In one aspect, there is provided a composition as described herein for use in treating diabetes or for use in controlling blood glucose levels in a diabetic subject.~~the use of the composition as described herein for treating diabetes or for controlling blood glucose levels in a subject.~~

In one aspect, there is provided the use of the composition as described herein for the manufacture of a medicament for treating diabetes in a subject.~~use of the composition as described herein for the manufacture of a medicament for treating diabetes or for controlling blood glucose levels in a subject.~~

DETAILED DESCRIPTION

A. Definitions

B. HYALURONAN DEGRADING ENZYME FORMULATIONS AND GENERATING INSULIN CO-FORMULATIONS

- **1. Hyaluronan-Degrading Enzyme Formulations**
- **2. Fast-Acting Insulin Formulations**
- **3. Hyaluronan-Degrading Enzyme and Insulin Co-formulations**
  - **a. Opposing requirements for stability**
    - **i. Preservatives**
    - **ii. NaCl and pH**
  - **b. Compatible Co-Formulation**

C. HYALURONAN DEGRADING ENZYMES

- **1. Hyaluronidases**
  - **a. Mammalian-type hyaluronidases**
    PH20
  - **b. Bacterial hyaluronidases**
  - **c. Hyaluronidases from leeches, other parasites and crustaceans**

CLAIMS:

1. A composition, comprising:

a hyaluronidase; and

lysyl lysine (Lys-Lys) in an amount to render the hyaluronidase stable.

2. The composition of claim 1, wherein the Lys-Lys concentration is between 5 mM to 120 mM, 10 mM to 100 mM, 5 mM to 50 mM, 10 mM to 50 mM, 30 mM to 110 mM, 30 mM to 80 mM, 50 mM to 100 mM or 100 mM to 120 mM.

3. The composition of claim 1, wherein the Lys-Lys concentration is 5 mM to 300 mM.

4. The composition of claim 1 or claim 3, wherein the Lys-Lys concentration is 50 mM to 150 mM, inclusive.

5. The composition of any of claims 1-4, wherein the concentration of hyaluronidase is at least 10 U/mL.

6. The composition of any of claims 1-5, wherein the amount of hyaluronidase is between 10 U/mL to 5000 U/mL, inclusive.

7. The composition of any of claims 1-6, wherein the formulation further comprises a stabilizing agent selected from among an amino acid, an amino acid derivative, an amine, a sugar, a polyol, a salt and a surfactant.

8. The composition of claim 7, wherein the stabilizing agent is a surfactant and the amount of surfactant, as a % of mass concentration (w/v) in the formulation, is between 0.0005% to 1.0%, 0.0005% to 0.005%, 0.001% to 0.01%, 0.01% to 0.5%, 0.01% to 0.1%, 0.01% to 0.05%, or 0.01% to 0.02%, inclusive.

9. The composition of claim 7 or claim 8, wherein the surfactant is selected from among a polypropylene glycol, polyethylene glycol, glycerin, sorbitol, poloxamer and polysorbate.

10. The composition of claim 7 or claim 8, wherein the surfactant is selected from among poloxamer 188, polysorbate 20 and polysorbate 80.

11. The composition of any of claims 1-10, wherein the composition comprises an antioxidant or a tonicity modifier.

12. The composition of any of claims 1-11, comprising a sufficient amount of a buffering agent to maintain the pH range of between 6.5 to 8.0, 6.8 to 7.8, 7.0 to 7.6, 6.5 to 7.2, or 6.8 to 7.4, inclusive, wherein the buffering agent is selected from among Tris, histidine, phosphate and citrate.

13. The composition of any of claims 1-12, wherein the hyaluronidase is a soluble hyaluronidase that is active at neutral pH.

14. The composition of claim 13, wherein the soluble hyaluronidase is a PH20 or a C-terminally truncated fragment thereof.

15. The composition of claim 13 or claim 14, wherein the soluble PH20 polypeptide is selected from ovine PH20, bovine PH20 and a soluble human PH20,

wherein the soluble human PH20 lacks all or a portion of the glycosylphosphatidylinositol (GPI) anchor attachement sequence.

