

ensure product stability, yet storage at -80°C impacts supply chain and distribution.

Crystallization of water during slow freezing can result in concentration of excipients which can impact the stability of biologics. Phase separation or pH shifts may also occur which can impact the stability of biologics. For the commercialization of any pharmaceutical product, it would be advantageous to identify formulations that offer stability for extended periods of time. It would be further advantageous to identify formulations that are stable under frozen storage at -20°C to account for freezer temperature excursions, variability, or temporary storage (up to 18 months) in a -20°C freezer, refrigerated conditions to allow for short-term storage (up to 12 months at 2-8°C) at the clinic before dosing, at room temperature to allow for manufacturing and labelling, or under multiple free-thaw cycles to allow for thawing of drug substance and drug product for filling and labelling operations.

2. SUMMARY OF THE INVENTION

[0006] The disclosure provides a pharmaceutical composition comprising a recombinant adeno-associated virus (AAV), buffering agent, ionic salt, sucrose, and surfactant such as poloxamer 188. Sucrose is provided at a concentration that prevents crystallization of the composition and maintains a pH between 6 and 9 during frozen and liquid states.

[0006a] In one aspect, provided herein is a pharmaceutical composition comprising an AAV8 viral vector, a salt excipient or buffer agent, a sugar and a surfactant, wherein said composition is stable after freeze and thaw, and after said freeze and thaw, a single dose of said composition is effective in treating a retinal disease in an eye of a human subject upon administration to said eye, and wherein said AAV8 viral vector comprises (1) AAV2 inverted terminal repeats that flank an expression cassette, (2) (a) a CB7 promoter comprising a CMV enhancer/chicken β -actin promoter, (b) a chicken β -actin intron, and (c) a rabbit β -globin poly A signal, and (3) nucleic acid sequences coding for a heavy chain and a light chain of anti-VEGF antigen-binding fragment, separated by a self-cleaving furin (F)/F2A linker, ensuring expression of equal amounts of the heavy and light chain polypeptides in said eye upon said administration.

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[0006b] In one aspect, provided herein is a use of the pharmaceutical composition as described herein in the manufacture of a medicament for the treatment of Neovascular Age-related Macular Degeneration (nAMD), dry AMD, retinal vein occlusion (RVO), diabetic macular edema (DME), or diabetic retinopathy (DR) in said human subject.

[0007] In some embodiments, the AAV comprises components from one or more adeno-associated virus serotypes selected from the group consisting of AAV1, AAV2, AAV2tYF, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAVrh10, AAV.rh20, AAV.rh39, AAV.Rh74, AAV.RHM4-1, AAV.hu37, AAV.Anc80, AAV.Anc80L65, rAAV.7m8, AAV.PHP.B, AAV.PHP.eB, AAV2.5, AAV2tYF, AAV3B, AAV.LK03, AAV.HSC1, AAV.HSC2, AAV.HSC3, AAV.HSC4, AAV.HSC5, AAV.HSC6, AAV.HSC7, AAV.HSC8, AAV.HSC9, AAV.HSC10, AAV.HSC11, AAV.HSC12, AAV.HSC13, AAV.HSC14, AAV.HSC15, or AAV.HSC16. In some embodiments, the rAAV comprises a capsid protein of the AAV8 or AAV9 serotype.

[0008] In some embodiments, the pharmaceutical composition further comprises an amino acid.

[0009] In some embodiments, the disclosure provides a pharmaceutical composition comprises a recombinant adeno-associated virus (AAV), ionic salt excipient or buffering agent, sucrose, and poloxamer 188. In some embodiments, the ionic salt excipient or buffering agent

WHAT IS CLAIMED:

1. A pharmaceutical composition comprising an AAV8 viral vector, a salt excipient or buffer agent, a sugar and a surfactant, wherein said composition is stable after freeze and thaw, and after said freeze and thaw, a single dose of said composition is effective in treating a retinal disease in an eye of a human subject upon administration to said eye, and wherein said AAV8 viral vector comprises (1) AAV2 inverted terminal repeats that flank an expression cassette, (2) (a) a CB7 promoter comprising a CMV enhancer/chicken β -actin promoter, (b) a chicken β -actin intron, and (c) a rabbit β -globin poly A signal, and (3) nucleic acid sequences coding for a heavy chain and a light chain of anti-VEGF antigen-binding fragment, separated by a self-cleaving furin (F)/F2A linker, ensuring expression of equal amounts of the heavy and light chain polypeptides in said eye upon said administration.
2. The pharmaceutical composition of claim 1, wherein said heavy chain comprising SEQ ID NO: 2, and said light chain comprises SEQ ID NO: 1 .
3. The pharmaceutical composition of claim 1 or 2, wherein said sugar is sucrose.
4. The pharmaceutical composition of claim 1-3, comprising both said salt excipient and said buffer agent.
5. The pharmaceutical composition of claim 1-4, wherein said salt excipient comprises potassium chloride and sodium chloride, and said buffer agent is phosphate buffer.
6. The pharmaceutical composition of claim 1-5, wherein the ionic strength of the pharmaceutical composition is in a range from 60 mM to 115 mM.
7. The pharmaceutical composition of claim 5 , wherein said potassium chloride is at a concentration of about 2.7 mmol/L, said sodium chloride is at a concentration of about 100 mmol/L, and said phosphate buffer comprises potassium

phosphate monobasic at a concentration of about 1.47 mmol/L and sodium phosphate dibasic at a concentration of about 8.10 mmol/L.

