

WHAT IS CLAIMED IS:

Claims

1.- ____ A pre-lyophilization formulation comprising:

~~(a) a Factor IX (FIX) polypeptide having FIX coagulation activity;~~

~~(b) a buffering agent;~~

~~(c) a stabilizing agent;~~

~~(d) a bulking agent; and~~

~~(e) a surfactant, wherein the (a) a Factor IX (FIX) polypeptide at a concentration between about 220 IU/vial to about 1,000 IU/vial, wherein the FIX polypeptide comprises the FIXFc-sc polypeptide of SEQ ID NO: 2, without the C-terminal lysine, and the Fc sc polypeptide of SEQ ID NO:4, without the C-terminal lysine, wherein the FIXFc-sc polypeptide and the Fc sc polypeptide are bound together through two disulfide bonds in the hinge region of Fc;~~

~~(b) L-histidine at a concentration between about 3 mg/mL and about 15 mg/mL;~~

~~(c) sucrose at a concentration between about 10 mg/mL and about 50 mg/mL;~~

~~(d) mannitol at a concentration between about 20 mg/mL and about 100 mg/mL;~~

~~and~~

~~(e) polysorbate 20 at a concentration between about 0.01 mg/mL and about 5 mg/mL,~~

~~wherein the formulation has a fill volume of less than about 5 mL, less than about 4mL, or less than about 3 mL 3 mL;~~ and wherein each of (a) - (e) are at an amount per vial (mg/vial) sufficient to allow

~~(1) improved stability of the FIX polypeptide when lyophilized;~~

~~(2) reduced reconstitution time when lyophilized;~~

~~(3) reduced splashing onto a stopper comprising the formulation;~~

~~(4) reduced lyophilization cycle time;~~

~~(5) increased shelf life of a lyophilate prepared from by lyophilizing the pre-lyophilization formulation at room temperature; or~~

~~(6) any combinations thereof,~~

compared to a reference pre-lyophilization formulation,

~~wherein wherein, for the purpose of comparison of reconstitution time, the volumes into which each of the lyophilate prepared by lyophilizing the formulation and the lyophilate prepared by lyophilizing the reference pre-lyophilization formulation are reconstituted respectively are the same,~~

~~wherein the reference pre-lyophilization~~ formulation comprises (a) - (e) at the amount per vial identical to the pre-lyophilization formulation, but has a ~~5 mL~~ 5 mL fill volume.

2.- The pre-lyophilization formulation of claim 1, wherein the ~~fill volume is about 4 mL, about 3.5 mL, about 3.0 mL, about 2.9 mL, about 2.8 mL, about 2.7 mL, about 2.65 mL, about 2.6 mL, about 2.5 mL, about 2.4 mL, about 2.3 mL, about 2.2 mL, about 2.1 mL, or about 2.0 mL.~~ volumes into which each of the lyophilate prepared by lyophilizing the formulation and the lyophilate prepared by lyophilizing the reference pre-lyophilization formulation are reconstituted respectively are about 5 mL.

3.- The pre-lyophilization formulation of claim 1 or claim 2, wherein the ~~fill volume is about 2.65 mL.~~

~~4. The pre-lyophilization formulation of any one of claims 1 to 3, wherein the reduced reconstitution time is less than 1.5 minute, less than 1 minute, less than 50 seconds, less than 40 seconds, less than 30 seconds, less than 20 seconds, or less than 10 seconds.~~

~~5. The pre-lyophilization formulation of any one of claims 1 to 4, wherein the reduced reconstitution time is less than 30 seconds.~~

~~6. The pre-lyophilization formulation of any one of claims 1 to 5, wherein the reduced lyophilization cycle time is about 4 days or less, about 3 days or less, about 2 days or less, or about a day or less.~~

~~7. The pre-lyophilization formulation of any one of claims 1 to 6, wherein the buffering agent is L-histidine.~~

~~8. The pre-lyophilization formulation of any one of claims 1 to 7, wherein the stabilizing agent is sucrose.~~

~~9. The pre-lyophilization formulation of any one of claims 1 to 8, wherein the bulking agent is mannitol.~~

~~10. The pre-lyophilization formulation of any one of claims 1 to 9, wherein the surfactant is polysorbate 20.~~

~~11. The pre-lyophilization formulation of any one of claims 1 to 10, wherein the formulation comprises at least 100 IU/vial of the FIX polypeptide.~~

