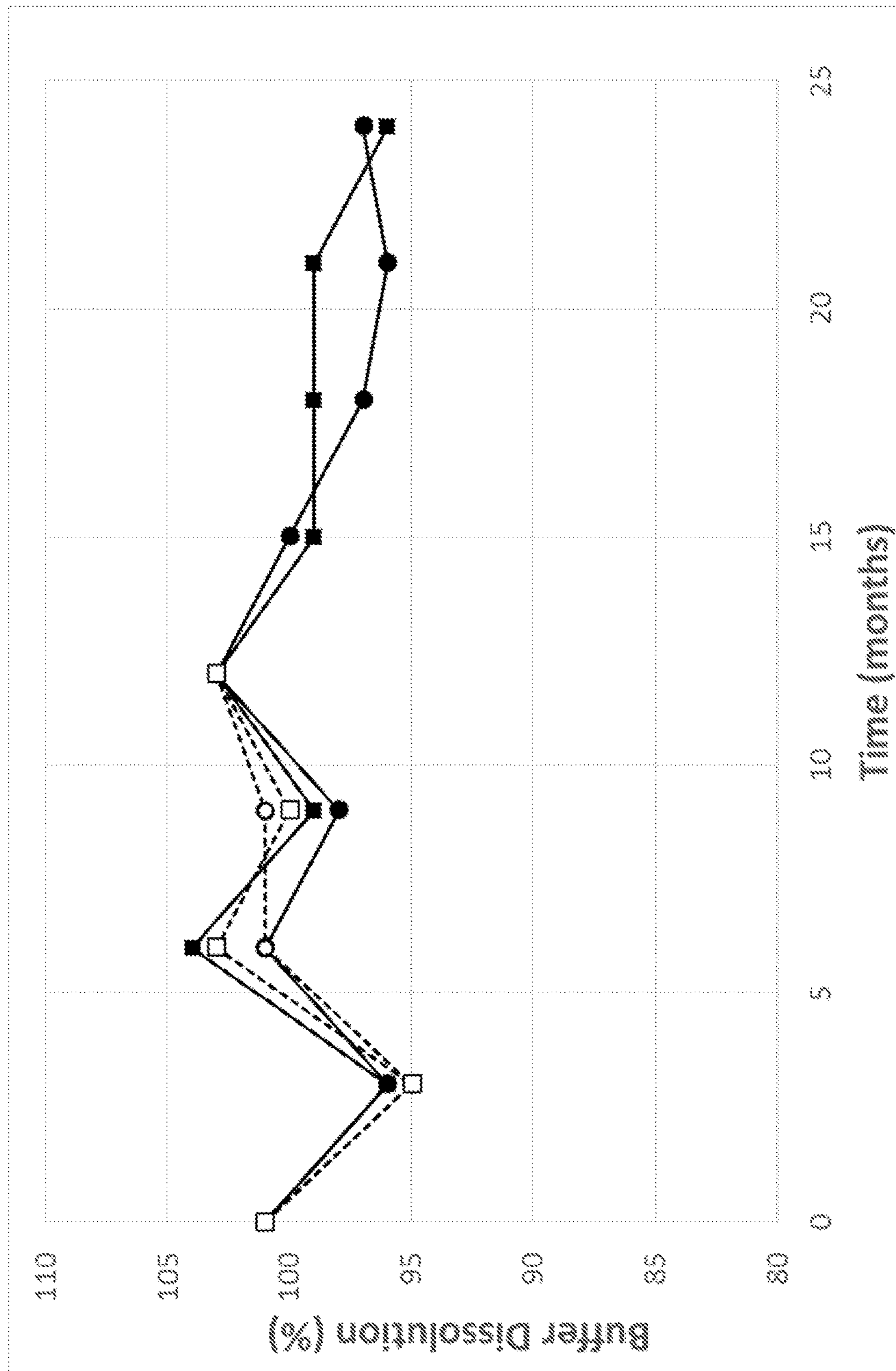

FIG. 1C

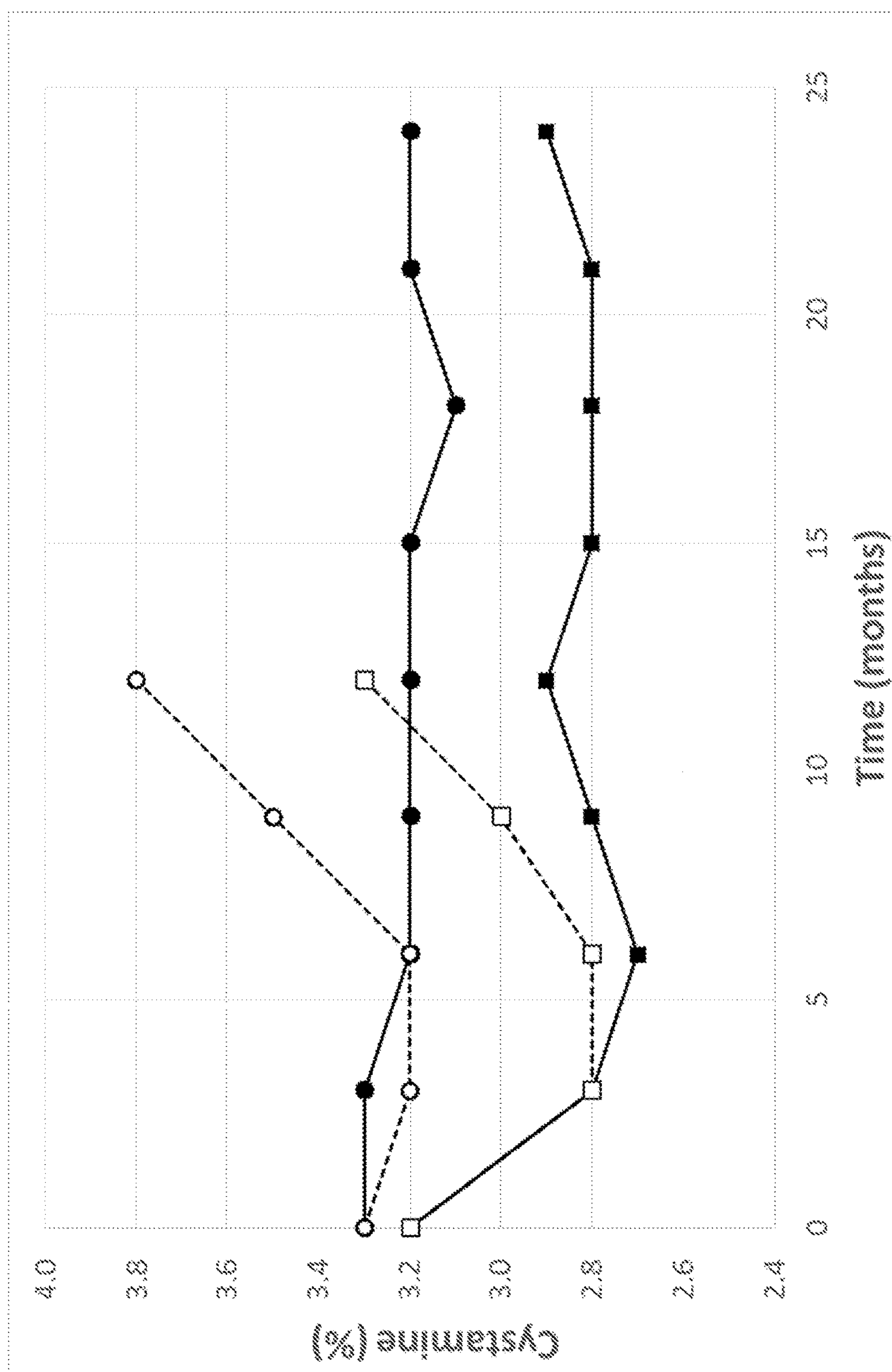

FIG. 1D

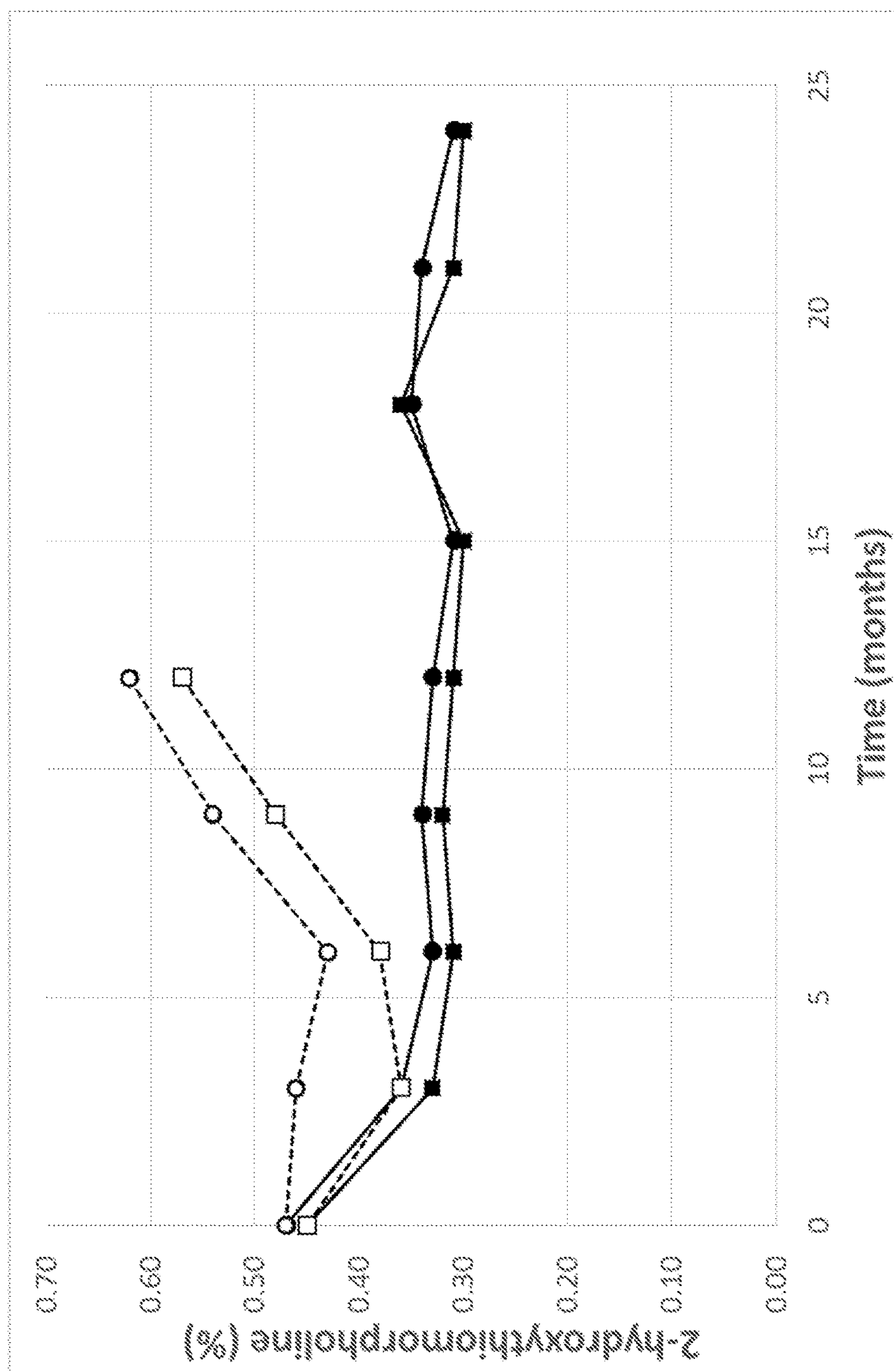

FIG. 1E

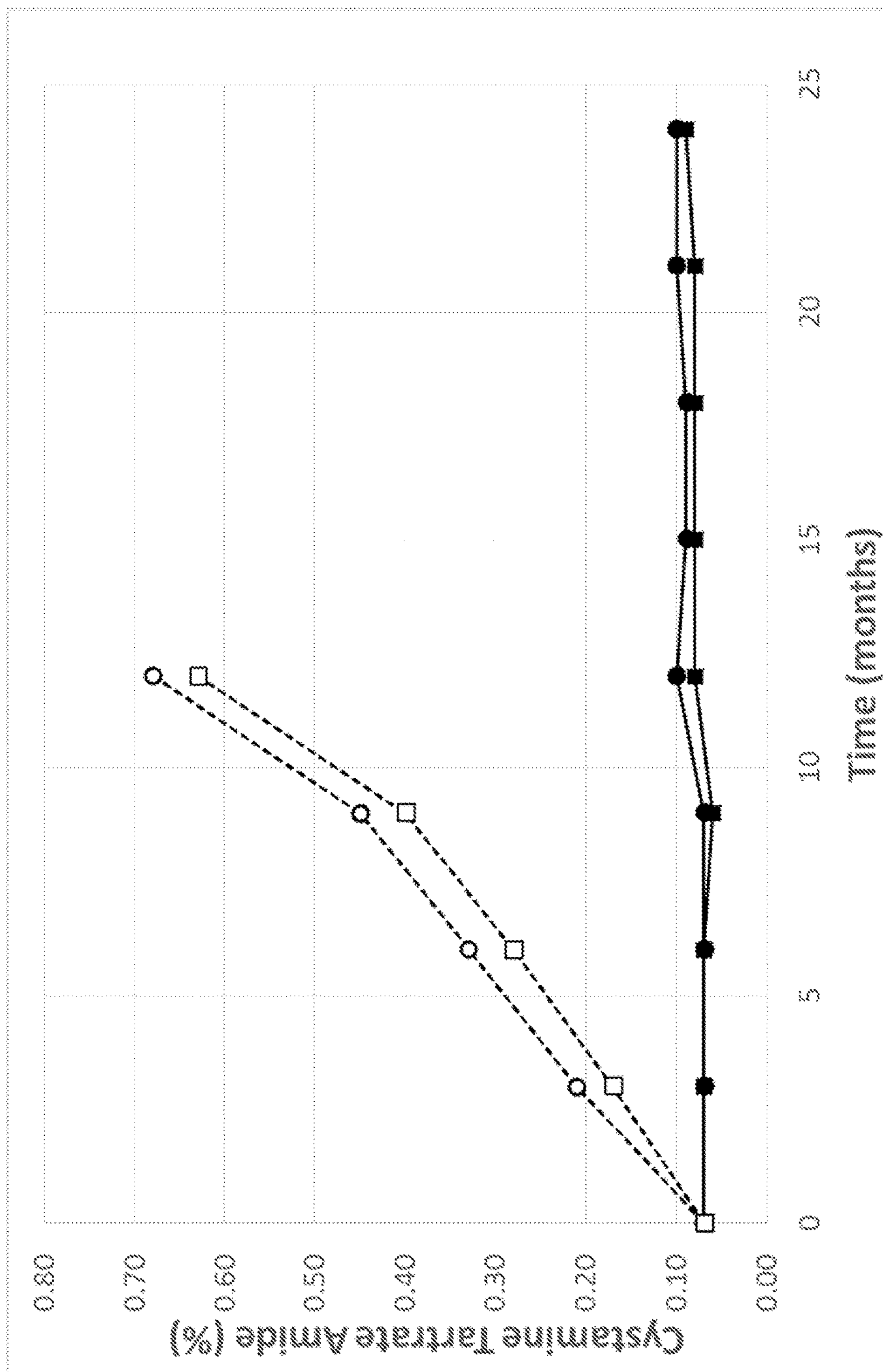

FIG. 1F

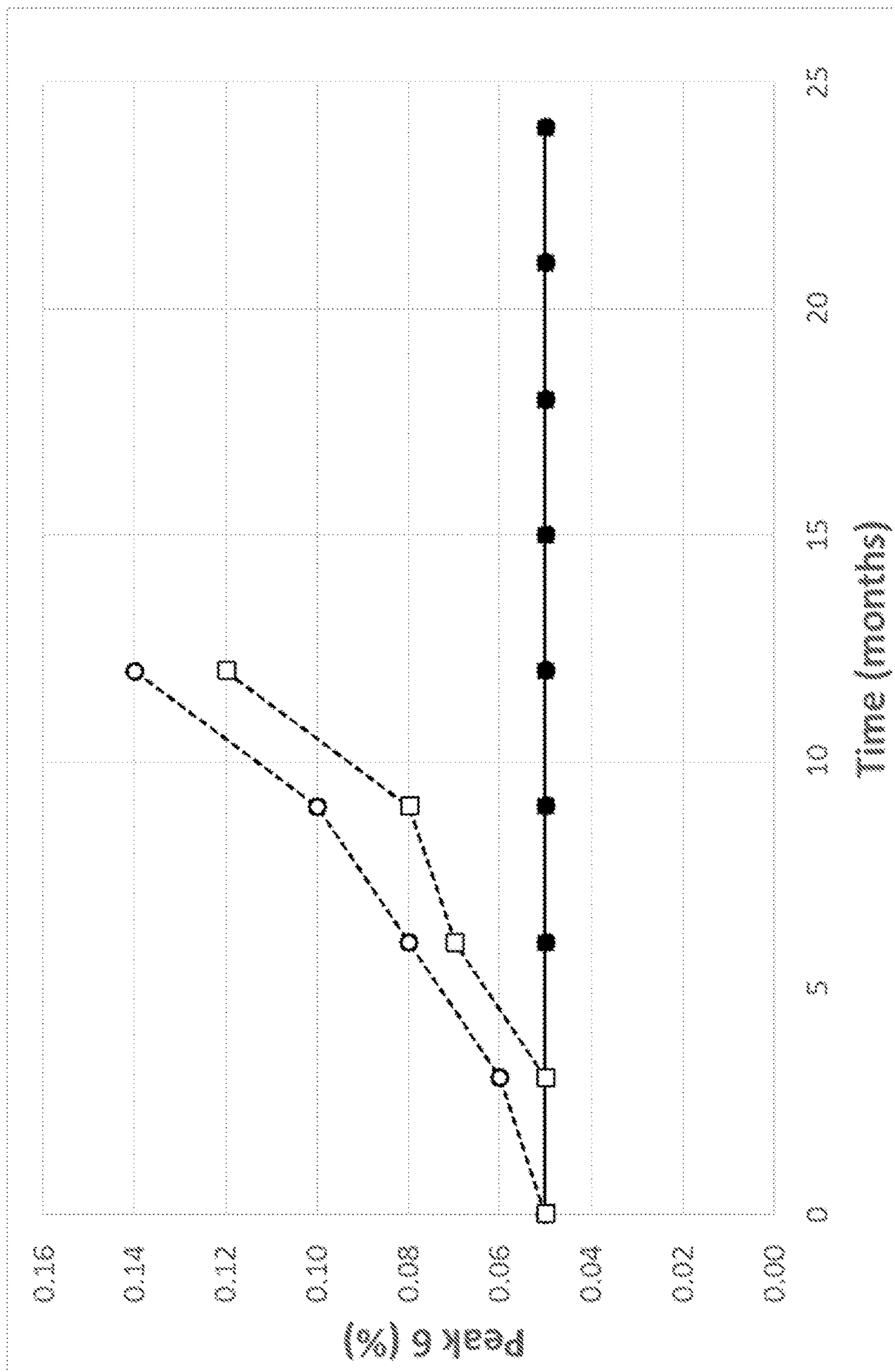

