HIGH CONCENTRATION VEGF RECEPTOR FUSION PROTEIN CONTAINING FORMULATIONS

[001] This application claims the benefit of U.S. Provisional Patent Application No. 62/813,882, filed March 5, 2019; U.S. Provisional Patent Application No. 62/769,876, filed November 20, 2018; U.S. Provisional Patent Application No. 62/752,127, filed October 29, 2018; and U.S. Provisional Patent Application No. 62/669,506, filed May 10, 2018; each of which is herein incorporated by reference in its entirety.

TECHNICAL FIELD

[002] Embodiments herein relate generally to high concentration VEGF receptor fusion protein containing formulations suitable for ocular administration. More particularly, embodiments herein provide liquid pharmaceutical formulations for intravitreal administration, where the formulations include more than 40 mg/ml VEGF receptor fusion protein, and show pharmaceutically acceptable potency, stability, viscosity and pH.

SEQUENCE LISTING

[003] An official copy of the sequence listing is submitted concurrently with the specification electronically via EFS-Web as an ASCII formatted sequence listing with a file name of "10430P1-US_SEQ_LIST_ST25.txt", a creation date of May 9, 2018, and a size of about 7 KB. The sequence listing contained in this ASCII formatted document is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[004] The development of a therapeutically useful liquid formulation requires a combination of ingredients, at differing amounts, to provide a functional and stable delivery vehicle for a drug of interest. This is particularly true where the drug is a protein, and furthermore, an antibody. Antibody formulations have long presented challenges to drug developers and manufacturers, as antibody activity and administration typically require a pharmaceutically acceptable potency, osmolality, protein stability, viscosity, and proper pH. These challenges are exacerbated when the antibody is at higher concentrations in the formulation, for example, beyond 20 to 40 mg/ml, the concentration of most pharmaceutically sold antibodies.

[005] Higher concentration antibody formulations allow for shorter injection times, smaller injection volumes, lower frequency of antibody administration, and more efficient manufacturing and storage utility. However, as noted above, the higher the antibody or protein concentration, the more difficult it is to maintain the proper activity and delivery parameters for the formulation. In particular, high concentration antibody and protein

formulations often contend with increased protein aggregation and viscosity, which results in lower overall antibody or protein potency, and lower manufacturing and poorer storage stability. Because of the issues related to high concentration protein or antibody formulations, few such pharmaceutically acceptable formulations have been developed. There is a need in the art for preparing high concentration, high stability, antibody and protein formulations that include the proper potency, stability, viscosity, osmolality and pH.

[006] One such protein in need of high concentration formulations is the vascular endothelial growth factor (VEGF) receptor fusion protein. VEGF receptor fusion proteins are used to block VEGF function in a number of ophthalmic formulations, for example, EYLEA® (Regeneron Pharmaceuticals, Inc.). High concentration VEGF receptor fusion protein containing formulations could allow for shorter ocular injection times, smaller injection volumes, fewer possible injections per administration cycle, and more efficient manufacturing and storage.

[007] The present invention is directed toward overcoming one or more of the problems discussed above.

SUMMARY OF THE INVENTION

[008] Various embodiments described herein encompass high concentration protein containing formulations (*e.g.*, which are suitable for intravitreal administration), and in particular, high concentration vascular endothelial growth factor (VEGF) receptor fusion protein containing formulations. High concentration VEGF receptor fusion protein containing formulations provide a number of therapeutic and economic benefits, including, pharmaceutically acceptable potency, long term manufacturing and storage stability, and a viscosity and pH compatible with ocular injection. High concentration VEGF receptor fusion protein containing formulations also allow for reduced ocular administration volumes, a benefit for avoiding undesired effects on the limited volume of the eye.

[009] Embodiments herein provide formulations having a VEGF receptor fusion protein, a buffer, a thermal stabilizer, a viscosity reducing agent, and a surfactant. In other embodiments, the formulations do not include a viscosity reducing agent. Formulations of the present invention have a pH and viscosity suitable for injection, and in particular, for therapeutic ocular injection.

[0010] In an embodiment of the invention, a pharmaceutical formulation of the present invention is provided having a VEGF receptor fusion protein at a concentration of at least 41 mg/ml or a concentration that contains a single dose of VEGF receptor fusion protein (which is discussed herein) in less than about 100 µl, less than about 50 µl, about 50 µl, about 57 µl, about 60 µl, about 70 µl, or about 75 µl, a buffer, optionally a thermal stabilizer and/or a

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viscosity reducing agent, and a surfactant, where the formulation has a pH of from about 5.0 to about 6.8 (*e.g.*, 5.8).