~~12. The pre-lyophilization formulation of any one of claims 1 to 11, wherein the formulation comprises about 200 IU/vial to about 10 is at a concentration between 220 IU/vial to 1,000 IU/vial of the FIX polypeptide.~~

~~13. The pre-lyophilization formulation of any one of claims 1 to 12, wherein the FIX polypeptide comprises about 220 IU/vial, about 250 IU/vial, about 300 IU/vial, about 400 IU/vial, about 500 IU/vial, about 600 IU/vial, about 700 IU/vial, about 800 IU/vial, about 900 IU/vial, about 1,000 IU/vial, about 1,100 IU/vial, about 1,200 IU/vial, about 1,300 IU/vial, about 1,400 IU/vial, about 1,500 IU/vial, about 2,000 IU/vial, about 2,500 IU/vial, about 3,000 IU/vial, about 4,000 IU/vial, about 5,000 IU/vial, about 5,500 IU/vial, about 6,000 IU/vial, about 6,500 IU/vial, about 7,000 IU/vial, about 7,500 IU/vial, about 8,000 IU/vial, about 8,500 IU/vial, about 9,000 IU/vial, about 9,500 IU/vial or about 10,000 IU/vial.~~

~~14. The pre-lyophilization formulation of any one of claims 1 to 13, wherein the buffering agent wherein the L-histidine is at a concentration ~~(mg/mL)~~ between ~~about~~ 3 mg/mL and ~~about~~ 15 mg/mL.~~

~~15. The pre-lyophilization formulation of any one of claims 1 to 14, wherein the buffering agent is at a concentration (mg/mL) between about 3.88 mg/mL and about 9.7 mg/mL.~~

~~16. The pre-lyophilization formulation of any one of claims 1 to 15, wherein the buffering agent wherein the sucrose is at a concentration ~~(between 10 mg/mL)~~ of about 7.76 and 50 mg/mL.~~

~~17. The pre-lyophilization formulation of any one of claims 1 to 16, wherein the stabilizing agent mannitol is at a concentration (mg/mL) between 1020 mg/mL and about 50100 mg/mL; or~~

~~18. The pre-lyophilization formulation of any one of claims 1 to 17, wherein the stabilizing agent wherein the polysorbate 20 is at a concentration (between 0.01 mg/mL) and 5 mg/mL.~~

~~4. The pre-lyophilization formulation of any one of claims 1 to 3, wherein the L-histidine is at a concentration between about 3.88 mg/mL and about 9.7 mg/mL, wherein the sucrose is at a concentration between about 17.85 mg/mL and about 29.95 mg/mL, or wherein the mannitol is at a concentration between about 35.7 mg/mL and about 59.5 mg/mL.~~

~~19. 5. The pre-lyophilization formulation of any one of claims 1 to 184, wherein the stabilizing agent is at a concentration (mg/mL) of about 23.8 mg/mL.~~

~~20. The pre-lyophilization formulation of any one of claims 1 to 19, wherein the bulking agent is at a concentration (mg/mL) between 20 mg/mL and about 100 mg/mL.~~

~~21. The pre-lyophilization formulation of any one of claims 1 to 20, wherein the bulking agent is at a concentration (mg/mL) between 35.7 mg/mL and 59.5 mg/mL.~~

~~22. The pre-lyophilization formulation of any one of claims 1 to 21, wherein the bulking agent L-histidine is at a concentration (of about 7.76 mg/mL) of 47.6 mg/mL.~~

~~23. The pre-lyophilization formulation of any one of claims 1 to 22, wherein the surfactant sucrose is at a concentration (mg/mL) between 0.01 mg/mL and of about 5 mg/mL.~~

~~24. The pre-lyophilization formulation of claim 23.8 mg/mL, wherein the surfactant mannitol is at a concentration of about 47.6 mg/mL, or wherein the polysorbate 20 is at a concentration (mg/mL) of about 0.2 mg/mL.~~

~~25. 6. The pre-lyophilization formulation of any one of claims 1 to 24, wherein the FIX polypeptide has a concentration (IU/mL) between about 80 IU/mL and about 2,750 IU/mL.~~