FIG. 1G

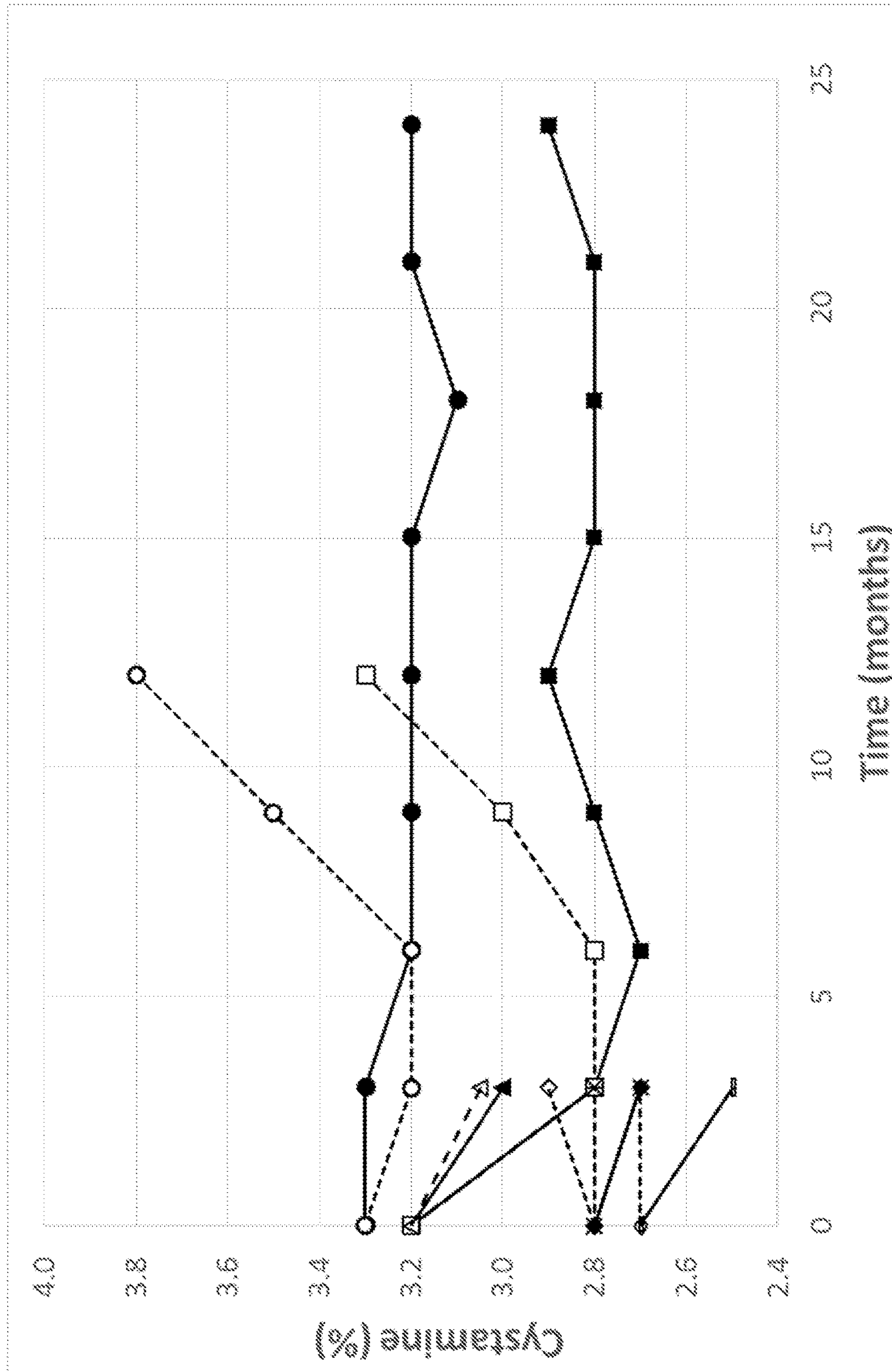

FIG. 2A

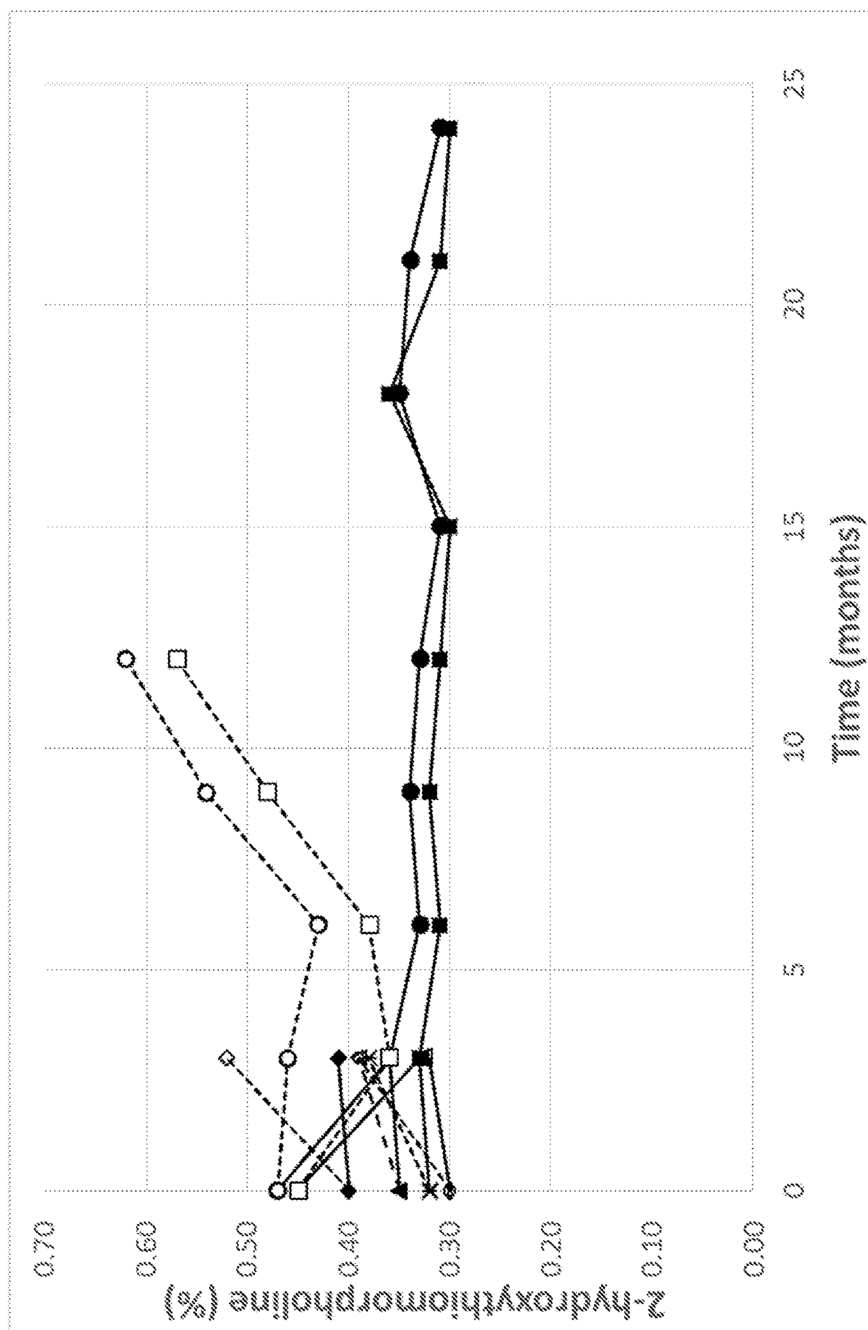

FIG. 2B

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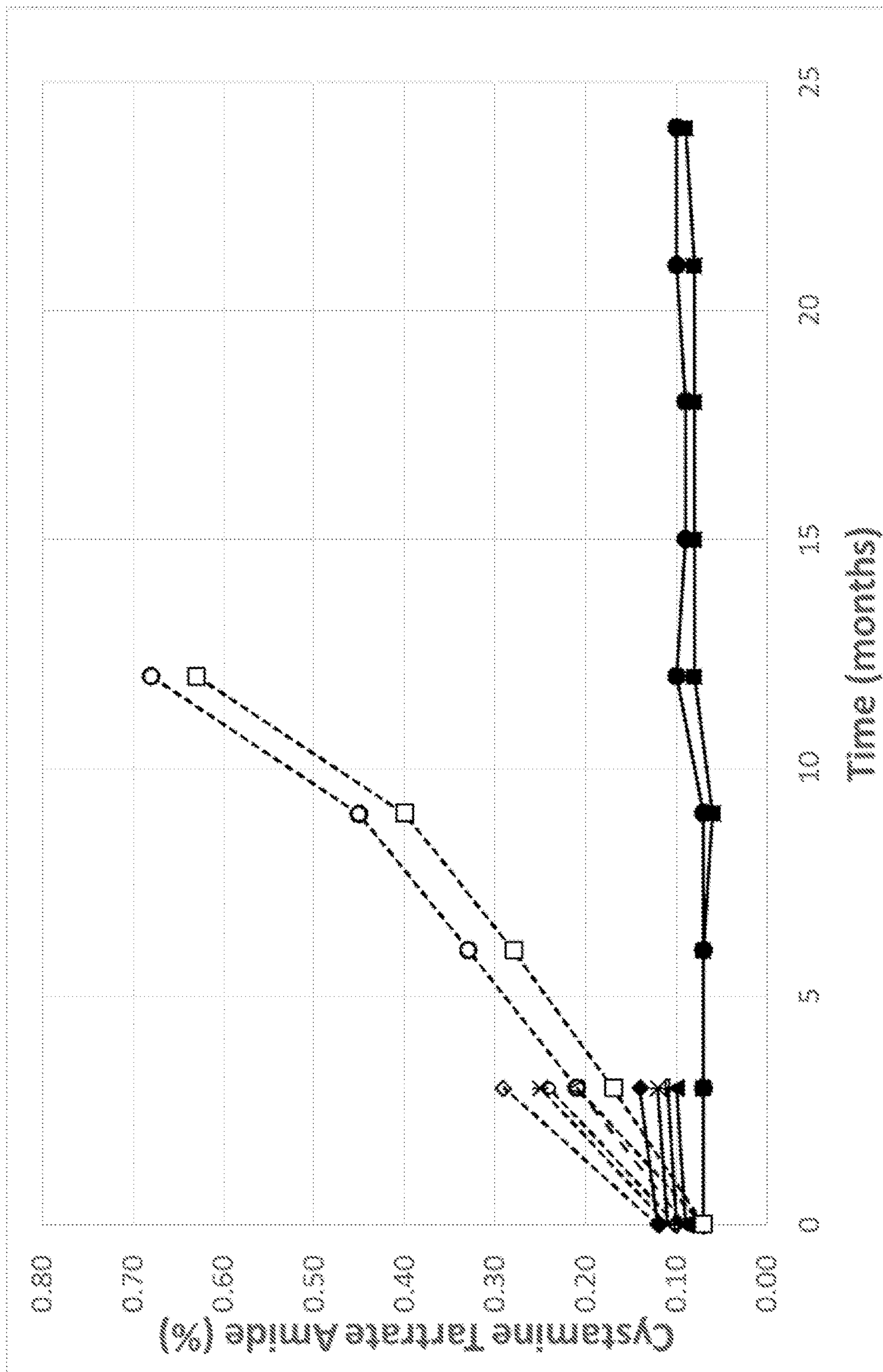