[0011] In an embodiment of the invention, the VEGF receptor fusion protein concentration is about 30 mg/ml, 60 mg/ml, 114 mg/ml, 120 mg/ml or 140 mg/ml.

[0012] In an embodiment of the invention, the surfactant can be a non-ionic surfactant (*e.g.*, about 0.02% to about 0.1%; or about 0.03% (w/v)). In an embodiment of the invention, the surfactant is a non-ionic surfactant having a polyoxyethylene moiety, for example, polysorbate 20 (PS20), polysorbate 80 (PS80), poloxamer 188, polyethylene glycol 3350 or mixtures thereof.

[0013] In an embodiment of the invention, the buffer is a histidine-based buffer (*e.g.*, 10 mM or 20 mM), such as histidine, histidine HCl or histidine acetate; a phosphate-based buffer, such as sodium phosphate (*e.g.*, 10 mM); an acetate-based buffer, such as sodium acetate and acetic acid, or a citrate-based buffer, such as sodium citrate or citric acid. In an embodiment of the invention, if the buffer is phosphate buffer, the pH is from about 5.7 to about 8.0, about 5.8 to about 8.0, about 5.7 to about 7.0, about 5.8 to about 7.0, about 5.9 to about 7.0 or about 6.0 to about 7.0; if the buffer is histidine buffer, the pH is from about 5.5 to about 6.5; if the buffer is citrate buffer, the pH is from about 3.0 to about 6.2 or about 5.0 to about 6.0; and if the buffer is acetate buffer, the pH is from about 3.7 to about 5.6 or about 5.0 to about 6.0.

[0014] In an embodiment of the invention, the thermal stabilizer is a sugar, such as sucrose (*e.g.*, about 2.5%, 5% or 8%, 10% or 20% (w/v), *e.g.*, about 2-20%), mannitol, sorbitol, or trehalose; L-proline (*e.g.*, about 2%, 3% or 4% (w/v)); glycine (*e.g.*, about 50 mM), glycerol, taurine (*e.g.*, about 50 mM) or propane sulfonic acid (*e.g.*, about 50 mM) or any combination of the above.

[0015] In some embodiments of the invention, the pharmaceutical formulations of the present invention include a viscosity reducing agent, for example, arginine hydrochloride (*e.g.*, L-arginine monohydrochloride) (*e.g.*, 50 mM), lysine, sodium chloride (*e.g.*, 40 mM or 50 mM), or magnesium chloride. Alternatively, in other embodiments, the formulations of the present invention specifically exclude substantially all, if not all, viscosity reducing agents.

[0016] In an embodiment of the invention, the pharmaceutical formulations of the present invention comprise, consists of or consists essentially of the constituents in any one of formulations A-KKKK as set forth herein.

[0017] In aspects herein, the pharmaceutical formulations of the present invention may include a VEGF receptor fusion protein (*e.g.*, aflibercept or conbercept) at a concentration of from about 41 mg/ml to about 275 mg/ml, from about 80 mg/ml to about 275 mg/ml, about 140 mg/ml to about 150 mg/ml, about 140 mg/ml to about 159 or 160 mg/ml; including about 60 mg/ml, about 80 mg/ml, about 100 mg/ml, about 113.3 mg/ml, about 114.3 mg/ml, about

120 mg/ml, about 133.3 mg/ml, about 140 mg/ml, about 150 mg/ml, about 200 mg/ml or about 250 mg/ml. In an embodiment of the invention, the formulation comprises about 40 mg/ml of the VEGF receptor fusion protein.

[0018] Some aspects of the pharmaceutical formulations of the present invention include a thermal stabilizer at a concentration of from about 2% (w/v) to about 9% (w/v) or from about 4% (w/v) to about 9% (w/v), with the proviso, that when the thermal stabilizer is taurine or propane sulfonic acid, the stabilizer is at a concentration of from about 25 mM to about 100 mM, surfactant is at a concentration of from about 0.02% (w/v) to about 0.1% (w/v) (*e.g.*, about 0.03%), and buffer is of from about 5 mM to about 15 mM.