16. The composition of any of claims 1-15, wherein the hyaluronidase is a PH20 polypeptide that has the sequence of amino acids set forth in any of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273, or a sequence of amino acids that exhibits at least 91%, or more sequence identity to any of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273.

17. The composition of any of claims 14-16, wherein the hyaluronidase is a C-terminal truncated PH20 that comprises the sequence of amino acids set forth in any of SEQ ID NOS: 4-9.

18. The composition of any of claims 1-4 and 7-17, wherein:

the amount of a hyaluronidase is between 10 U/mL to 20,000 U/mL, 50 U/mL to 15,000 U/mL, 50 U/mL to 10,000 U/mL, 50 U/mL to 5000 U/mL, 50 U/mL to 4000 U/mL, 100 U/mL to 10000 U/mL, 100 U/mL to 5000 U/mL, 100 U/mL to 2000 U/mL, 300 U/mL to 5000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL to 5000 U/mL, 600 U/mL to 2000 U/mL, 100 U/mL to 1000 U/mL, 200 U/mL to 800 U/mL, 100 U/mL to 500 U/mL, or 150 U/mL to 300 U/ml, inclusive; or

the amount of a hyaluronidase is at least or is 30 U/mL, 35 U/mL, 40 U/mL, 50 U/mL, 100 U/mL, 150 U/mL, 200 U/mL, 250 U/mL, 300 U/mL, 350 U/mL, 400 U/mL, 450 U/mL, 500 U/mL, 600 U/mL, 700 U/mL, 800 U/mL, 900 U/mL, 1000 U/mL, 2000 U/mL, 3000 U/mL, 4000 U/mL, 5000 U/mL, 10000 U/mL, 15000 U/mL or 20000 U/mL.

19. The composition of any of claims 1-18, wherein:

the pH of the composition is between 6.5 to 7.2;

and the composition comprises:

a hyaluronidase in an amount that is between 100 U/mL to 500 U/mL, inclusive;

Lys-Lys at a concentration that is between 5 mM to 30 mM, inclusive;

NaCl at a concentration less than 140 mM NaCl;

a surfactant that is polysorbate 80 at a percentage (%) of mass concentration (w/v) of between 0.01% to 0.05%, inclusive;

methionine at a concentration that is between 5 mM to 20 mM, inclusive; and

sodium phosphate at a concentration that is between 5 mM to 50 mM, inclusive.

20. The composition of any of claims 1-19, further comprising a fast-acting insulin.

21. The composition of claim 20, wherein the concentration of Lys-Lys is 30 mM to 120 mM, 50 mM to 105 mM or 80 mM to 100 mM, inclusive.

22. The composition of claim 20 or claim 21, wherein the fast-acting insulin is a regular insulin or a fast-acting insulin analog.

23. The composition of claim 22, wherein the fast-acting insulin is a regular insulin and the regular insulin is a human insulin or pig insulin.

24. The composition of claim 22, wherein the fast-acting insulin is an insulin analog and the insulin analog is selected from among insulin aspart, insulin lispro and insulin glulisine.

25. The composition of claim 24, wherein the insulin analog is selected from among an insulin having an A chain with a sequence of amino acids set forth in SEQ NOS:103 and a B chain having a sequence of amino acids set forth in any of SEQ NOS:147-149.

26. The composition of any of claims 20-25, wherein the amount of fast-acting insulin is between 10 U/mL to 1000 U/mL, 20 U/mL to 500 U/mL, 50 U/mL to 300 U/mL or 200 U/mL to 800 U/mL, inclusive.

27. The composition of any of claims 1-26 that is for single dosage administration or is for multiple dosage administration.

28. The composition of claim 27, wherein the composition is for multiple dosage administration and comprises an anti-microbially effective amount of a preservative or mixture of preservatives.