FIG. 2C

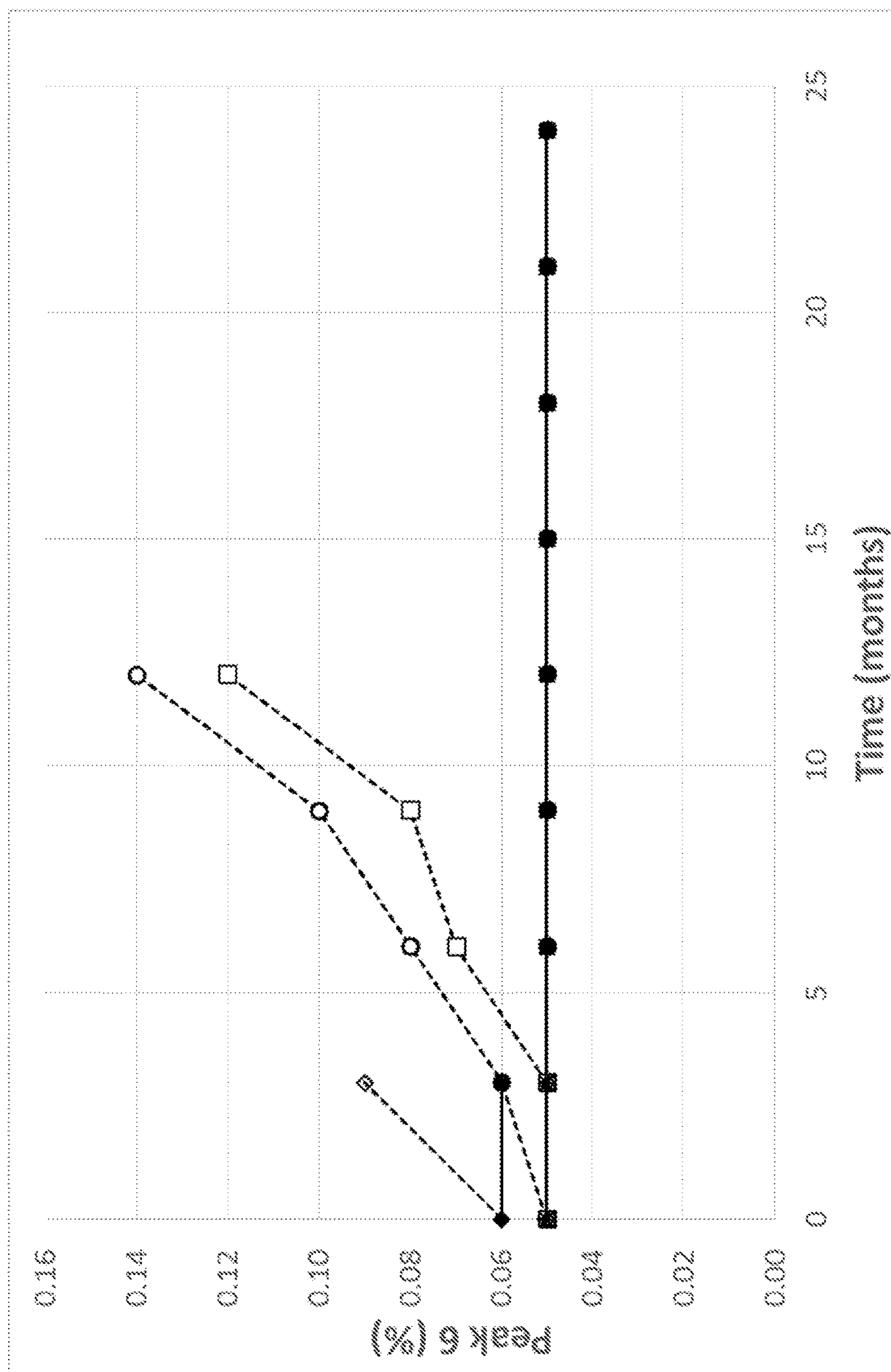

FIG. 2D

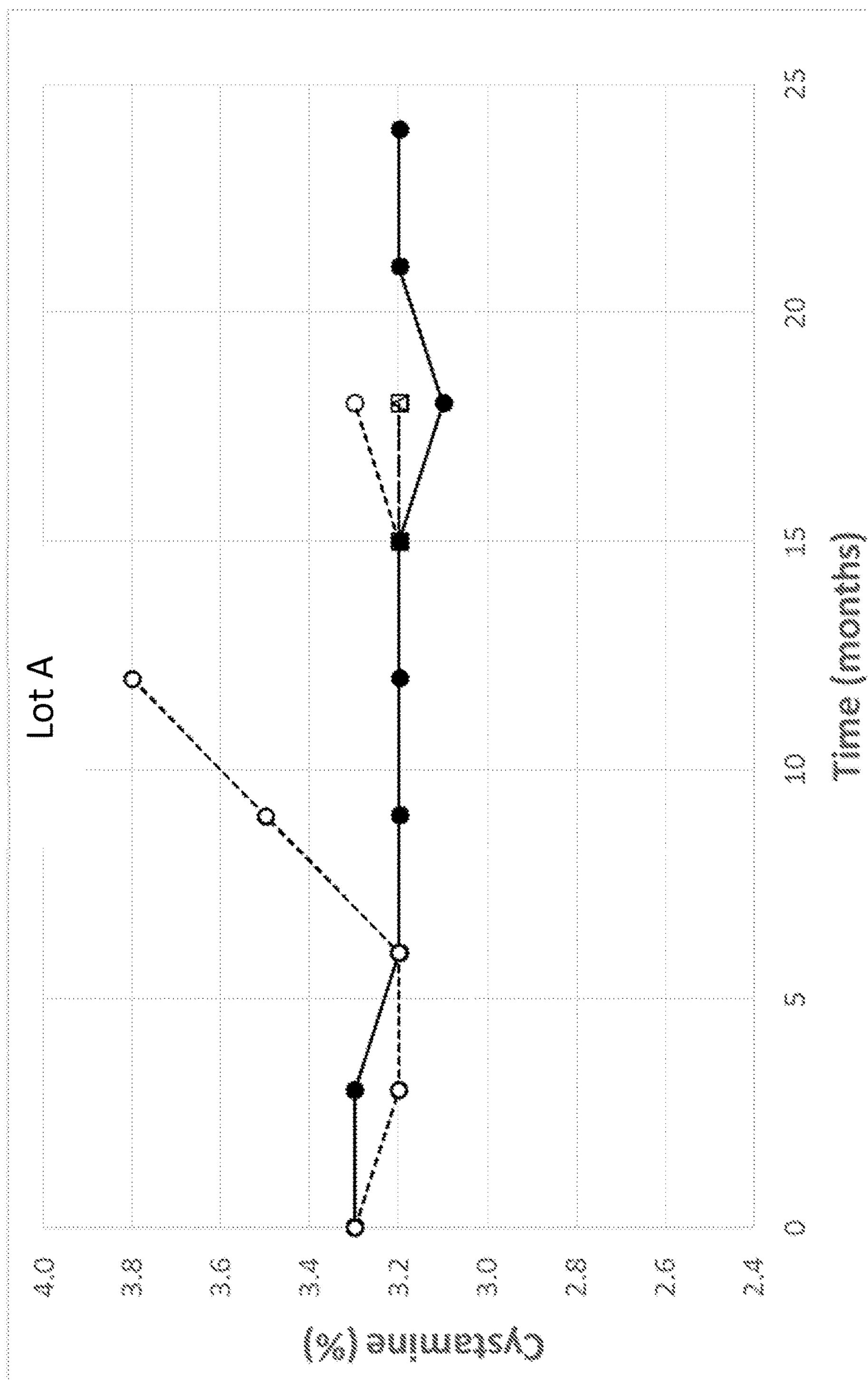

FIG. 3A

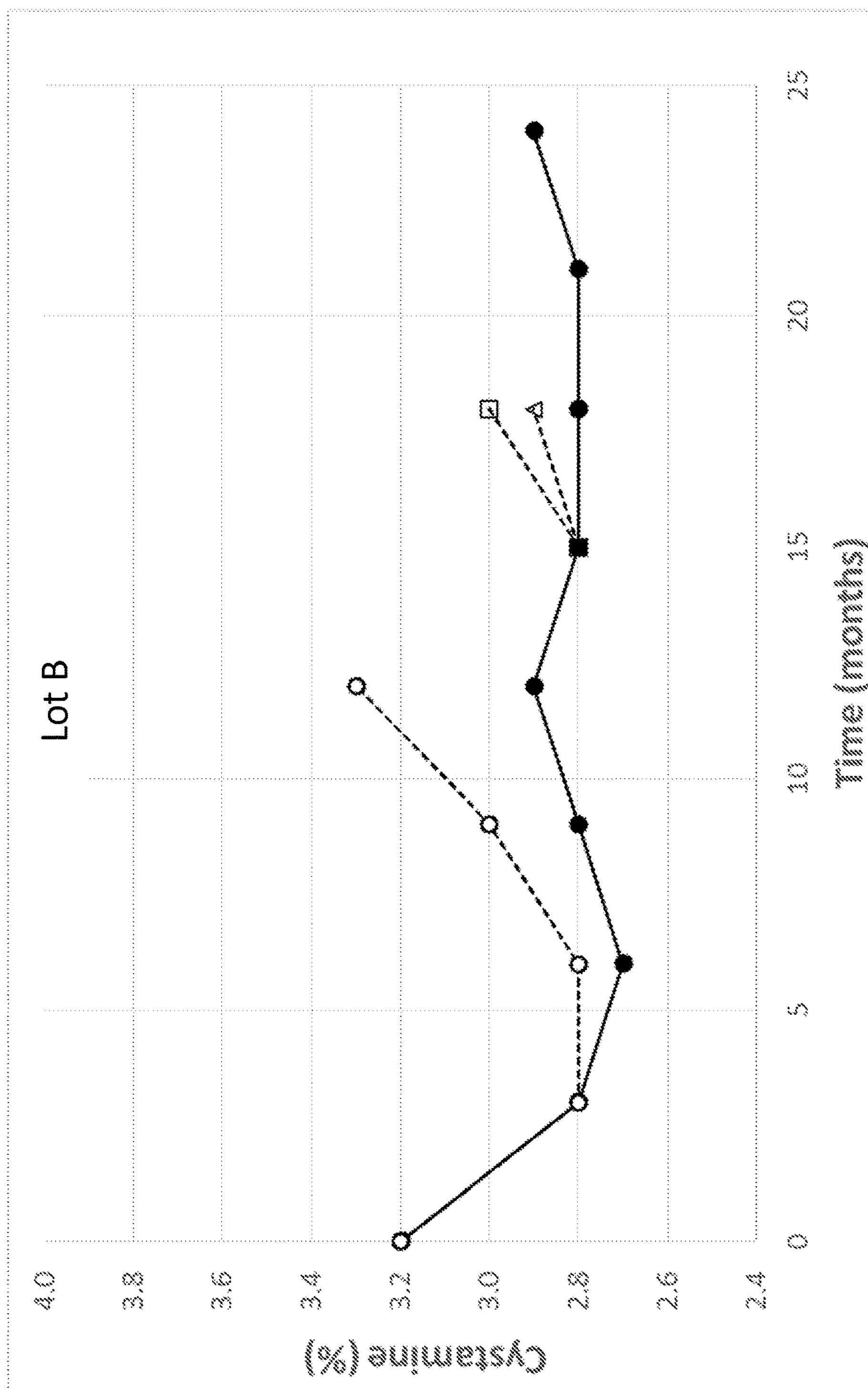

FIG. 3B

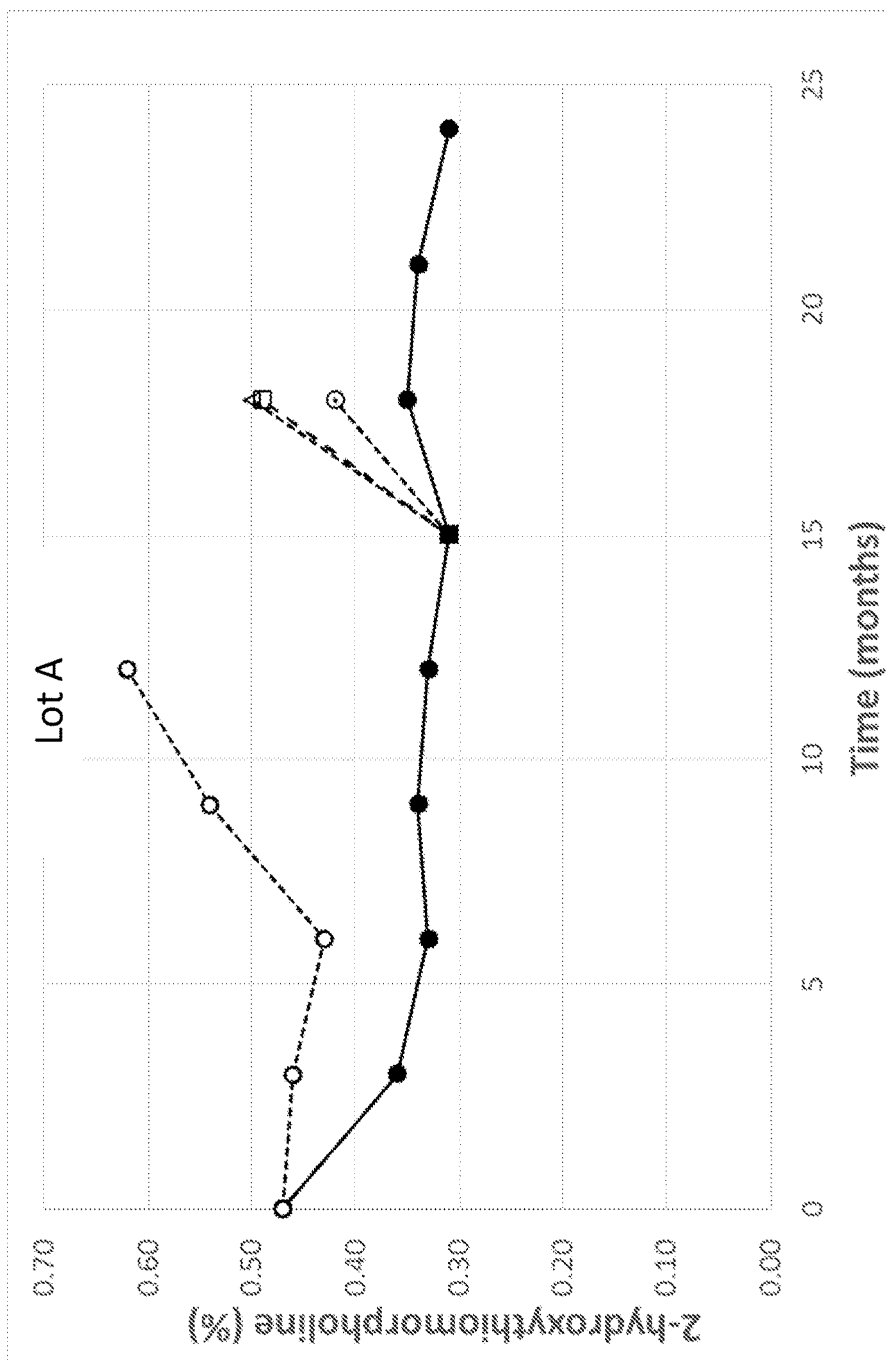

FIG. 3C

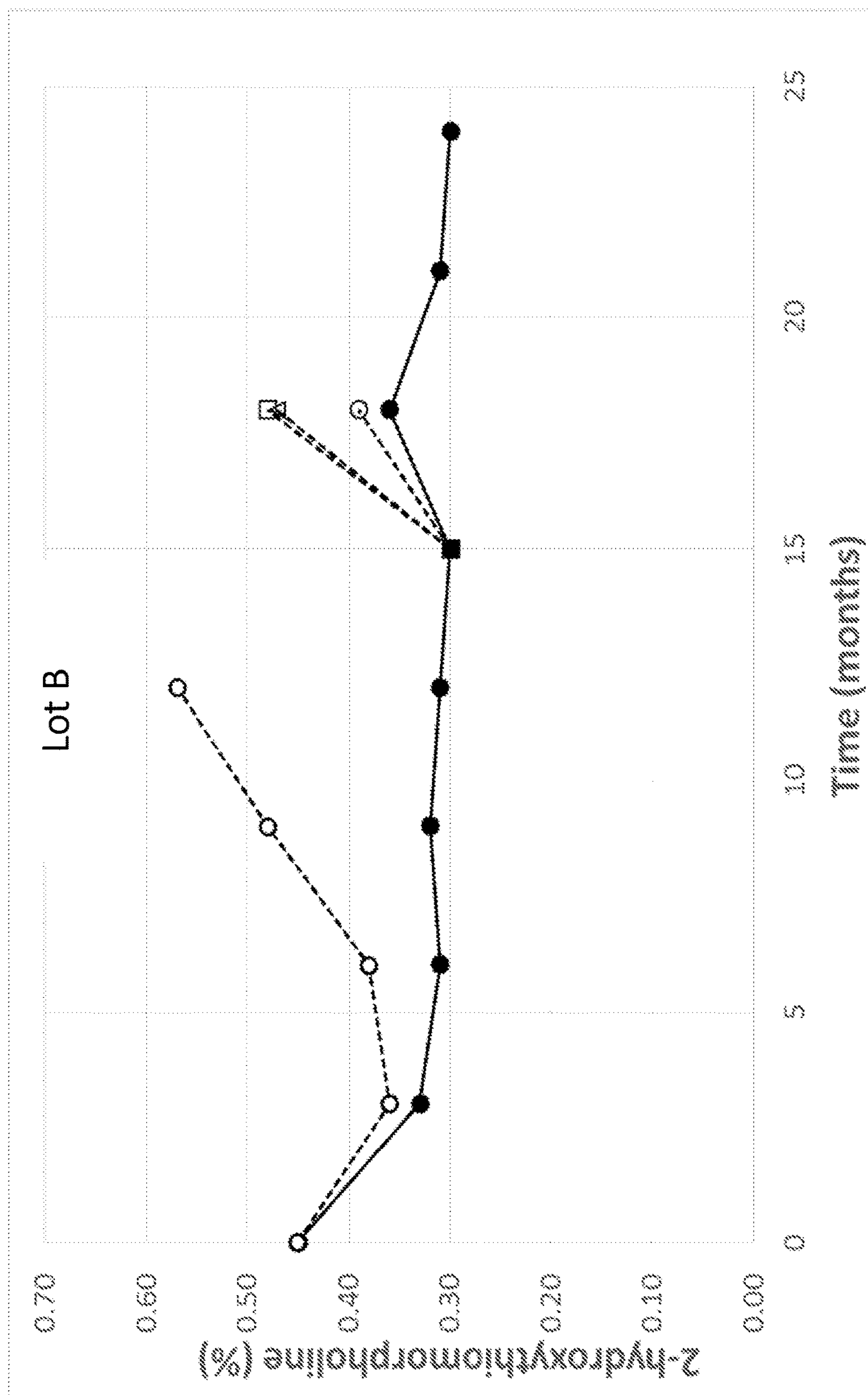

FIG. 3D

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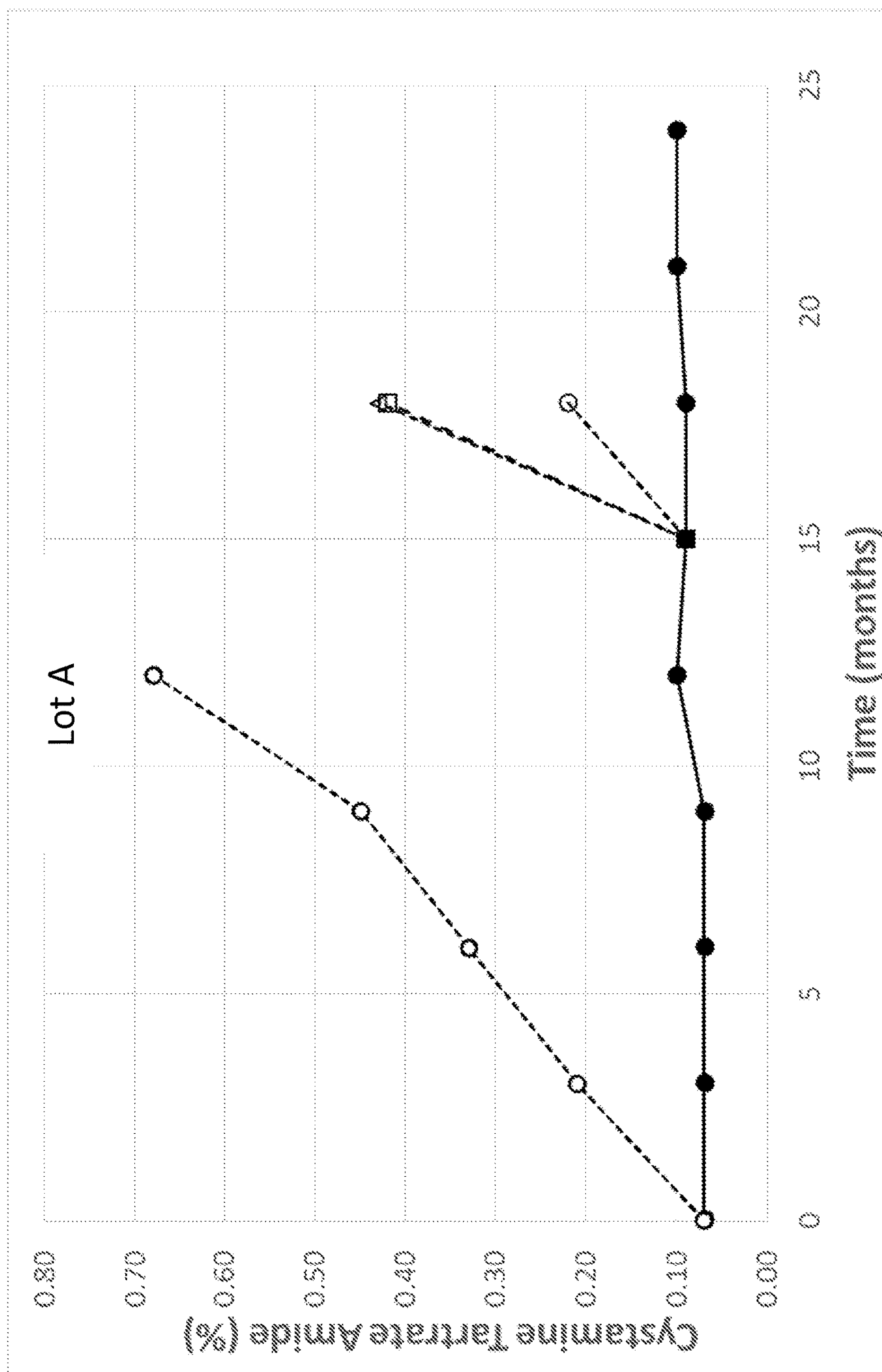