~~26. A pre-lyophilization formulation comprising:~~

~~(a) about 80 to about 2,750 IU/mL of rFIXFc;~~

~~(b) about 7.76 mg/mL of L-histidine;~~

~~(c) about 47.6 mg/mL of mannitol;~~

~~(d) about 23.8 mg/mL of sucrose; and,~~

~~(e) about 0.2 mg/mL of polysorbate 20.~~

~~27. The pre-lyophilization formulation of claim 265, wherein the formulation has a fill volume per vial of about 3 mL, about 2.9 mL, about 2.8 mL, about 2.7 mL, about 2.65 mL, about 2.6 mL, about 2.5 mL, about 2.4 mL, about 2.3 mL, about 2.2 mL, about 2.1 mL, or about 2.0 mL. 2.65 mL.~~

~~28. The pre-lyophilization formulation of claim 27, wherein the formulation has a fill volume per vial of about 2.65 mL.~~

~~29. 7. A lyophilate powder, which is lyophilized from the pre-lyophilization formulation of any one of claims 1 to 286.~~

~~30. The 8. A vial comprising the lyophilate powder of claim 29, which has a residual moisture level below 1%.~~

~~31. The lyophilate powder of claim 29 or 30, comprising a buffering agent, a stabilizing agent, a bulking agent, a surfactant, or any combinations thereof.~~

~~32. The lyophilate powder of claim 31, wherein the buffering agent is L-histidine.~~

~~33. The lyophilate powder of claim 31, wherein the stabilizing agent is sucrose.~~

~~34. The lyophilate powder of claim 31, wherein the surfactant is polysorbate 20.~~

~~35. The lyophilate powder of any one of claims 31 to 34, wherein the buffering agent is at a concentration between about 8 mg and about 39 mg per vial.~~

~~36. The lyophilate powder of claim 35, wherein the buffering agent is at a concentration of about 20.6 mg per vial.~~

~~37. The lyophilate powder of any one of claims 31 to 36, wherein the stabilizing agent is at a concentration between about 27 mg and about 132 mg per vial.~~

~~38. The lyophilate powder of claim 37, wherein the stabilizing agent is at a concentration of about 63.1 mg per vial.~~

~~39. The lyophilate powder of any one of claims 31 to 38, wherein the bulking agent is at a concentration between about 53 mg per vial and about 265 mg per vial.~~

~~40. The lyophilate powder of claim 39, wherein the bulking agent is at a concentration of about 126.1 mg per vial.~~

~~41. The lyophilate powder of any one of claims 31 to 40, wherein the surfactant is at a concentration between about 0.03mg and about 13 mg per vial.~~

~~42. The lyophilate powder of claim 41, wherein the surfactant is at a concentration of about 0.53 mg per vial.~~

~~43. A lyophilate powder comprising:~~

~~(a) a FIX polypeptide at an amount between about 2 mg per vial and about 150 mg per vial;~~

~~(b) a buffering agent at an amount between 10 mg per vial and about 30 mg per vial;~~

~~(c) a bulking agent at an amount between 70 mg vial and about 200 mg per vial;~~

~~(d) a stabilizing agent at an amount between 30 mg per vial and 100 mg per vial; and~~

~~(e) a surfactant at an amount between 0.05 mg per vial and about 5 mg per vial.~~

~~44. The lyophilate powder of claim 43, comprising:~~

~~(a) the lyophilized FIX polypeptide at an amount between about 2.2 mg per vial and about 125 mg per vial;~~

~~(b) the buffering agent at an amount between about 12.5 mg per vial and 25 mg per vial;~~

~~(c) the stabilizing agent at an amount between about 32.5 mg per vial and 80 mg per vial;~~

~~(d) the bulking agent at an amount between about 75 mg per vial and 150 mg per vial; and~~

~~(e) the surfactant at an amount between about 0.1 mg/mL and about 2 mg/mL.~~

~~45. The lyophilate powder of claim 44 comprising:~~

~~(a) about 2.2 to about 125 mg/vial of the FIX polypeptide;~~

~~(b) about 20.6 mg/vial of L-histidine.~~

~~(c) about 126.1 mg/vial of mannitol;~~

~~(d) about 63.1 mg/vial of sucrose; and,~~

~~(e) about 0.53 mg/vial of polysorbate-20;~~

~~46. A reconstituted formulation comprising the lyophilate power of any one of claims 29 to 45 reconstituted by a reconstitution buffer.~~

~~47. The reconstituted formulation of claim 46, which is used to treat hemophilia B.~~

~~48. The reconstitution formulation of claim 46 or 47, which is suitable for parenteral administration.~~

~~49. The reconstituted formulation of claim 48, wherein the parenteral administration is intravenous or subcutaneous administration.~~

~~50. The reconstituted formulation of any one of claims 47 to 49, comprising:~~

~~(a) the FIX polypeptide at a concentration between about 0.9 mg/mL and about 50 mg/mL;~~