8. The pharmaceutical composition of claim 1-7, wherein said sugar is at a concentration in a range from 3% (weight/volume, 30 g/L) to 18% (weight/volume, 180 g/L).
9. The pharmaceutical composition of claim 1-7, wherein said sugar is at a concentration of 4% (weight/volume, 40 g/L or about 117 mmol/L).
10. The pharmaceutical composition of claim 1-9, wherein said surfactant comprises poloxamer 188, polysorbate 20, or polysorbate 80 and is at a concentration in a range from 0.0005% (weight/volume, 0.005 g/L) to 0.05% (weight/volume, 0.5 g/L) or at a concentration of 0.001% (weight/volume, 0.01 g/L).
11. The pharmaceutical composition of claim 1-10, wherein the pH of the pharmaceutical composition is in a range from 6.9 to 7.9.
12. The pharmaceutical composition of claim 11, wherein the pH of the pharmaceutical composition is 7.4.
13. The pharmaceutical composition of claim 1-12, wherein the osmolality of the pharmaceutical composition is in a range from 295 mOsm/kg H₂O to 395 mOsm/kg H₂O.
14. The pharmaceutical composition of claim 1 or 2, wherein the pharmaceutical composition comprises:
 - (b) potassium chloride at a concentration of about 2.7 mmol/L,
 - (c) potassium phosphate monobasic at a concentration of about 1.47 mmol/L,
 - (d) sodium chloride at a concentration of about 100 mmol/L,
 - (e) sodium phosphate dibasic at a concentration of about 8.1 mmol/L,
 - (f) sucrose at a concentration of about 117 mmol/L,
 - (g) poloxamer 188, polysorbate 20, or polysorbate 80 at a concentration of 0.001%

weight/volume (0.01 g/L), and

(h) water.

15. The pharmaceutical composition of claim 1-14, wherein the AAV8 viral vector is at a concentration of about 3×10^9 GC/mL, about 1×10^{10} GC/mL, about 1.2×10^{10} GC/mL, about 1.6×10^{10} GC/mL, about 4×10^{10} GC/mL, about 6×10^{10} GC/mL, about 2×10^{11} GC/mL, about 2.4×10^{11} GC/mL, about 2.5×10^{11} GC/mL, about 3×10^{11} GC/mL, about 3.2×10^{11} GC/mL, about 6.2×10^{11} GC/mL, about 6.5×10^{11} GC/mL, about 1×10^{12} GC/mL, about 3×10^{12} GC/mL, about 2×10^{13} GC/mL or about 3×10^{13} GC/mL.
16. The pharmaceutical composition of claim 1-15, wherein each of the light chain and the heavy chain comprises an exogenous leader sequence comprising the amino acid sequence of SEQ ID NO: 52.
17. Use of a pharmaceutical composition according to any one of claims 1 to 16 in the manufacture of a medicament for the treatment of Neovascular Age-related Macular Degeneration (nAMD), dry AMD, retinal vein occlusion (RVO), diabetic macular edema (DME), or diabetic retinopathy (DR) in said human subject .
18. The use of claim 17, wherein the pharmaceutical composition is suitable for suprachoroidal or subretinal injection.
19. The use of any one of claims 17-18 , wherein the treatment comprises:
 - (i) preparing the pharmaceutical composition, storing the pharmaceutical composition at -60 °C or below for a first period of time; and
 - (ii) thawing the pharmaceutical composition;wherein the first period of time is up to: about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, about 11 months, about 12 months, about 15 months, about 18 months, or about 24 months.
20. The use of claim 19, further comprising storing the thawed pharmaceutical composition at 2-

8 °C for a second period of time, the second period of time is up to: about 1 week, about 2 weeks, about 3 weeks, about 4 weeks, about 1 month, or about 2 months.

21. The pharmaceutical composition of claim 1-16, wherein said single dose comprises at least 0.2 mL of the pharmaceutical composition.
22. The pharmaceutical composition of claim 1-16, wherein said single dose comprises at least 0.8 mL of the pharmaceutical composition.
23. The pharmaceutical composition of claim 1-16, wherein said single dose comprises about 0.95 mL of the pharmaceutical composition.