FIG. 3E

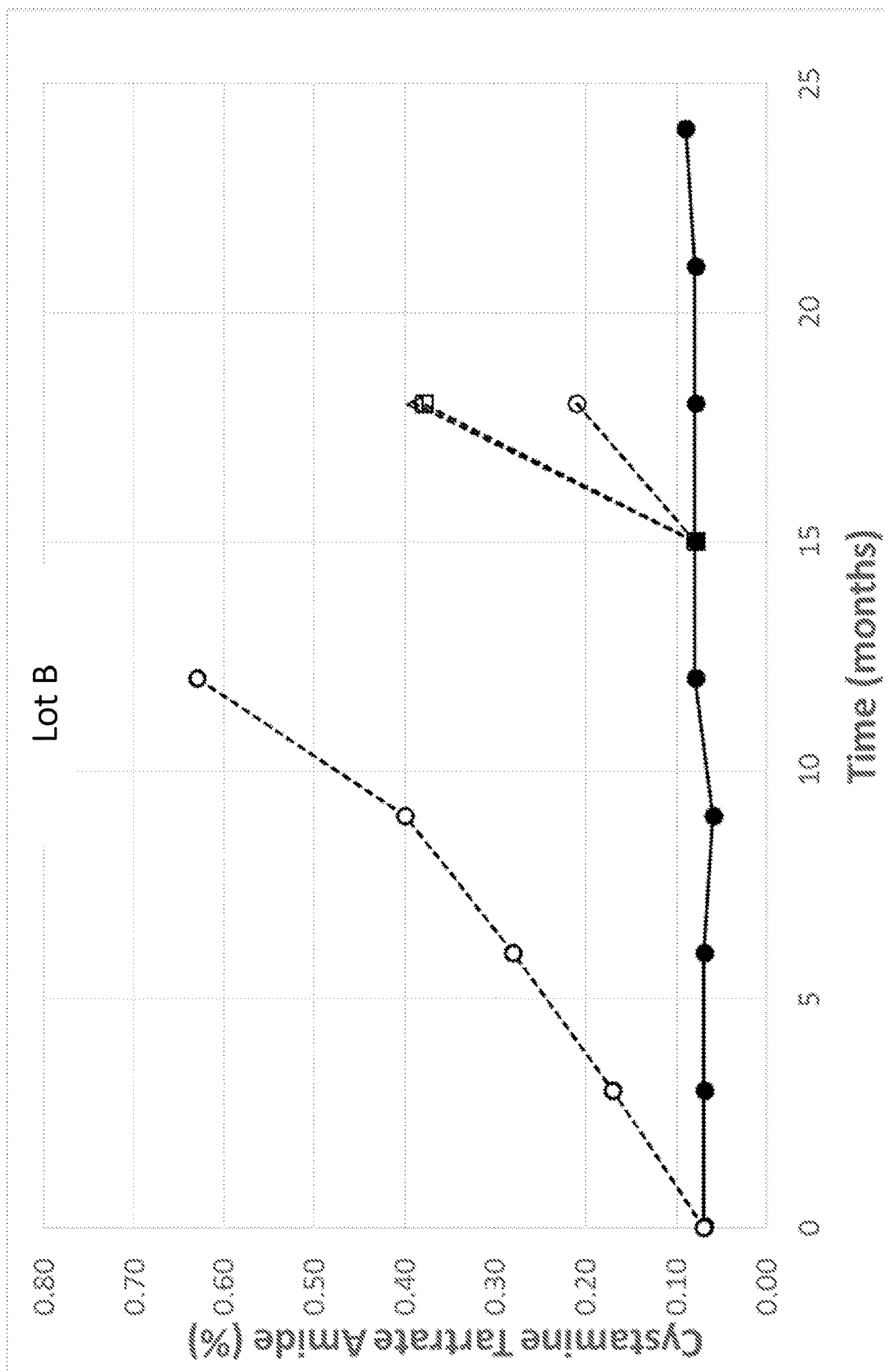

FIG. 3F

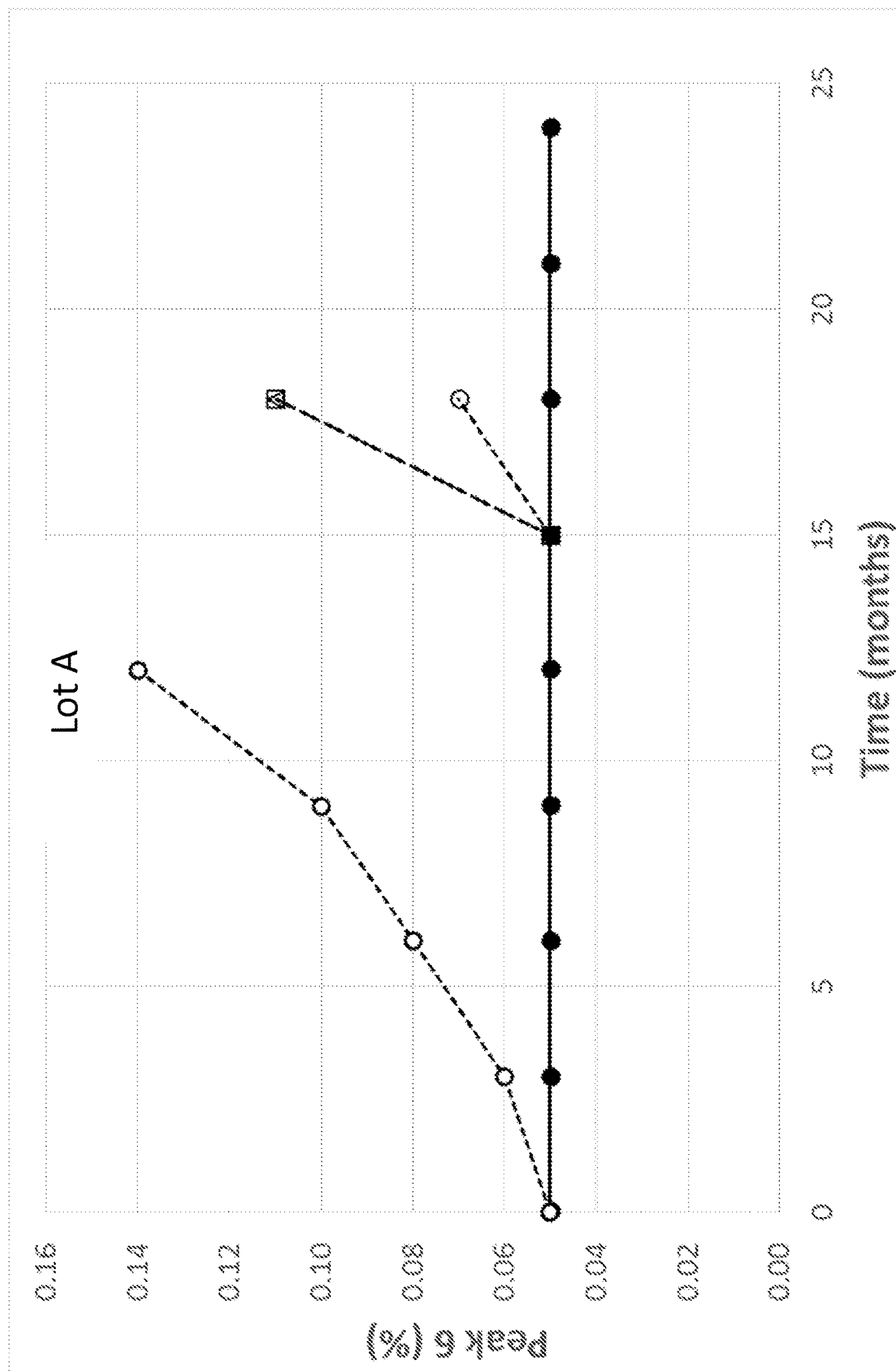

FIG. 3G

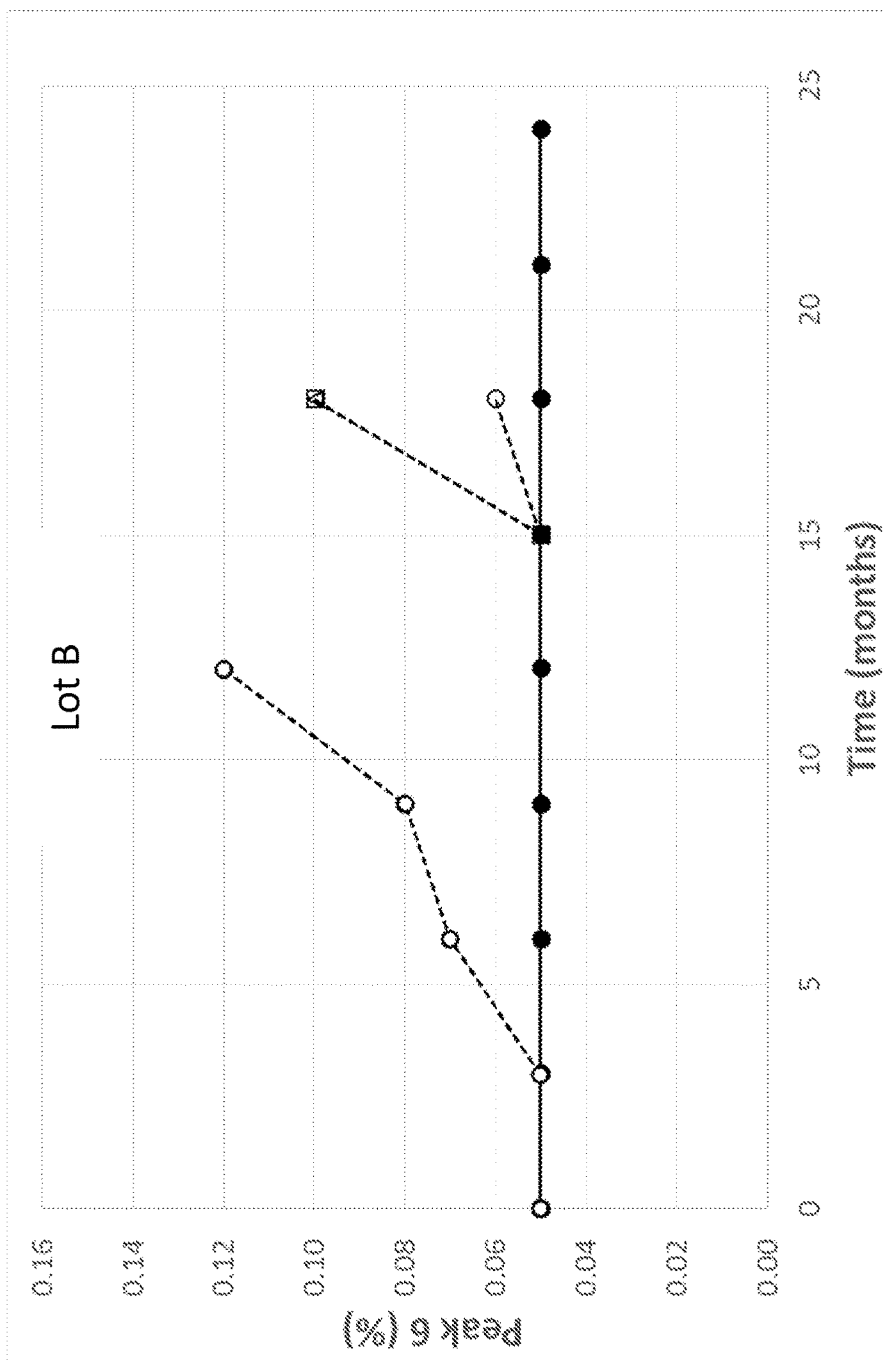

FIG. 3H

METHODS FOR STORING CYSTEAMINE FORMULATIONS AND RELATED METHODS OF TREATMENT

This application claims priority to U.S. Application No. 62/256,613 filed Nov. 17, 2015.

BACKGROUND

Cystinosis is a rare, autosomal recessive disease caused by intra-lysosomal accumulation of the amino acid cysteine within various tissues, including the spleen, liver, lymph nodes, kidney, bone marrow, and eyes. Nephropathic cystinosis is associated with kidney failure that necessitates kidney transplantation. A specific treatment for nephropathic cystinosis is the sulfhydryl agent cysteamine. Cysteamine has been shown to lower intracellular cystine levels, thereby reducing the rate of progress of kidney failure in children.

Cysteamine, and pharmaceutically acceptable salts thereof, may also be administered for the treatment of other metabolic and neurodegenerative diseases, including non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), Huntington's disease, Parkinson's disease, Rett Syndrome, cystic fibrosis, and others; used as free radical and radioprotectants; used as hepato-protectant agents.

Enterically-coated cysteamine compositions for increasing delivery of cysteamine to the small intestine and resulting in less frequent dosing compared to non-enteric-coated cysteamine have been described. See, e.g., WO 2014/204881, WO 2007/089670, and U.S. Pat. Nos. 8,026,284, 9,198,882, 9,192,590, 9,173,851, and 9,233,077.

In some cysteamine pharmaceutical compositions, cysteamine is not chemically stable and degrades into several impurities over time.

SUMMARY OF THE DISCLOSURE

The present disclosure provides methods of storing pharmaceutical compositions comprising cysteamine, or a pharmaceutically acceptable salt thereof, wherein the pharmaceutical composition is stored at refrigerated temperatures (e.g., 2° C.-8° C.) up to 24 months, or longer, and compositions that have been stored in such a manner that have fewer impurities than when stored at 25° C., and methods for using the same. In various embodiments, the disclosure provides a method of storing a pharmaceutical composition, comprising storing the pharmaceutical composition at a temperature of between about 2° C. and about 8° C., wherein the pharmaceutical composition comprises cysteamine, or a pharmaceutically acceptable salt thereof.

In one embodiment, the disclosure provides a method of stabilizing a pharmaceutical composition, comprising storing the pharmaceutical composition at a temperature of between about 2° C. and about 8° C., wherein the pharmaceutical composition comprises cysteamine, or a pharmaceutically acceptable salt thereof.

Also provided is a method of distributing a pharmaceutical composition, comprising storing the pharmaceutical composition at a temperature of between about 2° C. and about 8° C. prior to dispensing to a health care provider or a patient, wherein the pharmaceutical composition comprises cysteamine, or a pharmaceutically acceptable salt thereof.

Further contemplated is a method of treating a disease or disorder, comprising administering a pharmaceutical composition comprising cysteamine, or a pharmaceutically acceptable salt thereof to a subject in need thereof, wherein the pharmaceutical composition has been stored at a temperature of between about 2° C. and about 8° C. prior to administration. Exemplary diseases or disorders contemplated herein include, but are not limited to, cystinosis, fatty liver disease, a thrombotic disease, an MECP-2 related disorder, an inherited mitochondrial disease, a neurological disease or disorder, inflammation and cancer. Additional indications contemplated are set out in the Detailed Description.

In various embodiments of the methods, the pharmaceutical composition further comprises one or more materials that provide increased delivery of cysteamine to the small intestine. In one embodiment, a material that provides increased delivery of cysteamine to the small intestine comprises an enteric coating. Exemplary enteric coatings contemplated comprise a coating selected from the group consisting of polymerized gelatin, shellac, methacrylic acid copolymer type CNF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers formed from methyl acrylate, ethyl acrylate, methyl methacrylate, and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters. In one embodiment, the enteric coating comprises poly(methacrylic acid co-ethyl acrylate) 1:1 (Eudragit L 30-D-55).

In various embodiments of the methods, the pharmaceutical composition comprises a pharmaceutically acceptable salt of cysteamine, and the pharmaceutically acceptable salt of cysteamine is cysteamine bitartrate.

It is also contemplated that the pharmaceutical composition comprises a solid composition. In one embodiment, the pharmaceutical composition comprises a unit dose of about 25 mg cysteamine or of about 75 mg cystemine.

In various embodiments, the pharmaceutical composition comprises enteric coated beads. In various embodiments, the pharmaceutical composition is a pharmaceutical dosage form that includes a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core particle, wherein the plurality of beads is characterized by a distribution of particle sizes.

In one embodiment, the particle sizes of the beads are in a range of about 0.7 mm to about 2.5 mm, or about 0.7 mm to about 2.8 mm, or about 0.8 mm to about 1.7 mm. For example, the target bead size can be up to 2.5 mm with no more than 10 percent variation over this size, to a maximum size of 2.8 mm. In one embodiment, the enteric membrane on the beads is present in an amount in a range of about 20% to 40%, or about 20% to about 25%, or about 25% to about 35% as measured by the weight gain compared to the uncoated particle cores, or in a range of about 25% to about 31% weight gain, or about 27% to about 31% weight gain, or about 28.5% to about 31% weight gain, based on the weight of the uncoated particle cores.

In various embodiments, the bead formulation comprises a plurality of cysteamine beads, the beads comprising a core particle comprising, cysteamine or a pharmaceutically acceptable salt thereof, such as cysteamine bitartrate, a filler, a binder and an enteric membrane surrounding the core, wherein the plurality of beads is characterized by a distri-

bution of particle sizes in a range of about 0.7 mm to about 2.8 mm; wherein the enteric membrane is present in an amount in a range of about 20% to about 40% based on the weight of the bead core particles; wherein the formulation is a delayed release formulation having an enteric membrane that begins to dissolve within a pH range of about 4.5 to about 6.5, and wherein the beads are disposed in a capsule shell.

In further embodiments of the methods above, the pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. for up to 1 month, between about 1 month and about 6 months, between about 6 months and about 12 months, between about 12 month and about 15 months, between about 15 months and about 18 months, between about 18 months and about 21 months, between about 21 months and about 24 months, between about 24 months and about 36 months, or between about 36 months and about 39 months. In various embodiments, the pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. for 1 month, 6 months, 12 months, 15 months, 18 months, 21 months, 24 months, 36 months, or 39 months.

The methods herein may further comprise storing the pharmaceutical composition at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for up to 4 months.

In various embodiments, the pharmaceutical composition is stable through 12, 15, 18, 21 or 24 months at 2° C.-8° C. storage. In various embodiments, the pharmaceutical composition is stable through 24 months at 2° C.-8° C. storage. In various embodiments, the pharmaceutical composition is stable after storage at a temperature of 2° C.-8° C. for up to 15 months followed by excursions of up to 3 months at 25° C./60% RH, 30° C./65% RH, or 30° C./75% RH (18 months of total storage time).

In various embodiments of the methods, the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less that 0.5%. In some embodiments, the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In various embodiments of the methods above, the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less that 4%. In certain embodiments, the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

It is contemplated that the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than 0.5%. In various embodiments, the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

The methods also provide that the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition is less than 0.05%. In various embodiments, the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In various embodiments, the total amount of 2-hydroxymethylthiazolidine, cystamine, cystamine tartrate amide, and 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount if cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the total amount of 2-hydroxymethylthiazolidine, cystamine, cystamine tartrate amide, and 2-hydroxymethylthiazolidine present in the pharmaceutical acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and relative humidity of 60% for the same duration.

In another aspect, the present disclosure provides methods of treating a disease or disorder by administering to a subject in need thereof a pharmaceutical composition comprising cysteamine, or a pharmaceutically acceptable salt thereof, wherein the pharmaceutical composition has been stored at a temperature of between about 2° C. and about 8° C. prior to administration. Disease or disorders contemplated herein are described in more detail in the Detailed Description.

Also provided is a method of treating cystinosis, comprising administering a pharmaceutical composition comprising cysteamine, or a pharmaceutically acceptable salt thereof, wherein the pharmaceutical composition has been stored at a temperature of between about 2° C. and about 8° C. prior to administration. In various embodiments, the cystinosis is nephropathic cystinosis.

In various embodiments, the pharmaceutical composition is formulated to provide white blood cell cystine suppression with a 12 hour level below 1 nmol½cystine/mg protein.

In various embodiments, each dose of cysteamine is about 0.5-1.0 g/m2 body surface area. In various embodiments, the total daily dose of cysteamine is about 1.3 g/m2 body surface area or less. In various embodiments, the composition is administered less than four times daily, e.g., one, two or three times a day. In various embodiments, the composition is administered twice daily, or every 12 hours.

In various embodiments, the composition increases delivery to the proximal small intestine, the mid-small intestine, the duodenum, the jejunum or the mid-ileum.

In various embodiments, the composition is in the form of a tablet or a capsule.

In various embodiments, the cysteamine salt is cysteamine bitartrate.

DESCRIPTION OF THE DRAWINGS

FIG. **1**A shows a comparison of cold storage (2° C. to 8° C.) to room temperature storage (25° C./60% RH) for the amount of cysteamine bitartrate (relative the label claim; expressed as a percent) in samples of 25-mg PROCYSBI® ("Assay"). Open circles and squares (○, □) with dotted lines represent 25° C./60% RH data for lots A and B, respectively. Closed circles and squares (●, ■) with solid lines represent 2° C.-8° C. data for lots A and B, respectively.

FIG. **1**B shows a comparison of cold storage (2° C.-8° C.) to room temperature storage (25° C./60% RH) for acid stage dissolution tests of samples of 25-mg PROCYSBI® (relative the label claim; expressed as a percent). Open circles and squares (○, □) with dotted lines represent 25° C./60% RH data for lots A and B, respectively. Closed circles and squares (●, ■) with solid lines represent 2° C.-8° C. data for lots A and B, respectively.

FIG. **1**C shows a comparison of cold storage (2° C.-8° C.) to room temperature storage (25° C./60% RH) for buffer stage dissolution tests of samples of 25-mg PROCYSBI® (relative the label claim; expressed as a percent). Open circles and squares (○, □) with dotted lines represent 25° C./60% RH data for lots A and B, respectively. Closed circles and squares (●, ■) with solid lines represent 2° C.-8° C. data for lots A and B, respectively.

FIG. **1**D shows comparisons of cold storage (2° C.-8° C.) to room temperature storage (25° C./60% RH) for cystamine (relative to cysteamine bitartrate; expressed as a percentage) in samples of 25-mg PROCYSBI®. Open circles and squares (○, □) with dotted lines represent 25° C./60% RH data for lots A and B, respectively. Closed circles and squares (●, ■) with solid lines represent 2° C.-8° C. data for lots A and B, respectively.

FIG. **1**E shows a comparison of cold storage (2° C.-8° C.) to room temperature storage (25° C./60% RH) for 2-hydroxythiomorpholine (relative to cysteamine bitartrate; expressed as a percentage) in samples of 25-mg PROCYSBI®. Open circles and squares (○, □) with dotted lines represent 25° C./60% RH data for lots A and B, respectively. Closed circles and squares (●, ■) with solid lines represent 2° C.-8° C. data for lots A and B, respectively.