~~(b) the buffering agent at a concentration between 1.5 mg/mL and about 7.5 mg/mL;~~

~~(c) the bulking agent at a concentration between 10 mg/mL and about 50 mg/mL;~~

~~(d) the stabilizing agent at a concentration between 5 mg/mL and 25 mg/mL per vial; and~~

~~(e) the surfactant at a concentration between 0.005 mg/mL and about 2.5 mg/mL.~~

~~51. The reconstituted formulation of claim 50, comprising:~~

~~(a) the FIX polypeptide at a concentration between about 0.9 mg/mL and about 50 mg/mL;~~

~~(b) the buffering agent at a concentration of about 3.88 mg/mL;~~

~~(c) the bulking agent at a concentration of about 23.8 mg/mL;~~

~~(d) the stabilizing agent at a concentration of about 11.9 mg/mL;~~

~~(e) the surfactant at a concentration of about 0.1 mg/mL; and~~

~~(f) the reconstitution buffer.~~

~~52. The reconstituted formulation of claim 49, comprising~~

~~(a) the FIX polypeptide at a concentration between about 80 IU/mL and about 2,750 IU/mL;~~

~~(b) the buffering agent at a concentration of about 25 mM;~~

~~(c) the bulking agent at a concentration of about 131 mM;~~

~~(d) the stabilizing agent at a concentration of about 35 mM;~~

~~(e) the surfactant at a concentration of 0.01 % (w/v); and~~

~~(f) the reconstitution buffer.~~

~~53. A vial comprising the pre-lyophilization formulation of any one of claims 1 to 28, the lyophilate power of any one of claims 29 to 45, or the reconstituted formulation of any one of claims 46 to 52.~~

~~54. The pre-lyophilization formulation of any one of claims 1 to 28, the lyophilate power of any one of claims 29 to 45, the reconstituted formulation of 46 to 52, or the vial of 53, wherein the FIX polypeptide comprises wild-type FIX.~~

~~55. The pre-lyophilization formulation of any one of claims 1 to 28, the lyophilate power of any one of claims 29 to 45, the reconstituted formulation of 46 to 52, or the vial of 53, wherein the FIX polypeptide further comprises a heterologous moiety fused to wild-type FIX.~~

~~56. The pre-lyophilization formulation of any one of claims 1 to 28, the lyophilate power of any one of claims 29 to 45, the reconstituted formulation of 46 to 52, or the vial of 53, wherein the heterologous moiety is a moiety extending half life of FIX.~~

~~57. The pre-lyophilization formulation of any one of claims 1 to 28, the lyophilate power of any one of claims 29 to 45, the reconstituted formulation of 46 to 52, or the vial of 53, wherein the heterologous moiety comprises a polypeptide or a non-polypeptide moiety.~~

~~58. The pre-lyophilization formulation of any one of claims 1 to 28, the lyophilate power of any one of claims 29 to 45, the reconstituted formulation of 46 to 52, or the vial of 53, wherein the moiety extending half life of FIX comprises an FcRn binding partner or an Fc region.~~

~~59. The pre-lyophilization formulation of any one of claims 1 to 28, the lyophilate power of any one of claims 29 to 45, the reconstituted formulation of 46 to 52, or the vial of 53, wherein~~

the FIX polypeptide is at least about 80%, at least about 85%, at least about 90%, at least about 95%, or 100% identical to SEQ ID NO: 2.

~~60-9.~~ A kit comprising a first container comprising the lyophilate ~~power of any one of claims 29 to 45~~ of claim 7 and a second container comprising a reconstitution buffer at a volume sufficient to produce a reconstituted formulation, when combined with the lyophilization formulation of the first container.

~~61. The kit~~ 10. A composition comprising the lyophilate of claim 60, which is used 7, for reconstituting to treat hemophilia B.

~~62. The kit of claim 60~~ prepare a pharmaceutical composition for preventing or 61, wherein the reconstitution buffer comprises NaCl.