29. The composition of any of claims 1-28, wherein:

the pH of the composition is between 6.8 to 7.4; and

the composition comprises:

a hyaluronidase that is a PH20 in an amount between 100 U/mL to 1000 U/mL, inclusive;

a fast-acting insulin analog that is insulin glulisine is an amount between 10 U/mL to 1000 U/mL, inclusive;

Lys-Lys at a concentration between 50 mM to 105 mM, inclusive;

NaCl at a concentration of less than 100 mM;

a surfactant that is polysorbate 20 at a percentage (%) of mass concentration (w/v) of between 0.0005% to 0.005%, inclusive;

methionine at a concentration between 5 mM to 20 mM, inclusive; and

a preservative(s) that comprises phenol at a percentage (%) of mass concentration (w/v) of between 0.1% to 0.25% and m-cresol at a % w/v of between 0.05% to 0.2%.

30. The composition of any of claims 1-28, wherein:

the pH of the composition is between 6.8 to 7.4; and

the composition comprises:

a hyaluronidase that is a PH20 in an amount between 100 U/mL to 1000 U/mL, inclusive;

a fast-acting insulin analog that is insulin aspart or insulin lispro is an amount between 10 U/mL to 1000 U/mL, inclusive;

Lys-Lys at a concentration between 80 mM to 100 mM, inclusive;

NaCl at a concentration of less than 30 mM;

a surfactant that is polysorbate 20 at a percentage (%) of mass concentration (w/v) of between 0.0005% to 0.005%, inclusive;

methionine at a concentration between 5 mM to 20 mM, inclusive; and

a preservative(s) that comprises phenol at a percentage (%) of mass concentration (w/v) of between 0.1% to 0.25% and m-cresol at a % w/v of between 0.05% to 0.2%.

31. A syringe or vial, comprising the stable composition of any of claims 1-30.

32. A closed loop system, comprising the stable composition of any of claims 1-30.

33. An insulin pump, comprising the stable composition of any of claims 1-30.

34. An insulin pen, comprising the stable composition of any of claims 1-30.

35. Use of the composition of any of claims 20-30 for treating diabetes or for controlling blood glucose levels in a subject.

36. The use of claim 35, wherein the diabetes is selected from among type 1 diabetes mellitus, type 2 diabetes mellitus and gestational diabetes

37. Use of the composition of any of claims 20-30 for the manufacture of a medicament for treating diabetes or for controlling blood glucose levels in a subject.

38. The use of claim 37, wherein the diabetes is selected from among type 1 diabetes mellitus, type 2 diabetes mellitus and gestational diabetes

CLAIMS:

1. A composition, comprising:

a hyaluronidase; and

lysyl lysine (Lys-Lys) in an amount to render the hyaluronidase stable.

2. The composition of claim 1, wherein the Lys-Lys concentration is between 5 mM to 120 mM, 10 mM to 100 mM, 5 mM to 50 mM, 10 mM to 50 mM, 30 mM to 110 mM, 30 mM to 80 mM, 50 mM to 100 mM or 100 mM to 120 mM.

3. The composition of claim 1, wherein the Lys-Lys concentration 5 mM to 300 mM.

4. The composition of claim 1 or claim 3, wherein the Lys-Lys concentration is 50 mM to 150 mM, inclusive.

5. The composition of any of claims 1-4, wherein the concentration of hyaluronidase is at least 10 U/mL.

6. The composition of any of claims 1-5, wherein the amount of hyaluronidase is between 10 U/mL to 5000 U/mL, inclusive.

7. The composition of any of claims 1-6, wherein the formulation further comprises a stabilizing agent selected from among an amino acid, an amino acid derivative, an amine, a sugar, a polyol, a salt and a surfactant.

8. The composition of claim 7, wherein the stabilizing agent is a surfactant and the amount of surfactant, as a % of mass concentration (w/v) in the formulation, is between 0.0005% to 1.0%, 0.0005% to 0.005%, 0.001% to 0.01%, 0.01% to 0.5%, 0.01% to 0.1%, 0.01% to 0.05%, or 0.01% to 0.02%, inclusive.