FIG. **1**F shows a comparison of cold storage (2° C.-8° C.) to room temperature storage (25° C./60% RH) for cystamine tartrate amide (relative to cysteamine bitartrate; expressed as a percentage) in samples of 25-mg PROCYSBI®. Open circles and squares (○, □) with dotted lines represent 25° C./60% RH data for lots A and B, respectively. Closed circles and squares (●, ■) with solid lines represent 2° C.-8° C. data for lots A and B, respectively.

FIG. **1**G shows a comparison of cold storage (2° C.-8° C.) to room temperature storage (25° C./60% RH) for Peak 6 (relative to cysteamine bitartrate; expressed as a percentage) in samples of 25-mg PROCYSBI®. Open circles and squares (○, □) with dotted lines represent 25° C./60% RH data for lots A and B, respectively. Closed circles and squares (●, ■) with solid lines represent 2° C.-8° C. data for lots A and B, respectively.

FIG. **2**A shows a comparison of cold storage (2° C.-8° C.) to room temperature storage (25° C./60% RH) for cystamine (relative to cysteamine bitartrate; expressed as a percentage) in samples of 25-mg or 75-mg PROCYSBI®. Open symbols with dotted lines represent 25° C./60% RH data and closed symbols with solid lines represent 2° C.-8° C. data. Data for lots A, B, C, D, E. and F are represented by circles (○, ●), squares (□, ■), triangles (Δ, ▲), diamonds (◇, ♦), stars (*) and horizontal - lines (-), respectively. Values below limit of quantitation (LOQ) and not detected (ND) are shown as 0.05%, the LOQ.

FIG. **2**B shows a comparison of cold storage (2° C.-8° C.) to room temperature storage (25° C./60% RH) for 2-hydroxythiomorpholine (relative to cysteamine bitartrate; expressed as a percentage) in samples of 25-mg or 75-mg PROCYSBI®. Open symbols with dotted lines represent 25° C./60% RH data and closed symbols with solid lines represent 2° C.-8° C. data. Data for lots A, B, C, D, E and F are represented by circles (○, ●), squares (□, ■), triangles (Δ, ▲), diamonds (◇, ♦), stars (*) and horizontal - lines (-), respectively. Values below limit of quantitation (LOQ) and not detected (ND) are shown as 0.05%, the LOQ.

FIG. **2**C shows a comparison of cold storage (2° C.-8° C.) to room temperature storage (25° C./60% RH) for cystamine tartrate amide (relative to cysteamine bitartrate; expressed as a percentage) in samples of 25-mg or 75-mg PROCYSBI®. Open symbols with dotted lines represent 25° C./60% RH data and closed symbols with solid lines represent 2° C.-8° C. data. Data for lots A, B, C, D, E and F are represented by circles (○, ●), squares (□, ■), triangles (Δ, ▲), diamonds (◇, ♦), stars (*) and horizontal - lines (-), respectively. Values below limit of quantitation (LOQ) and not detected (ND) are shown as 0.05%, the LOQ.

FIG. **2**D shows a comparison of cold storage (2° C.-8° C.) to room temperature storage (25° C./60% RH) for Peak 6 (relative to cysteamine bitartrate expressed as a percentage) in samples of 25-mg or 75-mg PROCYSBI®. Open symbols with dotted lines represent 25° C./60% RH data and closed symbols with solid lines represent 2° C.-8° C. data. Data for lots A, B, C, D, E and F are represented by circles (○, ●), squares (□, ■), triangles (Δ, ▲), diamonds (◇, ♦), stars (*) and horizontal - lines (-), respectively. Values below limit of quantitation (LOQ) and not detected (ND) are shown as 0.05%, the LOQ.

FIG. **3**A shows levels of cystamine (relative to cysteamine bitartrate; expressed as a percentage) for samples from Lot A (25-mg PROCYSBI®) after storage at 25° C./60% RH, 30° C./65% RH or 30° C./75% RH for 3 months, following 15 months of initial storage at 2° C.-8° C. Closed circles (●) with solid lines represent 2° C.-8° C. data. Open circles (○) with dotted lines represent 25° C./60% RH data for 0-12 month consecutive storage as well as 15-18 months storage at 25° C./60% RH after 15 months storage at 2° C.-8° C. Open squares (□) with dotted lines represent storage at 30° C./65% RH after 15 months storage at 2° C.-8° C. Open triangles (Δ) with dotted lines represent storage at 30° C./75% RH after 15 months storage at 2° C.-8° C.

FIG. **3**B shows levels of cystamine (relative to cysteamine bitartrate; expressed as a percentage) for samples from Lot B (25-mg PROCYSBI®) after storage at 25° C./60% RH, 30° C./65% RH or 30°/75% RH for 3 months, following 15 months of initial storage at 2° C.-8° C. Closed circles (●) with solid lines represent 2° C.-8° C. data. Open circles (○) with dotted lines represent 25° C./60% RH data for 0-12 month consecutive storage as well as 15-18 months storage at 25° C./60% RH after 15 months storage at 2° C.-8° C. Open squares (□) with dotted lines represent storage at 30° C./65% RH after 15 months storage at 2° C.-8° C. Open

triangles (Δ) with dotted lines represent storage at 30° C./75% RH after 15 months storage at 2° C.-8° C.

FIG. 3C shows levels of 2-hydroxythiomorpholine (relative to cysteamine bitartrate; expressed as a percentage) for samples form Lot A (25-mg PROCYSBI®) after storage at 25° C./60% RH, 30° C./65% RH or 30°/75% RH for 3 months, following 15 months of initial storage at 2° C.-8° C. Closed circles (●) with solid lines represent 2° C.-8° C. data. Open circles (○) with dotted lines represent 25° C./60% RH data for 0-12 month consecutive storage as well as 15-18 months storage at 25° C./60% RH after 15 months storage at 2° C.-8° C. Open squares (□) with dotted lines represent storage at 30° C./65% RH after 15 months storage at 2° C.-8° C. Open triangles (Δ) with dotted lines represent storage at 30° C./75% RH after 15 months storage at 2° C.-8° C.

FIG. 3D shows levels of 2-hydroxythiomorpholine (relative to cysteamine bitartrate; expressed as a percentage) for samples from Lot B (25-mg PROCYSBI®) after storage at 25° C./60% RH, 30° C./65% RH or 30°/75% RH for 3 months, following 15 months of initial storage at 2° C.-8° C. Closed circles (●) with solid lines represent 2° C.-8° C. data. Open circles (○) with dotted lines represent 25° C./60% RH data for 0-12 month consecutive storage as well as 15-18 months storage at 25° C./60% RH after 15 months storage at 2° C.-8° C. Open squares (□) with dotted lines represent storage at 30° C./65% RH after 15 months storage at 2° C.-8° C. Open triangles (Δ) with dotted lines represent storage at 30° C./75% RH after 15 months storage at 2° C.-8° C.

FIG. 3E shows levels of cystamine tartrate amide (relative to cysteamine bitartrate; expressed as a percentage) for samples from Lot A (25-mg PROCYSBI®) after storage at 25° C./60% RH, 30° C./65% RH or 30°/75% RH for 3 months, following 15 months of initial storage at 2° C.-8° C. Closed circles (●) with solid lines represent 2° C.-8° C. data. Open circles (○) with dotted lines represent 25° C./60% RH data for 0-12 month consecutive storage as well as 15-18 months storage at 25° C./60% RH after 15 months storage at 2° C.-8° C. Open squares (□) with dotted lines represent storage at 30° C./65% RH after 15 months storage at 2° C.-8° C. Open triangles (Δ) with dotted lines represent storage at 30° C./75% RH after 15 months storage at 2° C.-8° C.

FIG. 3F shows levels of cystamine tartrate amide (relative to cysteamine bitartrate; expressed as a percentage) for samples from Lot B (25-mg PROCYSBI®) after storage at 25° C./60% RH, 30° C./65% RH or 30°/75% RH for 3 months, following 15 months of initial storage at 2° C.-8° C. Closed circles (●) with solid lines represent 2° C.-8° C. data. Open circles (○) with dotted lines represent 25° C./60% RH data for 0-12 month consecutive storage as well as 15-18 months storage at 25° C./60% RH after 15 months storage at 2° C.-8° C. Open squares (□) with dotted lines represent storage at 30° C./65% RH after 15 months storage at 2° C.-8° C. Open triangles (Δ) with dotted lines represent storage at 30° C./75% RH after 15 months storage at 2° C.-8° C.

FIG. 3G shows levels of Peak 6 (relative to cysteamine bitartrate; expressed as a percentage) for samples from Lot A (25-mg PROCYSBI®) after storage at 25° C./60% RH, 30° C./65% RH or 30°/75% RH for 3 months, following 15 months of initial storage at 2° C.-8° C. Closed circles (●) with solid lines represent 2° C.-8° C. data. Open circles (○) with dotted lines represent 25° C./60% RH data for 0-12 month consecutive storage as well as 15-18 months storage at 25° C./60% RH after 15 months storage at 2° C.-8° C. Open squares (□) with dotted lines represent storage at 30° C./65% RH after 15 months storage at 2° C.-8° C. Open triangles (Δ) with dotted lines represent storage at 30° C./75% RH after 15 months storage at 2° C.-8° C.

FIG. 3H shows levels of Peak 6 (relative to cysteamine bitartrate; expressed as a percentage) for samples from Lot B (25-mg PROCYSBI®) after storage at 25° C./60% RH, 30° C./65% RH or 30°/75% RH for 3 months, following 15 months of initial storage at 2° C.-8° C. Closed circles (●) with solid lines represent 2° C.-8° C. data. Open circles (○) with dotted lines represent 25° C./60% RH data for 0-12 month consecutive storage as well as 15-18 months storage at 25° C./60% RH after 15 months storage at 2° C.-8° C. Open squares (□) with dotted lines represent storage at 30° C./65% RH after 15 months storage at 2° C.-8° C. Open triangles (Δ) with dotted lines represent storage at 30° C./75% RH after 15 months storage at 2° C.-8° C.

DETAILED DESCRIPTION

In some aspects, the present disclosure provides methods of storing pharmaceutical compositions comprising cysteamine, or a pharmaceutically acceptable salt thereof, wherein the pharmaceutical composition is stored at refrigerated temperatures (e.g., 2° C.-8° C.) up to 24 months, or longer, and methods for using the same. In particular embodiments, pharmaceutical compositions handled according to the teachings provided herein provide greater chemical stability of cysteamine compared to compositions stored at room temperature. More specifically, the relative amounts of impurities in pharmaceutical compositions handled according to the present disclosure, measured at specific time points, are less than or equal to the corresponding amounts of impurities measured in the compositions when stored at room temperature. Methods for distributing cysteamine compositions are also provided. The present disclosure further provides methods of treating cystinosis comprising administering a pharmaceutical composition comprising cysteamine, or a pharmaceutically acceptable salt thereof, wherein the pharmaceutical composition has been stored under refrigerated temperatures prior to administration.

DEFINITIONS

It should be understood that the terms "a" and "an" as used herein refer to "one or more" of the enumerated components. The use of the alternative (e.g., "or") should be understood to mean either one, both, or any combination thereof of the alternatives.

In addition, it should be understood that the individual features, or groups of features, derived from the various combinations of the methods, pharmaceutical compositions, and substituents described herein, are disclosed by the present application to the same extent as if each feature or group of features was set forth individually. Thus, selection of particular features is within the scope of the present disclosure.

As used herein, the terms "include," "have," and "comprise" are used synonymously, which terms and variants thereof are intended to be construed as non-limiting.

The term "consisting essentially of" limits the scope of a claim to the specified materials or steps, or to those that do not materially affect the basic characteristics of a claimed invention.

As used herein, the term "about" means±20% of the indicated range, value, or structure, unless otherwise indicated.

All ranges set forth herein include all possible subsets of ranges and any combinations of such subset ranges. Unless otherwise stated, ranges are inclusive of the stated end-points. Where a range of values is provided, it is understood that each intervening value between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also contemplated to be part of the disclosure. For example, "between 2 and 4" includes, but is not limited to 2, 3, 4, 2 to 3, 3 to 4, and any number falling between 2 and 4.

PROCYSBI® (cysteamine bitartrate; also known as ethanethiol, 2-amino, (2R,3R)-2,3-dihydroxybutanedioate) delayed-release capsules (Raptor Pharmaceuticals, Inc.) is a prescription medicine used for the treatment of nephropathic cystinosis. PROCYSBI® comprises enteric coated cysteamine bitartrate beads encapsulated in gelatin capsules, and currently is available to patients in 25 mg and 75 mg strengths. PROCYSBI® contains the following inactive ingredients: microcrystalline cellulose, Eudragit® L 30 D-55, hypromellose, talc, triethyl citrate, sodium lauryl sulfate, and purified water.

An "enterically coated" drug or tablet refers to a drug, granule, granulation, powder or dosage form, including for example, a tablet, a caplet, and a capsule, that is coated with a substance—i.e., with an "enteric coating"—that remains intact in the stomach but dissolves and releases the drug once the small intestine is reached.

As used herein "enteric coating", is a material, a polymer material or materials which encase the medicament core (e.g., cysteamine, Cystagon®). Typically, a substantial amount or all of the enteric coating material is dissolved before the medicament or therapeutically active agent is released from the dosage form, so as to achieve delayed dissolution of the medicament core. In one embodiment, a suitable pH-sensitive polymer is one which will dissolve in intestinal juices at a higher pH level (pH greater than 4.5), such as within the small intestine and therefore permit release of the pharmacologically active substance in the regions of the small intestine, and not in the upper portion of the GI tract, such as the stomach.

By "pharmaceutically acceptable carrier" or "pharmaceutically acceptable vehicle" are meant materials that are suitable for oral administration and not biologically, or otherwise, undesirable, i.e., that may be administered to a subject along with an active ingredient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of a pharmaceutical composition in which it is contained.

Similarly, a "pharmaceutically acceptable" salt, ester, or other derivative of an active agent comprise, for example, salts, esters, or other derivatives which are not biologically or otherwise undesirable.

"Stabilizing agents" refer to compounds that lower the rate at which a pharmaceutical product degrades, particularly an oral pharmaceutical formulation under environmental conditions of storage.

By the terms "effective amount" or "therapeutically effective amount" of a formulation of cysteamine refers to a nontoxic but sufficient amount of the agent to provide the desired therapeutic effect. The exact amount required will vary from subject to subject, depending on the age, weight, and general condition of the subject, the severity of the condition being treated, and the like.

A "solid composition" as used herein refers to any solid-state composition that is, or can be made into, a solid pharmaceutical dosage form. Solid compositions include, for example, bulk powders, granules and granulations (including coated granules), and dosage forms suitable for oral administration to a subject, such as tablets or capsules, including compressed or extruded compositions. Moreover, the term, "solid" does not necessarily imply a complete absence of liquid or gaseous media. For example, solids can have various interstices, which may partially or fully fill with other gaseous and/or liquid media. Thus, the term "solid composition" includes compositions that are suspended (i.e., remain at least partially, if not substantially, insoluble) in liquid media, such as syrups, elixirs, and the like.

"Storage" refers to maintaining a pharmaceutical composition under a set of physical conditions for a period of time. For example, storage can include maintaining a pharmaceutical composition at a particular temperature, humidity, or both (e.g., 25° C./60% RH) for a given duration (e.g., 4 weeks up to 24 months, or longer). As used herein, "storage" can include, for example, storage by a manufacturer, a distributor, a pharmacy, or a hospital prior to dispensing the pharmaceutical composition to a patient or health care provider. "Storage" can also include handling by a patient, wherein the patient maintains a pharmaceutical composition under a set of physical conditions for a period of time.

Addition definitions are set forth throughout this disclosure.

Methods of Storing and Stabilizing Pharmaceutical Compositions

In certain aspects, the present disclosure provides methods of storing or distributing pharmaceutical compositions comprising cysteamine, or a pharmaceutically acceptable salt thereof, wherein the pharmaceutical compositions are stored under refrigerated conditions (e.g., 2° C.-8° C.). When compositions comprising cysteamine are stored at room temperature (e.g., 25° C.), impurities can increase over time as cysteamine degrades. The inventors have found that solid compositions including cysteamine as an active ingredient (or agent) exhibit unexpectedly high levels of impurities associated with cysteamine degradation when stored at 25° C./60% RH for 12 months. Impurities that may increase over time include cystamine, 2-hydroxythiomorpholine, cystamine tartrate amide, 2-hydroxymethylthiazolidine, and Peak 6. In contrast, when cysteamine-containing compositions are stored at temperatures of about 2° C. to about 8° C. for 12 months, or even 24 months, the level of each impurity is less than or equal to that found in the compositions stored at 25° C./60% RH for 12 months.

In one embodiment, the methods as described herein relate to methods involving pharmaceutical compositions comprising cysteamine, or a pharmaceutically acceptable salt thereof, and one or more materials that provide increased delivery of cysteamine to the small intestine. In one embodiment, a material that provides increased delivery of cysteamine to the small intestine comprises an enteric coating. Typically, a substantial amount of all of the enteric coating material is dissolved before the medicament or therapeutically active agent is released from the dosage form, so as to achieve delayed dissolution of the medicament core. For example, a suitable pH-sensitive polymer is one which will dissolve in intestinal juices at a higher pH level (pH greater than 4.5), such as within the small intestine, and therefore permit release of the pharmacologically active

substance in the regions of the small intestine and not in the upper portion of the GI tract, such as the stomach. In one embodiment, the enteric material begins to dissolve in an aqueous solution at pH between about 4.5 to about 5.5. In another embodiment, the enteric material begins to dissolve in an aqueous solution at pH between about 5.5 to about 6.5. In another embodiment, the enteric material rapidly dissolves in an aqueous solution at pH between of about 5. In still another embodiment, the enteric material rapidly dissolves in an aqueous solution at pH between of about 5.5. In specific embodiments, the cysteamine-containing composition may be a delayed release solid dosage form containing cysteamine, such as, for example, PROCYSBI® (Raptor Pharmaceuticals, Inc.).

For example, pH-sensitive materials will not undergo significant dissolution until the dosage form has emptied from the stomach. The pH of the small intestine gradually increases from about 4.5 to about 6.5 in the duodenal bulb to about 7.2 in the distal portions of the small intestine (ileum). In order to provide predictable dissolution corresponding to the small intestine transit time of about 3 hours (e.g., 2-3 hours) and permit reproducible release therein, the membrane should begin to dissolve within the pH range of the duodenum, and continue to dissolve at the pH range within the small intestine. Therefore, in one embodiment, the amount (thickness) of enteric membrane should be sufficient to be substantially dissolved during the approximate three hour transit time within the small intestine (e.g., the proximal and mid-small intestine).