~~63. A method of administering a FIX polypeptide to a hemophilia B patient in need thereof, comprising administering to the patient the reconstituted formulation of any one of 46 to 52, wherein the administration prevents or reduces~~ reducing the frequency or severity of bleeding episodes in ~~the patient.~~

~~64. A method of preventing, treating, ameliorating, or managing hemophilia B in a patient in need thereof by administering the reconstituted formulation of any one of claims 46 to 52.~~

~~65. A method of producing a lyophilate powder comprising a FIX polypeptide comprising lyophilizing the pre-lyophilization formulation of any one of claims 1 to 28.~~

~~66. A method of lyophilizing a FIX polypeptide comprising:~~

~~(a) a "freezing step" comprising freezing a pre-lyophilization formulation comprising the FIX polypeptide and an aqueous solvent;~~

~~(b) a "vacuum step" comprising reducing the pressure of the frozen pre-lyophilization formulation by an amount effective to remove the aqueous solvent from the frozen pre-lyophilization formulation; and,~~

~~(c) a single "drying step" comprising increasing the temperature of the frozen pre-lyophilization formulation above the collapse temperature,~~

~~thereby producing a lyophilate powder.~~

~~67. The method of claim 66, wherein the collapse temperature is about -1.5 °C.~~

~~68. The method of claim 66 or 67, wherein the pre-lyophilization formulation is frozen to a freezing temperature of about -65 to about -40°C during the freezing step.~~

~~69. The method of any one of claims 66 to 68, wherein the pre-lyophilization formulation is frozen to a freezing temperature of about -55°C during the freezing step.~~

~~70. The method of any one of claims 66 to 69, wherein the freezing temperature is ramped down from about 5°C to about -55°C during the freezing step.~~

~~71. The method of any one of claims 66 to 70, wherein the freezing temperature is held from about 30 minutes to about 5 hours during the freezing step.~~

~~72. The method of any one of claims 66 to 71, wherein the freezing temperature is held for about 2 hours during the freezing step.~~

~~73. The method of any one of claims 66 to 72, wherein the frozen pre-lyophilization formulation of step (a) is further subject to an "annealing step" (a') prior to the "vacuum step" (b).~~

~~74. The method of claim 73 wherein the temperature of the frozen pre-lyophilization formulation of step (a) is ramped up to an annealing temperature of about -15°C to about -2°C during the annealing step.~~

~~75. The method of claim 74, wherein the temperature of the frozen pre-lyophilization formulation of step (a) is ramped up to an annealing temperature of about -6°C during the annealing step.~~

~~76. The method of any one of claims 73 to 75, wherein the annealing temperature is held for about 30 minutes to about 5 hours during the annealing step.~~

~~77. The method of claim 76, wherein the annealing temperature is held for about 3 hours during the annealing step.~~

~~78. The method of any one of claims 73 to 77, wherein the temperature of the frozen pre-lyophilization formulation is ramped down from the annealing temperature to a temperature of about -65°C to about -40°C during the annealing step.~~

~~79. The method of claim 78, wherein the temperature of the frozen pre-lyophilization formulation is ramped down from the annealing temperature to a temperature of -55°C during the annealing step.~~

~~80. The method of any one of claims 66 to 79, wherein the "vacuum step" comprises subjecting the frozen pre-lyophilization formulation to a vacuum between about 0.05 and about 1 mbar.~~

- ~~81. The method of claim 80, wherein the vacuum in the "vacuum step" is about 0.33 mbar.~~
- ~~82. The method of any one of claims 66 to 81, wherein the vacuum is held in the "vacuum step" for about 2 hours.~~
- ~~83. The method of any one of claims 66 to 82, wherein the "drying step" comprises ramping up the temperature of the frozen pre-lyophilization formulation from about -55°C to a drying temperature of about 40°C.~~
- ~~84. The method of any one of claims 66 to 83, wherein the drying step further comprises holding the drying temperature for about 10 hours to about 40 hours.~~
- ~~85. The method of claim 84, wherein the drying temperature is held for about 25 hours.~~
- ~~86. The method of any one of claims 66 to 85, wherein the drying step is carried out at a pressure of about 0.05 mbar to about 1 mbar.~~
- ~~87. The method of claim 86, wherein the pressure is held at about 0.33 mbar during the drying step.~~
- ~~88. A method of producing a lyophilate powder comprising a FIX polypeptide, comprising:~~
- ~~(a) a "freezing step" comprising freezing a pre-lyophilization formulation comprising a FIX polypeptide by ramping down the temperature for about 2 hours to a freezing temperature of about -55°C, and holding the freezing temperature for about 2 hours; (a') an "annealing step" comprising ramping up for about 1.5 hours the temperature of the frozen pre-lyophilization formulation of step (a) to an annealing temperature of about -6°C, holding the annealing temperature for about 3 hours, and ramping down the temperature for about 1.5 hours to about -55°C;~~
- ~~(b) a "vacuum step" comprising holding the frozen pre-lyophilization formulation of step (a') at about -55°C for two hours at atmospheric pressure and ramping down the pressure for about 2 hours to about 0.33 mbar; and,~~
- ~~(c) a single "drying step" comprising ramping up for 3 hours the temperature of the frozen pre-lyophilization formulation of step (b) to about 40°C, while holding the pressure at about 0.33 mbar, and holding the temperature of the frozen pre-lyophilization formulation at about 40°C for about 25 hours, while holding the pressure at about 0.33 mbar,~~
- ~~thereby producing the lyophilate powder.~~
- ~~89. The method of any one of claims 66 to 88, wherein the pre-lyophilization formulation is the formulation of any one of claims 1 to 28.~~