[0019] The VEGF receptor fusion protein can, for example:

- be encoded by the nucleic acid sequence of SEQ ID NO:1 or nucleotides 79-1374 or 79-1371 of SEQ ID NO: 1;
- comprise amino acids of SEQ ID NO: 2 or amino acids 27-457 or 27-458 of SEQ ID NO: 2;
- comprise:
  (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2;
  (2) a VEGFR2 component comprising amino acids 130 to 231 of SEQ ID NO:2;
  (3) a multimerization component ("FcΔC1(a)") comprising amino acids 232 to 457 or 458 of SEQ ID NO:2 (the C-terminal amino acids of SEQ ID NO:2, *i.e.*, K458, may or may not be included in the VEGF receptor fusion proteins). Note that amino acids 1 to 26 of SEQ ID NO:2 are the signal sequence;
- comprise an immunoglobin-like (Ig) domain 2 of a first VEGF receptor (*e.g.*, VEGFR1) and Ig domain 3 of a second VEGF receptor (*e.g.*, VEGFR2), optionally further including an Ig domain 4 of the second VEGF receptor (*e.g.*, VEGFR2) and a multimerizing component (*e.g.*, Fc domain of IgG);
- be conbercept; or
- be aflibercept.

VEGF Trap

atggtcagctactgggacaccggggtcctgctgtgcgcgctgctcagctgtctgcttctc
acaggatctagttccggaagtgataccggtagacctttcgtagagatgtacagtgaaatc
cccgaaattatacacatgactgaaggaagggagctcgtcattccctgccgggttacgtca
cctaacatcactgttactttaaaaaagtttccacttgacactttgatccctgatggaaaa
cgcataatctgggacagtagaaagggcttcatcatatcaaatgcaacgtacaaagaaata
gggcttctgacctgtgaagcaacagtcaatgggcatttgtataagacaaactatctcaca
catcgacaaaccaatacaatcatagatgtggttctgagtccgtctcatggaattgaacta
tctgttggagaaaagcttgtcttaaattgtacagcaagaactgaactaaatgtggggatt

gacttcaactgggaataccttcttcgaagcatcagcataagaaacttgtaaaccgagac
ctaaaaacccagtctgggagtgagatgaagaaatttttgagcaccttaactatagatggt
gtaacccggagtgaccaaggattgtacacctgtgcagcatccagtgggctgatgaccaag
aagaacagcacatttgtcagggtccatgaaaaggacaaaactcacacatgcccaccgtgc
ccagcacctgaactcctgggggggaccgtcagtcttcctcttcccccaaaacccaaggac
accctcatgatctcccggacccctgaggtcacatgcgtggtggtggacgtgagccacgaa
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aactacaagaccacgcctcccgtgctggactccgacggctccttcttcctctacagcaag
ctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatgctccgtgatgcat
gaggctctgcacaaccactacacgcagaagagcctctccctgtctccgggtaaatga
(SEQ ID NO: 1)

MVSYWDTGVLLCALLSCLLLTGSSSGSDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNI
TVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNTII
DVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEM
KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK
(SEQ ID NO: 2)

AAs 1-26 = Signal Sequence

AAs 27-129 = Flt1-D2 (VEGFR1-D2)

AAs 130-231 = Flk1-D3 (VEGFR2-D3)

AAs 232-458 = FcΔC1

[0020] In another embodiment of the present invention, a container containing an aqueous solution of from about 5 mM to about 25 mM pharmaceutically acceptable buffer, from about 4% (w/v) to about 9% (w/v) of a pharmaceutically acceptable thermal stabilizer, from about 0.02% (w/v) to about 0.1% (w/v) of a pharmaceutically acceptable surfactant, and from about 41 mg/ml to about 275 mg/ml VEGF receptor fusion protein, is provided. When the thermal stabilizer includes either taurine or propane sulfonic acid, the taurine or propane sulfonic