Enteric (gastro-resistant) materials can include, but are not limited to, one or more of the following: cross-linked polyvinyl pyrrolidone; non-cross linked polyvinylpyrrolidone; hydroxypropylmethyl cellulose phthalate, hydroxypropylmethyl cellulose acetate succinate, cellulose acetate succinate; cellulose acetate phthalate, hydroxypropylmethyl cellulose acetate succinate, cellulose acetate trimellitate; starch acetate phthalate; polyvinyl acetate phthalate; carboxymethyl cellulose; methyl cellulose phthalate; methyl cellulose succinate; methyl cellulose phthalate succinate; methyl cellulose phthalic acid half ester; ethyl cellulose succinate; carboxymethylamide; potassium methacrylated-vinylbenzene copolymer; polyvinylalcohols; polyoxyethyleneglycols; polyethylene glycol; sodium alginate; galactomannone; carboxypolymethylene; sodium carboxymethyl starch; copolymers of acrylic acid and/or methacrylic acid with a monomer selected from the following: methyl methacrylate, ethyl methacrylate, ethyl acrylate, butyl methacrylate, hexyl methacrylate, decyl methacrylate, lauryl methacrylate, phenyl methacrylate, methyl acrylate, isopropyl acrylate, isobutyl acrylate, or octadecyl acrylate, e.g., EUDRAGIT-L and -S series, including L 100-55, L 30 D-55, L 100, S 100, L 12.5, and S 12.5, available from Evonik Industries; polyvinyl acetate; fats; oils; waxes, fatty alcohols; shellac; zein; gluten; ethylacrylate-maleic acid anhydride copolymer; maleic acid anhydride-vinyl methyl ether copolymer; styrol-maleic acid copolymer; 2-ethyl-hexyl-acrylate maleic acid anhydride; crotonic acid-vinyl acetate copolymer; glutaminic acid/glutamic acid ester copolymer; carboxymethylethylcellulose glycerol monooctanoate; polyarginine; poly(ethylene); poly(propylene); poly(ethylene oxide); poly(ethylene terephthalate); poly(vinyl isobutyl ether); poly(vinyl chloride); and polyurethane. A combination of enteric materials may also be used. In one embodiment, the enteric material rapidly dissolves at pH 5.5 and higher, to provide fast dissolution in the upper bowel. For example, the enteric material can be selected from a copolymer of methacrylic acid and methyl methacrylate, and a copolymer of methacrylic acid and ethyl acrylate. For example, an enteric polymer is poly(methacrylic acid co-ethyl acrylate) 1:1 (EUDRAGIT L 30 D-55 and EUDRAGIT L100-55).

Examples of some enteric coatings are disclosed in U.S. Pat. No. 5,225,202, including beeswax and glyceryl monostearate; beeswax, shellac and cellulose; and cetyl alcohol, mastic and shellac, as well as shellac and stearic acid (U.S. Pat. No. 2,809,918); polyvinyl acetate and ethyl cellulose (U.S. Pat. No. 3,835,221); and neutral copolymer of polymethacrylic acid esters (Eudragit L30D) (F. W. Goodhart et al., Pharm. Tech., pp. 64-71, April 1984); copolymers of methacrylic acid and methacrylic acid methylester (Eudragits), as a neutral copolymer of polymethacrylic acid esters containing metallic stearates (Mehta et al., U.S. Pat. Nos. 4,728,512 and 4,794,001). Such coatings comprise mixtures of fats and fatty acids, shellac and shellac derivatives and the cellulose acid phthalates, e.g., those having a free carboxyl content. See also Remington's Pharmaceutical Sciences, A. Osol, ed., Mack Pub. Co., Easton, Pa. (16th ed. 1980) at pages 1590-1593, and Zeitova et al. (U.S. Pat. No. 4,432,966), for descriptions of suitable enteric coating compositions.

One or more plasticizers can be added to enteric polymers in order to increase their pliability and reduce brittleness, as it is known in the art. Suitable plasticizers are known in the art and include, for example, butyl citrates, triethyl citrate, diethyl phthalate, dibutyl sebacate, PEGs (e.g., PEG 6000), acetyl triethyl citrate, and triacetin. In one type of embodiment, the plasticizer is triethyl citrate. While some enteric materials are flexible and do not require addition of plasticizers, more brittle polymers (e.g., Eudragit L/S types, Eudragit RL/RS, and Eudragit FS 30 D) benefit from plasticizers, e.g. in the range of 5 wt. % to 30 wt. % based on the dry polymer mass, e.g. about 8 wt. % to about 12 wt. % triethyl citrate with poly(methacrylic acid co-ethyl acrylate) 1:1.

One or more anti-tacking agents (antiadherents) can also be added to an enteric coating mixture in order to reduce the tackiness of the film and prevent agglomeration, as it is known in the art. Anti-tacking agents include talc, and glyceryl monostearate, fumed silica (e.g., AEROSIL 200), precipitated silica (e.g., SIPERNAT PQ), and magnesium stearate, for example. Anti-tacking agents can be used in any suitable quantity, for example in a range of about 10 wt. % to 100 wt. % based on dry polymer mass, or about 10 wt. % to about 50 wt. %, or about 10 wt. % to about 30 wt. %, or about 15 wt. % to about 30 wt. %. For example, in one embodiment the amount of talc is in a range of 15 wt. % to about 30 wt. %, based on dry polymer mass.

One or more surfactants can also be added to an enteric coating mixture in order to improve substrate wettability and/or stabilize suspensions, as it is known in the art. Surfactants include Polysorbate 80, sorbitan monooleate, and sodium dodecyl sulfate, for example.

The enteric membrane can be formed by any suitable process. Coating processes include pan coating, fluid bed coating, and dry coating (e.g., heat dry coating and electrostatic dry coating), for example. Pan coating and fluid bed coating using solvent are well established processes. In liquid coating, the enteric material and optional excipients (e.g., pigments, plasticizers, anti-tacking agents) are mixed in an organic solvent or water to form a solution or dispersion. The coating solution or dispersion is sprayed into solid dosage forms in a pan coater or a fluid bed dryer and dried by hot air. For example, in a Wurster fluid bed coating process, the coating fluid is sprayed from the bottom of the

fluid bed apparatus, whereas in an alternative the coating fluid is applied by top spraying, and in another alternative tangential spray is applied.

The amount of enteric material applied is sufficient to achieve desired acid resistance and release characteristics. For example, in one embodiment the amount of enteric membrane will be sufficient to meet United States Pharmacopeia (USP) <711> requirements (USP 36-NF 31) for delayed-release dosage forms, thereby not releasing 10.0 wt. % drug after 2 hours in 0.1N HCl. In another aspect, a formulation will be sufficient to release at least 80% of the active in 20 minutes in pH 6.8 buffer solution, e.g., using the dissolution method of USP 36-NF 31 section <711>.

In one embodiment, an enteric membrane is present in an amount in a range of about 20% to 40%, or 25% to about 35% as measured by the weight gain compared to the uncoated particle cores, or in a range of about 25% to about 31% weight gain, or about 27% to about 31% weight gain, or about 28.5% to about 31% weight gain, based on the weight of the uncoated particle cores.

In pharmaceutical compositions used in the methods described herein, the cysteamine is present in the compositions in a therapeutically effective amount; in one embodiment, the composition is in unit dosage form. The amount of cysteamine administered will be dependent on the age, weight, and general condition of the subject, the severity of the condition being treated, and the judgment of the prescribing physician. In one embodiment, the dose is administered twice per day at about 0.5-1.0 g/m^2 (e.g., 0.7-0.8 g/m^2) body surface area. Current non-enterically coated doses are about 1.35 g/m^2 body surface area and are administered 4-5 times per day. In another embodiment, the dose is about 0.2-1.95 g/m^2 body surface area.

In one aspect, a method of storing a pharmaceutical composition is provided, comprising storing the pharmaceutical composition at a temperature of between about 2° C. and about 8° C., wherein the pharmaceutical composition comprises cysteamine, or a pharmaceutically acceptable salt thereof. In another aspect, a method of stabilizing a pharmaceutical composition is provided, comprising storing the pharmaceutical composition at a temperature of between about 2° C. and about 8° C., wherein the pharmaceutical composition comprises cysteamine, or a pharmaceutically acceptable salt thereof.

In any of the aforementioned methods, the pharmaceutical composition may further comprise one or more materials that provide increased delivery of cysteamine to the small intestine. For example, in one embodiment, the material that provides increased delivery of cysteamine to the small intestine comprises an enteric coating, such as a coating selected from the group consisting of polymerized gelatin, shellac, methacrylic acid copolymer type CNF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers formed from methyl acrylate, ethyl acrylate, methyl methacrylate, and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters. In one embodiment, the enteric coating comprises poly(methacrylic acid co-ethyl acrylate) 1:1 (Eudragit L 30-D-55).

In any of the aforementioned embodiments, the pharmaceutical composition may comprise a pharmaceutically acceptable salt of cysteamine. In one embodiment, the pharmaceutically acceptable salt of cysteamine is cysteamine bitartrate.

In any of the aforementioned embodiments, the pharmaceutical composition may comprise a solid composition. In one embodiment, the pharmaceutical composition comprises a unit dose of about 25 mg cysteamine. In another embodiment, the pharmaceutical composition comprises a unit dose of about 75 cysteamine. Exemplary pharmaceutical compositions comprising cysteamine are disclosed in International Patent Publication WO 2014/204881.

In various embodiments, the pharmaceutical composition comprises enteric coated beads. In various embodiments, the pharmaceutical composition is a pharmaceutical dosage form that includes a plurality of cysteamine beads, the beads including a core particle including cysteamine or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable excipient, and an enteric membrane surrounding the core particle, wherein the plurality of beads is characterized by a distribution of particle sizes.

In one embodiment, the particle sizes of the beads are in a range of about 0.7 mm to about 2.5 mm, or about 0.7 mm to about 2.8 mm, or about 0.8 mm to about 1.7 mm. For example, the target bead size can be up to 2.5 mm with no more than 10 percent variation over this size, to a maximum size of 2.8 mm. In one embodiment, the enteric membrane on the beads is present in an amount in a range of about 20% to 40%, or about 20% to about 25%, or about 25% to about 35% as measured by the weight gain compared to the uncoated particle cores, or in a range of about 25% to about 31% weight gain, or about 27% to about 31% weight gain, or about 28.5% to about 31% weight gain, based on the weight of the uncoated particle cores.

In various embodiments, the bead formulation comprises a plurality of cysteamine beads, the beads comprising a core particle comprising cysteamine, optionally cysteamine bitartrate, a filler, a binder and an enteric membrane surrounding the core, wherein the plurality of beads is characterized by a distribution of particle sizes in a range of about 0.7 mm to about 2.8 mm; wherein the enteric membrane is present in an amount in a range of about 20% to about 40% based on the weight of the bead core particles; wherein the formulation is a delayed release formulation having an enteric membrane that begins to dissolve within a pH range of about 4.5 to about 6.5, and wherein the beads are disposed in a capsule shell.

In one embodiment, a method of storing a pharmaceutical composition is provided, comprising storing the pharmaceutical composition at a temperature of between about 2° C. and about 8° C., wherein the pharmaceutical composition comprises cysteamine bitartrate. In a further embodiment, the pharmaceutical composition comprises PROCYSBI®. In another embodiment, a method of stabilizing a pharmaceutical composition is provided, comprising storing the pharmaceutical composition at a temperature of between about 2° C. and about 8° C., wherein the pharmaceutical composition comprises cysteamine bitartrate. In a further embodiment, the pharmaceutical composition comprises PROCYSBI®.

In any of the aforementioned embodiments, the pharmaceutical composition may be stored at a temperature of between about 2° C. and about 8° C. for up to 1 month, between about 1 month and about 6 months, between about 6 months and about 12 months, between about 12 months and about 15 months, between about 15 months and about 18 months, between about 18 months and about 21 months, between about 21 months and about 24 months, between

about 24 months and about 36 months, or between about 36 months and about 39 months. In one embodiment, the pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. for 1 month, 6 months, 12 months, 15 months, 18 months, 21 months, 24 months, 36 months, or 39 months.

In any of the aforementioned embodiments, the method may further comprise storing the pharmaceutical composition at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for up to 4 months.

In any of the aforementioned embodiments, the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 0.5%. For example, in one embodiment, a method of storing a pharmaceutical composition is provided, wherein (a) the pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. for up to 24 months, (b) the pharmaceutical composition comprises cysteamine bitartrate, and (c) the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine bitartrate present in the pharmaceutical composition, is less than 0.5%. In another embodiment, a method of storing or stabilizing a pharmaceutical composition is provided, wherein the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any one of the aforementioned embodiments, the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 4%. For example, in one embodiment, a method of storing a pharmaceutical composition is provided, wherein (a) the pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. for up to 24 months, (b) the pharmaceutical composition comprises cysteamine bitartrate, and (c) the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine bitartrate present in the pharmaceutical composition, is less than 4%. In another embodiment, a method of storing or stabilizing a pharmaceutical composition is provided, wherein the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 0.5%. For example, in one embodiment, a method of storing a pharmaceutical composition is provided, wherein (a) the pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. for up to 24 months, (b) the pharmaceutical composition comprises cysteamine bitartrate, and (c) the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine bitartrate present in the pharmaceutical composition, is less than 0.5%. In another embodiment, a method of storing or stabilizing a pharmaceutical composition is provided, wherein the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 0.5%. For example, in one embodiment, a method of storing a pharmaceutical composition is provided, wherein (a) the pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. for up to 24 months, (b) the pharmaceutical composition comprises cysteamine bitartrate, and (c) the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine bitartrate present in the pharmaceutical composition, is less than 0.5%. In another embodiment, a method of storing or stabilizing a pharmaceutical composition is provided, wherein the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the total amount of 2-hydroxythiomorpholine, cystamine, cystamine tartrate amide, and 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than or equal to the total amount of 2-hydroxythiomorpholine, cystamine, cystamine tartrate amide, and 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

Methods of Distributing Pharmaceutical Compositions

In another aspect, a method of distributing a pharmaceutical composition is provided. In one embodiment, the method comprises storing the pharmaceutical composition at a temperature of between about 2° C. and about 8° C. prior to dispensing to a health care provider or a patient, wherein the pharmaceutical composition comprises cysteamine, or a pharmaceutically acceptable salt thereof. In another embodi-

ment, a pharmaceutical composition comprising cysteamine, or a pharmaceutically acceptable salt thereof, is shipped from a manufacture to a distributor under refrigerated conditions, and is then stored by the distributor prior to dispensing to a health care provider or a patient. In one embodiment, a pharmaceutical composition comprising cysteamine, or a pharmaceutically acceptable salt thereof, is stored by a distributor at a temperature of between about 2° C. and about 8° C. for between about 4 weeks and about 24 months, or longer.

In any of the aforementioned methods of distributing, the pharmaceutical composition may further comprise one or more materials that provide increased delivery of cysteamine to the small intestine. For example, in one embodiment, the material that provides increased delivery of cysteamine to the small intestine comprises an enteric coating, such as a coating selected from the group consisting of polymerized gelatin, shellac, methacrylic acid copolymer type CNF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers formed from methyl acrylate, ethyl acrylate, methyl methacrylate, and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters. In one embodiment, the enteric coating comprises poly(methacrylic acid co-ethyl acrylate) 1:1 (Eudragit L 30-D-55).

In any of the aforementioned embodiments, the pharmaceutical composition may comprise a pharmaceutically acceptable salt of cysteamine. In one embodiment, the pharmaceutically acceptable salt of cysteamine is cysteamine bitartrate.

In any of the aforementioned embodiments, the pharmaceutical composition may comprise a solid composition. In one embodiment, the pharmaceutical composition comprises a unit dose of about 25 mg cysteamine. In another embodiment, the pharmaceutical composition comprises a unit dose of about 75 mg cysteamine.

In one embodiment, a method of distributing a pharmaceutical composition is provided, comprising storing the pharmaceutical composition at a temperature of between about 2° C. and about 8° C. prior to dispensing to a health care provider or a patient, wherein the pharmaceutical composition comprises cysteamine bitartrate. In a further embodiment, the pharmaceutical composition comprises PROCYSBI®.

In any of the aforementioned embodiments, the pharmaceutical composition may be stored at a temperature of between about 2° C. and about 8° C. for up to 1 month, between about 1 month and about 6 months, between about 6 months and about 12 months, between about 12 months and about 15 months, between about 15 months and about 18 months, between about 18 months and about 21 months, between about 21 months and about 24 months, between about 24 months and about 36 months, or between about 36 months and about 39 months. In one embodiment, the pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. for 1 month, 6 months, 12 months, 15 months, 18 months, 21 months, 24 months, 36 months, or 39 months.

In any of the aforementioned embodiments, the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 0.5%. In another embodiment, a method of distributing a pharmaceutical composition is provided, wherein the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 4%. In another embodiment, a method of distributing a pharmaceutical composition is provided, wherein the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25 C° and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 0.5%. In another embodiment, a method of distributing a pharmaceutical composition is provided, wherein the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 0.5%. In another embodiment, a method of distributing a pharmaceutical composition is provided, wherein the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the total amount of 2-hydroxythiomorpholine, cystamine, cystamine tartrate amide, and 2-hydroxymethylthiazolidine present in

the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than or equal to the total amount of 2-hydroxythiomorpholine, cystamine, cystamine tartrate amide, and 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° and about 25° and a relative humidity of 60% for the same duration.

Method of Treatment

In another aspect, the present disclosure provides methods of treating a disease or disorder by administering to a subject in need thereof a pharmaceutical composition comprising cysteamine, or a pharmaceutically acceptable salt thereof, wherein the pharmaceutical composition has been stored at a temperature of between about 2° C. and about 8° C. prior to administration. In various embodiments, the disease or disorder is cystinosis, fatty liver disease, a thrombotic disease, an MECP-2 related disorder, an inherited mitochondrial disease, a neurological disease or disorder, inflammation and cancer.

In various embodiments, the fatty liver disease is selected from the group consisting of non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), fatty liver disease resulting from hepatitis, fatty liver disease resulting from obesity, fatty liver disease resulting from diabetes, fatty liver disease resulting from insulin resistance, fatty liver disease resulting from hypertriglyceridemia, Abetalipoproteinemia, glycogen storage diseases, Weber-Christian disease, Wolmans disease, acute fatty liver of pregnancy, and lipodystrophy.

In various embodiments, the thrombotic disease is selected from the group consisting of sickle cell disease, deep vein thrombosis, pulmonary embolism, cardiac embolism, hypercoagulable state, thrombophilia, Factor V Leiden, Antithrombin III deficiency, Protein C deficiency, Protein S deficiency, Prothrombin gene mutation (G20210A), Hyperhomcysteinemia, antiphospholipid antibody syndrome (APS), anticardiolipin antibody (ACLA) thrombosis syndrome, or lupus anticoagulant (LA) syndrome.

In various embodiments, the neurological disease or disorder is selected from the group consisting of Huntington's Disease, Parkinson's Disease, amyotrophic lateral sclerosis, multiple sclerosis, Alzheimer's disease spinal muscle atrophy, concussion, stroke, and traumatic brain injury (TBI).

In various embodiments, the MECP-2 related disease is selected from the group consisting of Rett syndrome, autism, pervasive development disorder, non-syndromic mental retardation, idiopathic neonatal encephalopathy and idiopathic cerebral palsy.