90. The method of any one of claims 65 to 89, wherein the lyophilate powder has one or more characteristics selected from the group consisting of:

(1) improved stability of the FIX polypeptide when lyophilized;

(2) reduced reconstitution time when lyophilized;

(3) reduced splashing onto a stopper comprising the formulation;

(4) reduced lyophilization cycle time;

(5) increased shelf life of a lyophilate prepared from the pre-lyophilization formulation at room temperature; or

(6) any combinations thereof, 91. The method of any one of claims 65 to 90, wherein the pre-lyophilization formulation is in a fill volume of less than 5 mL.

92. The method of claim 91, wherein the fill volume of the pre-lyophilization formulation is about 4 mL, about 3.5 mL, about 3.0 mL, about 2.9 mL, about 2.8 mL, about 2.7 mL, about 2.65 mL, about 2.6 mL, about 2.5 mL, about 2.4 mL, about 2.3 mL, about 2.2 mL, about 2.1 mL, or about 2.0 mL.

93. The method of claim 91 or 92, wherein the fill volume of the pre-lyophilization formulation is about 2.65 mL.

94. The method of any one of claims 90 to 93, wherein the reduced reconstitution time is less than 1.5 minutes, less than 1 minute, less than 50 seconds, less than 40 seconds, less than 30 seconds, less than 20 seconds, or less than 10 seconds.

95. The method of any one of claims 90 to 94, wherein the reduced reconstitution time is less than 30 seconds.

96. The method of any one of claims 90 to 95, wherein the reduced lyophilization cycle time is about 4 days or less, about 3 days or less, about 2 days or less, or about a day or less.

97. The method of any one of claims 66 to 96, wherein the pre-lyophilization formulation is aseptically filtered and aseptically filled into a vial prior to step (a).

98. The method of any one of claims 65 to 97, wherein the lyophilate powder is produced from the pre-lyophilization formulation in about 45 hours or less.

99. The method of any one of claims 65 to 98, wherein the residual moisture in the lyophilate powder is less than 0.7%.

~~100. The method of any one of claims 65 to 99, wherein the residual moisture in the lyophilate powder is about 0.5%.~~

~~101. A method of stabilizing a lyophilate powder comprising a FIX polypeptide, comprising lyophilizing a pre-lyophilization formulation according to the method of any one of claims 65 to 100, wherein the lyophilate powder is stabilized as measured by Size Exclusion Chromatography (SEC) with respect to a lyophilate powder prepared by using a lyophilization method comprising more than one drying step.~~

~~102. A method of increasing the shelf life of a lyophilate powder comprising a FIX polypeptide, comprising lyophilizing a pre-lyophilization formulation according to the method of any one of claims 65 to 100, wherein the shelf life of the lyophilate powder is increased as measured by SEC and/or FIX clotting activity assay with respect to the shelflife of a lyophilate powder prepared by using a lyophilization method comprising more than one drying step.~~

~~103. A method to decrease the reconstitution time of a lyophilate powder comprising a FIX polypeptide, comprising lyophilizing a pre-lyophilization formulation according to the method of any one of claims 65 to 100, wherein the reconstitution time of the lyophilate powder is decreased with respect to the reconstitution time of a lyophilate powder prepared by using a lyophilization method comprising more than one drying step.~~

~~104. A method to reduce lyophilization process time of producing a lyophilate powder comprising a FIX polypeptide, comprising lyophilizing a pre-lyophilization formulation according to the method of any one of claims 65 to 100, wherein the lyophilization process time of the pre-lyophilization formulation is reduced with respect to the lyophilization process time of producing a lyophilate powder using a lyophilization method comprising more than one drying steps~~a hemophilia B patient.