9. The composition of claim 7 or claim 8, wherein the surfactant is selected from among a polypropylene glycol, polyethylene glycol, glycerin, sorbitol, poloxamer and polysorbate.

10. The composition of claim 7 or claim 8, wherein the surfactant is selected from among poloxamer 188, polysorbate 20 and polysorbate 80.

11. The composition of any of claims 1-10, wherein the composition comprises an antioxidant or a tonicity modifier.

12. The composition of any of claims 1-11, comprising a sufficient amount of a buffering agent to maintain the pH range of between 6.5 to 8.0, 6.8 to 7.8, 7.0 to 7.6, 6.5 to 7.2, or 6.8 to 7.4, inclusive, wherein the buffering agent is selected from among Tris, histidine, phosphate and citrate.

13. The composition of any of claims 1-12, wherein the hyaluronidase is a soluble hyaluronidase that is active at neutral pH.

14. The composition of claim 13, wherein the soluble hyaluronidase is a PH20 or a C-terminally truncated fragment thereof.

15. The composition of claim 13 or claim 14, wherein the soluble PH20 polypeptide is selected from ovine PH20, bovine PH20 and a soluble human PH20, wherein the soluble human PH20 lacks all or a portion of the glycosylphosphatidylinositol (GPI) anchor attachment sequence.

16. The composition of any of claims 1-15, wherein the hyaluronidase is a PH20 polypeptide that has the sequence of amino acids set forth in any of SEQ ID NOS: 4-9, 47-48,

234-254, and 267-273, or a sequence of amino acids that exhibits at least 91%, or more sequence identity to any of SEQ ID NOS: 4-9, 47-48, 234-254, and 267-273.

17. The composition of any of claims 14-16, wherein the hyaluronidase is a C-terminal truncated PH20 that comprises the sequence of amino acids set forth in any of SEQ ID NOS: 4-9.

18. The composition of any of claims 1-4 and 7-17, wherein:

the amount of a hyaluronidase is between 10 U/mL to 20,000 U/mL, 50 U/mL to 15,000 U/mL, 50 U/mL to 10,000 U/mL, 50 U/mL to 5000 U/mL, 50 U/mL to 4000 U/mL, 100 U/mL to 10000 U/mL, 100 U/mL to 5000 U/mL, 100 U/mL to 2000 U/mL, 300 U/mL to 5000 U/mL, 300 U/mL to 2000 U/mL, 600 U/mL to 5000 U/mL, 600 U/mL to 2000 U/mL, 100 U/mL to 1000 U/mL, 200 U/mL to 800 U/mL, 100 U/mL to 500 U/mL, or 150 U/mL to 300 U/ml, inclusive; or

the amount of a hyaluronidase is at least or is 30 U/mL, 35 U/mL, 40 U/mL, 50 U/mL, 100 U/mL, 150 U/mL, 200 U/mL, 250 U/mL, 300 U/mL, 350 U/mL, 400 U/mL, 450 U/mL, 500 U/mL, 600 U/mL, 700 U/mL, 800 U/mL, 900 U/mL, 1000 U/mL, 2000 U/mL, 3000 U/mL, 4000 U/mL, 5000 U/mL, 10000 U/mL, 15000 U/mL or 20000 U/mL.

19. The composition of any of claims 1-18, wherein:

the pH of the composition is between 6.5 to 7.2;

and the composition comprises:

a hyaluronidase in an amount that is between 100 U/mL to 500 U/mL, inclusive;

Lys-Lys at a concentration that is between 5 mM to 30 mM, inclusive;

NaCl at a concentration less than 140 mM NaCl;

a surfactant that is polysorbate 80 at a percentage (%) of mass concentration (w/v) of between 0.01% to 0.05%, inclusive;

methionine at a concentration that is between 5 mM to 20 mM, inclusive; and

sodium phosphate at a concentration that is between 5 mM to 50 mM, inclusive.