In various embodiments, the inherited mitochondrial disease is selected from the group consisting of Friedreich's ataxia, Leber's hereditary optic neuropathy (LHON), myoclonic epilepsy and ragged-red fibers, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like syndrome (MELAS), Kearn-Sayre syndrome and subacute necrotizing encephalopathy (Leigh's Syndrome).

In various embodiments, the cancer is selected from the group consisting of breast cancer, melanoma, prostate cancer, pancreatic cancer, head and neck cancer, lung cancer, non small-cell lung carcinoma, renal cancer, colorectal cancer, colon cancer, ovarian cancer, liver cancer and gastric cancer.

In one aspect, the cysteamine composition is administered in dosage form, wherein the dose is administered either one time per day or multiple times per day. The cysteamine composition may be administered less than four times per day, e.g., one, two or three times per day. In some embodiments, an effective dosage of cysteamine composition may be within the range of 0.01 mg to 1000 mg per kg (mg/kg) of body weight per day. Further, the effective dose may be 0.5 mg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg/25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 55 mg/kg, 60 mg/kg, 70 mg/kg, 75 mg/kg, 80 mg/kg, 90 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 225 mg/kg, 250 mg/kg, 275 mg/kg, 300 mg/kg, 325 mg/kg, 350 mg/kg, 375 mg/kg, 400 mg/kg, 425 mg/kg, 450 mg/kg, 475 mg/kg, 500 mg/kg, 525 mg/kg, 550 mg/kg, 575 mg/kg, 600 mg/kg, 625 mg/kg, 650 mg/kg, 675 mg/kg, 700 mg/kg, 725 mg/kg, 750 mg/kg, 775 mg/kg, 800 mg/kg, 825 mg/kg, 850 mg/kg, 875 mg/kg, 900 mg/kg, 925 mg/kg, 950 mg/kg, 975 mg/kg or 1000 mg/kg, or may range between any two of the foregoing values. In some embodiments, the dose above may be the total daily dose, or may be the dose administered in one of the one, two or three daily administrations. In some embodiments, the cysteamine composition is administered at a total daily dose of from approximately 0.25 g/m2 to 4.0 g/m2 body surface area, e.g., at least about 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2 g/m2, or up to about 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.2, 2.5, 2.7, 3.0, or 3.5 g/m2 or may range between any two of the foregoing values. In some embodiments, the cysteamine composition may be administered at a total daily dose of about 0.5-2.0 g/m^2 body surface area, or 1-1.5 g/m^2 body surface area, or 0.5-1 g/m^2 body surface area, or about 0.7-0.8 g/m^2 body surface area, or about 1.3 g/m^2 body surface area (e.g., about 1.35 g/m^2 body surface area), or about 1.3 to about 1.95 $grams/m^2/day$, or about 0.5 to about 1.5 $grams/m^2/day$, or about 0.5 to about 1.0 $grams/m^2/day$, preferably at a frequency of fewer than four times per day, e.g. three, two or one times per day. Salts or esters of the same active ingredient may vary in molecular weight depending on the type and weight of the salt or ester moiety. For administration of enteric dosage form, e.g., a tablet or capsule or other oral dosage form comprising the enterically coated cysteamine product, a total weight in the range of approximately 100 mg to 1000 mg is used. In certain embodiments, the amount of cysteamine active ingredient in a tablet or capsule is approximately 15, 20, 25, 50, 75, 100, 125, 150, 175, 200, 250, 300, 400 or 500 mg.

In one embodiment, provided is a method of treating cystinosis, comprising administering a therapeutically effective amount of a pharmaceutical composition comprising cysteamine, or a pharmaceutically acceptable salt thereof, wherein the pharmaceutical composition has been stored at a temperature of between about 2° C. and about 8° C. prior to administration.

In various embodiments, the pharmaceutical composition is formulated to provide white blood cell cystine suppression with a 12 hour level below 1 nmol ½ cystine/mg protein.

In various embodiments, each dose of cysteamine is about 0.5-1.0 g/m2 body surface area. In various embodiments, the total daily dose of cysteamine is about 1.30 g/m2 body surface area or less.

In various embodiments, the composition increases delivery to the proximal small intestine, the mid-small intestine, the duodenum, the jejunum or the mid-ileum.

In various embodiments, the composition is in the form of a tablet or a capsule.

In various embodiments, the cysteamine salt is cysteamine bitartrate.

The methods of treatment of the disclosure can be used in combination with other therapies useful for treating cystinosis and neurodegenerative diseases and disorders. For example, indomethacin therapy (Indocid® or Endol®) is an anti-inflammatory used to treat rheumatoid arthritis and lumbago, but it can be used to reduce water and electrolyte urine loss. In children with cystinosis, indomethacin reduces the urine volume and therefore liquid consumption by about 30%, sometimes by half. In most cases this is associated with an appetite improvement. Indomethacin treatment is generally followed for several years.

Other therapies can be combined with the methods and compositions of the disclosure to treat diseases and disorders that are attributed to or result from cystinosis. Urinary phosphorus loss, for example, entails rickets, and it may be necessary to give a phosphorus supplement. Carnitine is lost in the urine and blood levels are low. Carnitine allows fat to be used by the muscles to provide energy. Hormone supplementation is sometimes necessary. Sometimes the thyroid gland will not produce enough thyroid hormones. This is given as thyroxin (drops or tables). Insulin treatment is sometimes necessary if diabetes appears, when the pancreas does not produce enough insulin. These treatments have become rarely necessary in children whom are treated with cysteamine, since the treatment protects the thyroid and the pancreas. Some adolescent boys require a testosterone treatment if puberty is late. Growth hormone therapy may be indicated if growth is not sufficient despite a good hydro electrolytes balance. Accordingly, such therapies can be combined with the enterically coated cysteamine and cystamine compositions and methods of the disclosure.

The effectiveness of a method of the disclosure can be assessed by measuring leukocyte cystine concentrations. Dosage adjustment and therapy can be made by a medical specialist depending upon, for example, the severity of cystinosis and/or the concentration of cystine. Additional therapies including the use of omeprazole (Prilosec®) can reduce these symptoms.

Accordingly, in one embodiment, the present disclosure provides a method of treating cystinosis, comprising administering a pharmaceutical composition comprising cysteamine, or a pharmaceutically acceptable salt thereof, wherein the pharmaceutical composition has been stored at a temperature of between about 2° C. and about 8° C. prior to administration. In another embodiment, a method of treating cystinosis is provided, wherein the pharmaceutical composition has been first stored at a temperature of between about 2° C. and about 8° C. prior to administration and is subsequently stored at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for up to 4 months prior to administration.

In any of the aforementioned methods of treatment, the pharmaceutical composition may further comprise one or more materials that provide increased delivery of cysteamine to the small intestine. For example, in one embodiment, the material that provides increased delivery of cysteamine to the small intestine comprises an enteric coating, such as a coating selected from the group consisting of polymerized gelatin, shellac, methacrylic acid copolymer type CNF, cellulose butyrate phthalate, cellulose hydrogen phthalate, cellulose proprionate phthalate, polyvinyl acetate phthalate (PVAP), cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT), hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate, dioxypropyl methylcellulose succinate, carboxymethyl ethylcellulose (CMEC), hydroxypropyl methylcellulose acetate succinate (HPMCAS), and acrylic acid polymers and copolymers formed from methyl acrylate, ethyl acrylate, methyl methacrylate, and/or ethyl methacrylate with copolymers of acrylic and methacrylic acid esters. In one embodiment, the enteric coating comprises poly(methacrylic acid co-ethyl acrylate) 1:1 (Eudragit L 30-D-55).

In any of the aforementioned embodiments, the pharmaceutical composition may comprise a pharmaceutically acceptable salt of cysteamine. In one embodiment, the pharmaceutically acceptable salt of cysteamine is cysteamine bitartrate.

In any of the aforementioned embodiments, the pharmaceutical composition may comprise a solid composition. In one embodiment, the pharmaceutical composition comprises a unit dose of about 25 mg cysteamine. In another embodiment, the pharmaceutical composition comprises a unit dose of about 75 mg cysteamine. In various embodiments, the pharmaceutical composition is a bead formulation as described herein.

In one embodiment, a method of treating cystinosis is provided, comprising administering a pharmaceutical composition comprising cysteamine bitartrate, wherein the pharmaceutical composition has been stored at a temperature of between about 2° C. and about 8° C. prior to administration. In another embodiment, a method of treating cystinosis is provided, comprising administering a pharmaceutical composition comprising cysteamine bitartrate, wherein the pharmaceutical composition has been first stored at a temperature of between about 2° C. and about 8° C. prior to administration and subsequently stored at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for up to 4 months prior to administration. In further embodiment, the pharmaceutical composition comprises PROCYSBI®.

In any of the aforementioned embodiments, the pharmaceutical composition may be stored at a temperature of between 2° C. and about 8° C. for up to 1 month, between about 1 month and about 6 months, between about 6 months and about 12 months, between about 12 months and about 15 months, between about 15 months and about 18 months, between about 18 months and about 21 months, between about 21 months and about 24 months, between about 24 months and about 36 months, or between about 36 months and about 39 months. In one embodiment, the pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. for 1 month, 6 months, 12 months, 15 months, 18 months, 21 months, 24 months, 36 months, or 39 months.

In any of the aforementioned embodiments, the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 0.5%. In another embodiment, a method of treating a cystinosis is provided, wherein the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of 2-hydroxythiomorpholine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the amount of cystamine present in the pharmaceutical composition, rela-

tive to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 4%. In another embodiment, a method of treating cystinosis is provided, wherein the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of cystamine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 0.5%. In another embodiment, a method of treating cystinosis is provided, wherein the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of cystamine tartrate amide present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than 0.5%. In another embodiment, a method of treating cystinosis is provided, wherein the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, is less than or equal to the amount of 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the total amount of 2-hydroxythiomorpholine cystamine, cystamine tartrate amide, and 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, may be less than or equal to the total amount of 2-hydroxythiomorpholine, cystamine, cystamine tartrate amide, and 2-hydroxymethylthiazolidine present in the pharmaceutical composition, relative to the amount of cysteamine, or a pharmaceutically acceptable salt thereof, present in the pharmaceutical composition, after storage at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for the same duration.

In any of the aforementioned embodiments, the cystinosis may be nephropathic cystinosis.

In another aspect, the present disclosure provides methods of treating a disease or disorder, comprising administering a therapeutically effective amount of a pharmaceutical composition comprising cysteamine, or a pharmaceutical acceptable salt thereof, wherein the pharmaceutical composition

has been stored at a temperature of between about 2° C. and about 8° C. prior to administration. In another embodiment, a method of treating a disease or disorder is provided, wherein the pharmaceutical composition has been first stored at a temperature of between about 2° C. and about 8° C. prior to administration and is subsequently stored at a temperature of between about 20° C. and about 25° C. and a relative humidity of 60% for up to 4 months prior to administration. In various embodiments, the disease or disorder is cystinosis, fatty liver disease, a thrombotic disease, an MECP-2 related disorder, an inherited mitochondrial disease, a neurological disease or disorder, inflammation and cancer. In one embodiment, the disease or disorder is a metabolic and neurodegenerative disease, such as non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), Huntington's disease, Parkinson's disease, Rett Syndrome, or cystic fibrosis. In another embodiment, the disease or disorder is a fibrotic disorder or an inflammatory disorder.

EXAMPLES

Example 1

Impurity Levels in PROCYSBI® after Storage at 25° C.

Nineteen impurities have been identified for PROCYSBI® (Table 1). Seven of these impurities do not increase during storage at 25° C./60% relative humidity (RH): Peak 3, Peak E, RRT 1.41-1.47, Peak F, Peak G, Peak H, and Peak I. Twelve impurities may increase when stored at 25° C./60% RH: cystamine, Peak A, Peak B, Peak C, 2-hydroxythiomorpholine, Peak D, cystamine tartrate amide, 2-hydroxymethylthiazolidine, Peak 5, Peak 6, Peak J, and Peak K.

TABLE 1

Impurities That Do and Do Not Increase on Stability When Stored at 25° C./60% RH

Impurities that do not increase when stored at 25° C./60% RH	Impurities that may increase when stored at 25° C./60% RH
Peak 3	Cystamine
Peak E	Peak A
RRT 1.41-1.47	Peak B
Peak F	Peak C
Peak G	2-hydroxythiomorpholine
Peak H	Peak D
Peak I	Cystamine tartrate amide
	2-hydroxymethylthiazolidine
	Peak 5
	Peak 6
	Peak J
	Peak K

Example 2

Storage at 2° C.-8° C. Reduces the Rate of Growth of Impurities in PROCYSBI®

Samples of PROCYSBI® were tested for chemical stability after storage under conditions of either 2° C.-8° C. (refrigerated)/ambient humidity, or 25° C./60% RH (room temperature), for up to 24 months. Samples were placed in controlled environmental chambers, which were maintained

at the described temperature (±2° C.) and relative humidity (±5%). At each scheduled time point, samples were removed from each storage condition within 1 week of the scheduled date, and held at ambient conditions until analyzed. Amounts of cysteamine bitartrate and the 12 impurities that may grow on stability at 25° C./60% RH (cystamine, Peak A, Peak B, Peak C, 2-hydroxythiomorpholine, Peak D, cystamine tartrate amide, 2-hydroxymethylthiazolidine, Peak 5, Peak 6, Peak J, and Peak K), as well as total related substances (TRS), were measured by HPLC (gradient pump; waters column; dimensions: 150-mm×4.6-mm (i.d.); Xbridge C_{18} packing, 3.5-μm particle size; detection: UV @ 210 nm; Injection Volume: 10 μL for assay, 100 μL for related substance (RS) testing; flow rate: 1.0 mL/min; column temperature: 40° C.; autosampler temperature: 4° C.; run time 40 minutes). The mobile phases were 23.6 mM 1-octanesulfonic acid sodium and 29.0 mM sodium phosphate pH 2.6/ACN/MeOH 85/3/12 (v/v/v) (mobile phase A) and 0.20 M 1-octanesulfonic acid sodium and 0.10 M sodium phosphate pH 2.6/ACN/MeOH 10/18/72 (v/v/v) (mobile phase B). Elution was performed using a gradient (linear) under the following parameters:

Time (min.)	% A	% B
0.0	100	0
2.0	100	0
20.0	60	40
25.0	60	40
25.1	100	0
40.0	100	0

The limit of quantitation (LOQ) was 0.05%. For graphical purposes, values at or below LOQ are presented at 0.05%. Data for the amount of cysteamine bitartrate in the samples are calculated relative to the label claim of 25 mg or 75 mg ("Assay"). Acid dissolution and buffer dissolution tests were conducted in accordance with the dissolution method of USP 36-NF 31 section <711>. In brief, in the acid dissolution test, the composition was mixed with 0.1M HCl for 2 hours, at which time a sample of the fluid was taken and assayed for the amount of cysteamine bitartrate present. In the buffer dissolution test, the composition was immediately transferred from the acid solution to a pH 6.8 buffer solution, and the liquid was sampled after either 20 minutes or 30 minutes. Graphical representation (e.g., the Y-axes) of acid dissolution and buffer dissolution data are shown relative to the label claim of 25 mg or 75 mg. Graphical (e.g., the Y-axes) and tabular representation of impurities data show the amount of a given chemical impurity relative to the amount of cysteamine bitartrate measured in that chromatogram (i.e., the ratio of the amount of the impurity to the amount of cysteamine bitartrate, expressed as a percent). Graphical and tabular representations of time in months were also rounded to the nearest integer.

FIGS. **1**A-**1**G show long-term stability data for two lots of 25 mg PROCYSBI® (Lots A and B) stored at 2° C.-8° C./ambient RH for 24 months or at 25° C./60% RH for 12 months. Samples stored at 2° C.-8° C. were tested after storage for 3, 6, 9, 12, 15, 18, 21, and 24 months. Samples stored at 25° C. were tested after storage for 0, 3, 6, 9, 12, 15, 18, 21, and 24 months. At each time point, three 60-count bottles were taken from storage and analyzed as described above. FIG. **1**A shows the amount of cysteamine bitartrate relative to the label claim of 25 mg ("Assay") (expressed as a percent), while FIGS. **1**B and **1**C, respectively, show acid stage dissolution and buffer stage dissolution relative to the label claim of 25 mg (expressed as a percent). FIGS. **1**D-**1**G show the amount of the impurities cystamine, 2-hydroxythiomorpholine, cystamine tartrate amide, and Peak 6 after storage at 2° C.-8° C./ambient RH for 24 months or at 25° C./60% RH for 12 months. At 25° C./60% RH, each of the impurities increased through 12-month storage. In sharp contrast, the levels of cystamine, 2-hydroxythiomorpholine, cystamine tartrate amide, and Peak 6 exhibited little to no increases through 24 months at 2° C.-8° C. storage.

Tables 2 and 3 compare 2° C.-8° C. drug product storage with storage at 25° C./60% RH for lots A and B, respectively, for the 12 impurities that may grow on stability (cystamine, Peak A, Peak B, Peak C, 2-hydroxythiomorpholine, Peak D, cystamine tartrate amide, 2-hydroxymethylthiazolidine, Peak 5, Peak 6, Peak J, and Peak K), as well as total related substances (TRS). Tables 2 and 3 show that the levels of each of these impurities at 2° C.-8° C. exhibited little to no growth through 24 months. Tables 2 and 3 also show that the impurity levels after 18 months storage at 2° C.-8° C. were all lower than or equal to levels after only 12 months storage of 25° C./60% RH. As Tables 2 and 3 illustrate, 2° C.-8° C. storage of DP markedly reduces the rate of growth impurities relative to storage at 25° C./60% RH.

TABLE 2

Comparison of Impurities Levels for Lot A After Storage at 2° C.-8° C. and at 25° C./60% RH for Impurities That May Increase o9n Stability

	Impurity Level for Lot A %			
	2° C.-8° C.			25° C./60% RH
Impurity	Initial	18 M	24 M	12 M
Cystamine	3.3	3.1	3.2	3.8
Peak A	ND	ND	ND	0.10
Peak B	0.05	0.05	0.07	0.06
Peak C	ND	ND	<0.05	0.05
2-hydroxythiomorpholine	0.47	0.35	0.31	0.62
Peak D	ND	ND	ND	0.07
Cystamine Tartrate Amide	0.07	0.09	0.10	0.68
2-hydroxymethylthiazolidine	<0.05	<0.05	ND	0.08
Peak 5	<0.05	<0.05	<0.05	0.11
Peak 6	<0.05	<0.05	<0.05	0.14
Peak J	ND	ND	ND	0.06
Peak K	ND	ND	ND	0.07
TRS	4.4	4.1	4.1	6.2

ND = not detected
<0.05 = detected but below LOQ (0.05%)

TABLE 3

Comparison of Impurities Levels for Lot B After Storage at 2° C.-8° C. and at 25° C./60% RH for impurities That May Increase on Stability

	Impurity Level for Lot B %			
	2° C.-8° C.			25° C./60% RH
Impurity	Initial	18 M	24 M	12 M
Cystamine	3.2	2.8	2.9	3.3
Peak A	ND	ND	ND	0.10
Peak B	<0.05	0.06	0.08	0.06
Peak C	ND	ND	ND	<0.05
2-hydroxythiomorpholine	0.45	0.36	0.30	0.57
Peak D	ND	ND	<0.05	0.06
Cystamine Tartrate Amide	0.07	0.08	0.09	0.63
2-hydroxymethylthiazolidine	<0.05	<0.05	ND	0.07
Peak 5	<0.05	<0.05	<0.05	0.11
Peak 6	<0.05	<0.05	<0.05	0.12

TABLE 3-continued

Comparison of Impurities Levels for Lot B After Storage at 2° C.-8° C. and at 25° C./60% RH for impurities That May Increase on Stability

Impurity	Impurity Level for Lot B % 2° C.-8° C. Initial	18 M	24 M	25° C./60% RH 12 M
Peak J	ND	ND	<0.05	<0.05
Peak K	ND	ND	ND	<0.05
TRS	4.2	3.8	4.0	5.5

ND = not detected
<0.05 = detected but below LOQ (0.05%)

FIGS. **2**A-**2**D show the relative amounts of impurities for the two lots as above, with the addition of stability data for 4 lots (a third lot of 25 mg PROCYSBI®, Lot C, and three 75 mg lots, Lots D, E, and F) stored at 2° C.-8° C. for 3 months (i.e., 3 lots of the 25 mg strength and 3 lots of the 75 mg strength). After three months of storage, three 60-count bottles of 25 mg strength PROCYSBI® and two 250-count bottles of 75 mg strength PROCYSBI® were taken from storage and analyzed as described above. FIGS. **2**A-**2**D show that impurity levels measured through three months in the additional samples are consistent with the trends observed in the 24-month data shown in FIGS. **1**D-**1**G and Tables 2 and 3. Stability data for the 75 mg strength lots stored at 2° C.-8° C. are expected to trend with data from the 25 mg strength lots at 2° C.-8° C. because (i) the enteric coated beads are the same (e.g., same API, formulation, components ratios), (ii) the manufacturing process is the same; in fact, beads for 25 mg strength capsules are also encapsulated at the 75 mg strength (i.e., split batches), (iii) the packaging and product-contacting materials are the same, and (iv) the degradation products and degradation mechanisms are the same for the two dosage strengths.

Taken together, these results show that the impurities growth rates during storage of PROCYSBI® at 2° C.-8° C. are dramatically lower than those observed at 25° C./60% RH. In fact, little to no growth of impurities was observed through 24-moth storage at 2° C.-8° C. At the same time, there was no degradation in product performance as measured by Assay, Acid Stage Dissolution, or Buffer Stage Dissolution after storage at 2° C.-8° C. Thus, the lower storage temperature provides a clear improvement in product quality.

Example 3

Predicted Shelf-Life Values for PROCYSBI® when Stored at 2° C.-8° C.

Predicted shelf-life values for PROCYSBI® for storage conditions of 2° C.-8° C. were calculated using the software package SLIMStat® for the 24 months of data obtained for two lots of 25 mg PROCYSBI® (Lots A and B) (see Example 2). Table 4 shows SLIMStat® predictions for Lots A and B individually. Numeric predictions for several impurities were not available due to limited data points with values about LOQ (0.05%) over 24 months (i.e., the confidence interval did not exceed the product stability specification); these results are represented in Table 4 by the start symbol (*).

The shortest predicted shelf-life for 25 mg PROCYSBI® stored at 2° C.-8° C. was 79 months, based on Peak B for Lot B. The longest predicted shelf-life was 2,608 months (i.e., 217 years), based upon the 2-hydroxythiomorpholine results for Lot B. The shelf-life predictions based upon Assay, and Acid Stage Dissolution and Buffer Stage Dissolution varied from 39 months up to 493 months. Notably, all SLIMStat® shelf-life predictions for 2° C.-8° C. storage were far beyond the currently approved expiry of 18 months at room temperature, again illustrating the marked decrease in DP degradation with 2° C.-8° C. storage.

TABLE 4

Predicted Shelf-Life at 2° C.-8° C. Storage

Test	Predicted Shelf-Life at 2° C.-8° C. (M) Lot A	Lot B
Cystamine	*	357
Peak A	266	180
Peak B	118	79
Peak C	*	*
2-hydroxythiomorpholine	*	2608
Peak D	*	*
Cystamine Tartrate Amide	584	941
2-hydroxymethylthiazolidine	*	*
Peak 5	*	*
Peak 6	*	*
Peak J	*	*
Peak K	764	1018
Total Related Substances	*	439
Assay[a]	48	39
Acid Stage Dissolution	493	*
Buffer Stage Dissolution[b]	69	69

* = Confidence interval of SLIMStat ® prediction does not exceed Specification.
[a]Based upon a lower Assay specification of 90.0%.
[b]Based upon a minimum dissolution specification of 80% within 20 minutes.

Example 4

Impurity Levels in PROCYSBI® Remain Low after Storage at 2° C.-8° C. for 15 Months Followed by Storage for 3 Months at 25° C.

Samples of PROCYSBI® were tested for chemical stability after storage at a temperature of 2° C.-8° C. for up to 15 months followed by excursions of up to 3 months at 25° C./60% RH, 30° C./65% RH, or 30° C./75% RH (18 months of total storage time). Samples were transferred from one condition to another on the same day. The methods used for storing and testing the samples were the same as those described above in Example 2.

FIGS. **3**A-**3**H show stability data for two lots of 25 mg PROCYSBI® (Lots A and B) at 2-8° C. for 15 months followed by excursions of up 3 months at 25° C./60% RH, 30° C./65% RH, or 30° C./75% RH. Levels of the impurities cystamine (FIGS. **3**A and **3**B), 2-hydroxythiomorpholine (FIGS. **3**C and **3**D), cystamine tartrate amide (FIGS. **3**E and **3**F), and Peak 6 (FIGS. **3**G and **3**H) are shown as amount of the chemical impurity relative to the amount of cysteamine bitartrate measured in that chromatogram (i.e., the ratio of the amount of the impurity to the amount of cysteamine bitartrate, expressed as a percent). The apparent growth rates of the impurities during the 3-month step-up period generally was similar to the growth rates observed for 25 mg PROCYSBI® stored exclusively at 25° C./60% RH. FIG. **4** also shows that the levels of each of the four impurities after the 3-month step-ups (18 months total) were lower than their respective levels after only 12 months storage exclusively at 25° C./60% RH.

Tables 5 and 6 show the stability date for 25 mg PROCYSBI® Lots A and B, respectively, for the 12 impurities

that may increase on stability for (a) 3-month storage at 25° C./60% RH, 30° C./65% RH and 30° C./75% RH after 15 months initial storage 2° C.-8° C., (b) 25° C./60% RH storage for 12 months, and (c) 2° C.-8° C. storage at both 15 and 18 months. As shown in Tables 5 and 6, levels of each of the impurities after the samples were transferred to and stored at higher temperatures were all nearly all lower than or equal to their respective levels after only 12 months storage exclusively at 25° C./60% RH.

TABLES 5

Results for Storage at 25° C./60% RH, 30° C./65% RH, and 30° C./75% RH After Initial Storage at 2° C.-8° C. for Lot A

	Impurity Levels for Lot A (%)						
	2° C.-8° C.			15M -> 18M Step-Ups*			25° C./60% RH
Test	Initial	15M	18M	25° C./60%	30° C./65%	30° C./75%	12M
Cystamine	3.3	3.2	3.1	3.3	3.2	3.2	3.8
Peak A	ND	<0.05	ND	ND	ND	ND	0.10
Peak B	0.05	0.06	0.05	0.05	0.06	0.06	0.06
Peak C	ND	ND	ND	ND	ND	ND	<0.05
2-hydroxythiomorpholine	0.47	0.31	0.35	0.42	0.49	0.50	0.62
Peak D	ND	ND	ND	ND	<0.05	<0.05	0.07
Cystamine Tartrate Amide	0.07	0.09	0.09	0.22	0.42	0.43	0.68
2-hydroxymethylthiazolidine	<0.05	ND	<0.05	ND	ND	ND	0.08
Peak 5	<0.05	<0.05	<0.05	0.05	0.07	0.08	0.11
Peak 6	<0.05	0.05	<0.05	0.07	0.11	0.11	0.14
Peak J	ND	ND	ND	ND	<0.05	<0.05	0.06
Peak K	ND	ND	ND	ND	ND	ND	0.07
TRS	4.4	4.1	4.1	4.5	4.8	4.8	6.2

*15M storage at 2° C.-8° C. followed by 3M storage at 25° C./60% RH, 30° C./65% RH or 30° C./75% RH
<0.05 = below LOQ
ND = not detected

TABLE 6

Results for Storage at 25° C./60% RH, 30° C./65% RH, and 30° C./75% RH After Initial Storage at 2° C.-8° C. for Lot B

	Impurity Levels for Lot B (%)						
	2° C.-8° C.			15M -> 18M Step-Ups*			25° C./60% RH
Test	Initial	15M	18M	25° C./60%	30° C./65%	30° C./75%	12M
Cystamine	3.2	2.8	2.8	2.8	3.0	2.9	3.3
Peak A	ND	<0.05	ND	ND	ND	ND	0.10
Peak B	<0.05	0.07	0.06	0.10	0.06	0.06	0.06
Peak C	ND	ND	ND	<0.05	ND	ND	<0.05
2-hydroxythiomorpholine	0.45	0.30	0.36	0.39	0.48	0.47	0.57
Peak D	ND	ND	ND	<0.05	<0.05	<0.05	0.06
Cystamine Tartrate Amide	0.07	0.08	0.08	0.21	0.38	0.39	0.63
2-hydroxymethylthiazolidine	<0.05	ND	<0.05	0.05	ND	ND	0.07
Peak 5	<0.05	<0.05	<0.05	0.06	0.07	0.08	0.11
Peak 6	<0.05	0.05	<0.05	0.06	0.10	0.10	0.12
Peak J	ND	ND	ND	ND	<0.05	<0.05	<0.05
Peak K	ND	ND	ND	ND	ND	ND	<0.05
TRS	4.2	3.8	3.8	4.1	4.5	4.4	5.5

*15M storage at 2° C.-8° C. followed by 3M storage at 25° C./60% RH, 30° C./65% RH or 30° C./75% RH
<0.05 = below LOQ
ND = not detected

Taken together, FIGS. **3**A-**3**G and Tables 5 and 6 show that 25 mg PROCYSBI® stored at 2° C.-8° C. for 15 months, followed by storage at 25° C./60% RH for 3 additional months (18 months total time) resulted in lower levels for nearly all of the 19 measured impurities relative to 12 months storage at 25° C./60% RH. Moreover, samples tested after storage for 3 months at more stringent conditions (e.g., 30° C./65% RH and 30° C./75% RH) had lower impurity levels than samples stored for 12 months at 25° C./60% RH.

Example 5

Predicted Shelf-Life Values for PROCYSBI® when Stored at 2° C.-8° C. Followed by Storage for 4 Months at 25° C.

An extrapolation analysis was performed to evaluate 4-month storage at room temperature following 2° C.-8° C. long-term storage. Specifically, the duration of 25° C./60% RH storage required for each impurity to increase from its assumed level at release to its stability specification level as calculated.

Degradation rates for each of the 12 impurities that may increase on stability at 25° C./60% RH were determined from a date set of 10 lots (G, H, I, J, K, L, M, N, O, and P) using SLIMStat® (Table 7).

TABLE 7

Degradation Rates for PROCYSBI ® at 25° C./60% RH

Impurity	Degradation Rates (%. month) (by lot)										
	G	H	I	J	K	L	M	N	O	P	Max.
Cystamine	0.0390	0.0460	0.0296	−0.0167	−0.0067	0.0621	0.0369	0.0927	0.0952	0.0705	0.0952
Peak A	0.0000	0.0007	0.0006	−0.0024	−0.0026	0.0017	0.0005	0.0028	0.0031	0.0005	0.0031
Peak B	0.0082	0.0056	0.0041	0.0030	0.0017	0.0023	0.0029	0.0043	0.0037	0.0050	0.0082
Peak C	0.0005	0.0008	0.0004	0.0000	0.0000	0.0010	0.0000	0.0042	0.0035	0.0010	0.0042
2-hydroxythiomorpholine	0.0226	0.0202	0.0145	0.0217	0.0127	0.0286	0.0182	0.0372	0.0343	0.0288	0.0372
Peak D	0.0052	0.0053	0.0052	0.0000	0.0000	0.0045	0.0019	0.0133	0.0105	0.0066	0.0133
Cystamine Tartrate Amide	0.0539	0.0566	0.0542	0.0543	0.0587	0.0700	0.0505	0.0690	0.0694	0.0487	0.0700
2-hydroxymethylthiazolidine	0.0087	0.0065	0.0056	0.0007	0.0000	0.0078	0.0052	0.0114	0.0110	0.0074	0.0114
Peak 5	0.0055	0.0047	0.0046	0.0013	0.0000	0.0087	0.0065	0.0095	0.0095	0.0060	0.0095
Peak 6	0.0093	0.0068	0.0068	0.0020	0.0033	0.0106	0.0051	0.0215	0.0181	0.0104	0.0215
Peak J	0.0029	0.0023	0.0012	0.0000	0.0000	0.0021	0.0020	0.0098	0.0072	0.0054	0.0098
Peak K	0.0026	0.0012	0.0002	0.0010	0.0000	0.0005	0.0000	0.0019	0.0015	0.0000	0.0026
TRS	0.1367	0.1367	0.1527	0.0367	0.0700	0.2190	0.1638	0.3053	0.2914	0.2373	0.3053

In calculating the degradation rates to be used in the extrapolation analysis, the largest individual degradation rate for each of the 12 impurities was used ("Highest Degradation Rate").

The level of each impurity at DP lot release was assumed to be close to the release specification (see ICH Guidance, Evaluation for Stability Data Q1E, 2003). Numerically, close to release specification was taken to be 80% of the release specification (e.g., for an impurity with a release specification of 0.15%, the level of that impurity upon release was taken to be 80% of 0.15%, or 0.12%). The release specifications currently approved in the US and EU are identical for the 12 impurities that may grow on stability at 25° C./60% RH. The release specifications and assumed values at release (i.e., "close" to release specifications) for the 12 impurities are shown in Table 8.

The rates of growth of impurities at 2° C.-8° C. were taken to be negligible, based upon the 24-month real-time stability results Example 2. That is, the assumed impurity levels at release were taken to be the same as values after storage at 2° C.-8° C. for up to 24 months.

Using this information, the duration of 25/60% RH storage required for each impurity to increase from its assumed level at release (i.e., 80% of the release specification to its stability specification level, $t_{Step\text{-}Up}$, was calculated with Equation 1 (assuming linear degradation kinetics).

$$t_{Step\text{-}Up} = \frac{(\text{Stability Specification}) - (\text{Assumed Release Level; "Close"})}{(\text{Highest Degradation Rate})} \quad \text{Equation 1}$$

Table 8 shows the calculated $t_{Step\text{-}Up}$ values. For each of the 12 impurities that may increase on stability, the $t_{Step\text{-}Up}$ value is greater than or equal to 4 months. In summary, the extrapolation analysis indicates that PROCYSBI® can be stored at 2° C.-8° C. for 0-24 months, followed by storage for 4 months at 25° C., without resulting in high levels of impurities. Accordingly, patients would be able to store PROCYSBI®, once they receive it, at room temperature conditions for up to four months. Patient storage at refrigerated conditions is also supported through a total storage duration of at least 24 months.

TABLE 8

Extrapolation Analysis of 4-Month Step-Ups To 25° C./60% RH Storage

Impurity	Specifications (%)			Highest Deg Rate	$t_{Step\text{-}Up}$	Supports 4M Step-
	Release	Close*	Stability	(%/month)	(months)	Up to 25° C.
Cystamine	4.0	3.2	5.0	0.0952	19	Yes
Peak A	0.20	0.16	0.20	0.0031	13	Yes
Peak B	0.15	0.12	0.15	0.0082	4	Yes
Peak C	0.10	0.08	0.10	0.0042	5	Yes
2-hydroxythiomorpholine	0.50	0.40	1.0	0.0372	16	Yes
Peak D	0.10	0.08	0.30	0.0133	17	Yes
Cystamine Tartrate Amide	0.15	0.12	1.3	0.0700	17	Yes
2-hydroxymethylthiazolidine	0.10	0.08	0.3	0.0114	19	Yes
Peak 5	0.10	0.08	0.30	0.0095	23	Yes
Peak 6	0.10	0.08	0.30	0.0215	10	Yes
Peak J	0.10	0.08	0.15	0.0098	7	Yes
Peak K	0.10	0.08	0.15	0.0026	27	Yes
TRS	5.0	4.0	8.0	0.3053	13	Yes

*Close to Release Specification = 80% * (Release Specification)

The various embodiments described above can be combined to provide further embodiments. All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet are incorporated herein by reference, in their entirety. Aspects of the embodiments can be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments.

These and other changes can be made to the embodiments in light of the above-detailed description. In general, in the following claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the disclosure.

The invention claimed is:

1. A method of treating cystinosis in a subject in need thereof, comprising administering twice daily to the subject an oral pharmaceutical composition comprising cysteamine bitartrate, wherein the oral pharmaceutical composition contains 2-hydroxythiomorpholine in an amount less than 0.5% relative to the amount of cysteamine bitartrate, and wherein the oral pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. prior to administration.

2. The method according to claim **1**, wherein the oral pharmaceutical composition further comprises an enteric coating wherein the enteric coating comprises poly(methacrylic acid co-ethyl acrylate) 1:1.

3. A method of treating cystinosis in a subject in need thereof, comprising administering twice daily to the subject an oral pharmaceutical composition comprising cysteamine bitartrate, wherein the oral pharmaceutical composition contains cystamine in an amount less than 4% relative to the amount of cysteamine bitartrate, and wherein the oral pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. prior to administration.

4. The method according to claim **3**, wherein the oral pharmaceutical composition further comprises an enteric coating, wherein the enteric coating comprises poly(methacrylic acid co-ethyl acrylate) 1:1.

5. A method of treating cystinosis in a subject in need thereof, comprising administering twice daily to the subject an oral pharmaceutical composition comprising cysteamine bitartrate, wherein the oral pharmaceutical composition contains cystamine tartrate amide in an amount less than 0.5% relative to the amount of cysteamine bitartrate, and wherein the oral pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. prior to administration.

6. The method according to claim **5**, wherein the oral pharmaceutical composition further comprises an enteric coating, wherein the enteric coating comprises poly(methacrylic acid co-ethyl acrylate) 1:1.

7. A method of treating cystinosis in a subject in need thereof, comprising administering twice daily to the subject an oral pharmaceutical composition comprising cysteamine bitartrate, wherein the oral pharmaceutical composition contains 2-hydroxymethylthiazolidine in an amount less than 0.05% relative to the amount of cysteamine bitartrate, and wherein the oral pharmaceutical composition is stored at a temperature of between about 2° C. and about 8° C. prior to administration.

8. The method according to claim **7**, wherein the oral pharmaceutical composition further comprises an enteric coating, wherein the enteric coating comprises poly(methacrylic acid co-ethyl acrylate) 1:1.

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