20. The composition of any of claims 1-19, further comprising a fast-acting insulin.

21. The composition of claim 20, wherein the concentration of Lys-Lys is 30 mM to 120 mM, 50 mM to 105 mM or 80 mM to 100 mM, inclusive.

22. The composition of claim 20 or claim 21, wherein the fast-acting insulin is a regular insulin or a fast-acting insulin analog.

23. The composition of claim 22, wherein the fast-acting insulin is a regular insulin and the regular insulin is a human insulin or pig insulin.

24. The composition of claim 22, wherein the fast-acting insulin is an insulin analog and the insulin analog is selected from among insulin aspart, insulin lispro and insulin glulisine.

25. The composition of claim 24, wherein the insulin analog is selected from among an insulin having an A chain with a sequence of amino acids set forth in SEQ NOS:103 and a B chain having a sequence of amino acids set forth in any of SEQ NOS:147-149.

26. The composition of any of claims 20-25, wherein the amount of fast-acting insulin is between 10 U/mL to 1000 U/mL, 20 U/mL to 500 U/mL, 50 U/mL to 300 U/mL or 200 U/mL to 800 U/mL, inclusive.

27. The composition of any of claims 1-26 that is for single dosage administration or is for multiple dosage administration.

28. The composition of claim 27, wherein the composition is for multiple dosage administration and comprises an anti-microbially effective amount of a preservative or mixture of preservatives.

29. The composition of any of claims 1-28, wherein:

the pH of the composition is between 6.8 to 7.4; and

the composition comprises:

a hyaluronidase that is a PH20 in an amount between 100 U/mL to 1000 U/mL, inclusive;

a fast-acting insulin analog that is insulin glulisine is an amount between 10 U/mL to 1000 U/mL, inclusive;

Lys-Lys at a concentration between 50 mM to 105 mM, inclusive;

NaCl at a concentration of less than 100 mM;

a surfactant that is polysorbate 20 at a percentage (%) of mass concentration (w/v) of between 0.0005% to 0.005%, inclusive;

methionine at a concentration between 5 mM to 20 mM, inclusive; and

a preservative(s) that comprises phenol at a percentage (%) of mass concentration (w/v) of between 0.1% to 0.25% and m-cresol at a % w/v of between 0.05% to 0.2%.

30. The composition of any of claims 1-28, wherein:

the pH of the composition is between 6.8 to 7.4; and

the composition comprises: a hyaluronidase that is a PH20 in an amount between 100 U/mL to 1000 U/mL, inclusive;

a fast-acting insulin analog that is insulin aspart or insulin lispro is an amount between 10 U/mL to 1000 U/mL, inclusive;

Lys-Lys at a concentration between 80 mM to 100 mM, inclusive;

NaCl at a concentration of less than 30 mM;

a surfactant that is polysorbate 20 at a percentage (%) of mass concentration (w/v) of between 0.0005% to 0.005%, inclusive;

methionine at a concentration between 5 mM to 20 mM, inclusive; and

a preservative(s) that comprises phenol at a percentage (%) of mass concentration (w/v) of between 0.1% to 0.25% and m-cresol at a % w/v of between 0.05% to 0.2%.

31. A syringe or vial, comprising the stable composition of any of claims 1-30.

32. A closed loop system, comprising the stable composition of any of claims 1-30.

33. An insulin pump, comprising the stable composition of any of claims 1-30.

34. An insulin pen, comprising the stable composition of any of claims 1-30.

35. A composition of any of claims 20-30 for use in treating diabetes or for use in controlling blood glucose levels in a diabetic subject.

36. The composition for use of claim 35, wherein the diabetes is selected from among type 1 diabetes mellitus, type 2 diabetes mellitus and gestational diabetes.

37. Use of the composition of any of claims 20-30 for the manufacture of a medicament for treating diabetes in a subject.

38. The use of claim 37, wherein the diabetes is selected from among type 1 diabetes mellitus, type 2 diabetes mellitus and gestational diabetes.