acid is at a concentration of from about 25 mM to about 100 mM. The aqueous solution has a pH of from about 5.0 to about 6.8, and can be from about 5.5 to about 6.2 (*e.g.*, 5.8). In some aspects, the container is a vial or syringe. In other aspects, the aqueous solution does not include an inorganic salt. When the thermal stabilizer is a sugar it can be sucrose, mannitol, sorbitol or trehalose, and can be present at between about 4% (w/v) and 9% (w/v); the surfactant can be a non-ionic surfactant, and can include a polyoxyethylene moiety, such as polysorbate 20, polysorbate 80, poloxamer 188, or polyethylene glycol 3350, at a concentration of from about 0.02% to about 0.1% weight per volume (w/v), and more typically, 0.02% to about 0.04% (w/v). In an embodiment of the invention, the VEGF receptor fusion protein is encoded by the nucleic acid sequence of SEQ ID NO:1 or nucleotides 79-1374 or 79-1371 of SEQ ID NO: 1; comprises amino acids of SEQ ID NO: 2 or amino acids 27-457 or 27-458 of SEQ ID NO: 2; comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130 to 231 of SEQ ID NO:2; and (3) a multimerization component ("FcΔC1(a)") comprising amino acids 232 to 457 of SEQ ID NO:2 (the C-terminal amino acids of SEQ ID NO:2, *i.e.*, K458, may or may not be included in the VEGF receptor fusion proteins); comprises an immunoglobin-like (Ig) domain 2 of a first VEGF receptor (*e.g.*, VEGFR1) and Ig domain 3 of a second VEGF receptor (*e.g.*, VEGFR2), optionally further including an Ig domain 4 of the second VEGF receptor (*e.g.*, VEGFR2) and a multimerizing component (*e.g.*, Fc domain of IgG); is conbercept or is aflibercept.

[0021] In still other embodiments, pharmaceutical formulations of the present invention are provided having a VEGF receptor fusion protein in an aqueous vehicle, where the aqueous vehicle has a viscosity of from about 10 cP to about 15 cP at 20^{0}C, more typically about 10 cP to about 13 cP at 20^{0}C, and most typically from about 11 cP to about 12 cP at 20^{0}C (*e.g.*, about 6.0, 7.3, 11.5 or 12.0 cP at 20°C). In an embodiment of the invention, the viscosity is about 12 cP to about 15 cP at 20^{0}C. The pH of the formulation can be from about 5.8 to about 6.5 (*e.g.*, about 5.8). In some cases, the formulations do not include a viscosity reducing agent, for example, do not include arginine hydrochloride, lysine, sodium chloride, or magnesium chloride. In other cases, the formulations include 10 mM histidine hydrochloride or 10 mM histidine-acetate. The sugar can be sucrose, mannitol, sorbitol or trehalose, and can be present at between about 4% (w/v) and 9% (w/v) (*e.g.*, about 5%); the surfactant can be a non-ionic surfactant, and can include a polyoxyethylene moiety, such as polysorbate 20, polysorbate 80, poloxamer 188, or polyethylene glycol 3350, at a concentration of from about 0.02% to about 0.1% weight per volume (w/v), and more typically, 0.02% to about 0.04% (w/v) (*e.g.*, 0.03%). In addition, the formulation can additionally include a thermal stabilizer selected from either taurine or propane sulfonic acid at a concentration of between about 25 mM to about 100 mM, and more typically about, 50

mM to about 70 mM. In an embodiment of the invention, a formulation comprises VEGF receptor fusion protein, such as aflibercept, (*e.g.*, at a concentration of about 41-275 mg/ml, about 50 mg/ml, about 100 mg/ml, about 115 mg/ml, about 125 mg/ml, about 150 mg/ml, about 200 mg/ml or any of the "high" concentrations discussed herein), about 10 mM histidine-based buffer, about 5% (w/v) sucrose, about 0.03% (w/v) non-ionic surfactant such as polysorbate *e.g.*, polysorbate 20, and about 50 mM arginine, L-arginine or L-arginine monohydrochloride, with a pH of about 5.8. In an embodiment of the invention, the VEGF receptor fusion protein is encoded by the nucleic acid sequence of SEQ ID NO:1 or nucleotides 79-1374 or 79-1371 of SEQ ID NO: 1; comprises amino acids of SEQ ID NO: 2 or amino acids 27-457 or 27-458 of SEQ ID NO: 2; comprises (1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2; (2) a VEGFR2 component comprising amino acids 130 to 231 of SEQ ID NO:2; and (3) a multimerization component ("FcΔC1(a)") comprising amino acids 232 to 457 of SEQ ID NO:2 (the C-terminal amino acids of SEQ ID NO:2, *i.e.*, K458, may or may not be included in the VEGF receptor fusion proteins); comprises an immunoglobin-like (Ig) domain 2 of a first VEGF receptor (*e.g.*, VEGFR1) and Ig domain 3 of a second VEGF receptor (*e.g.*, VEGFR2), optionally further including an Ig domain 4 of the second VEGF receptor (*e.g.*, VEGFR2) and a multimerizing component (*e.g.*, Fc domain of IgG); is conbercept or is aflibercept.

[0022] In yet another embodiment, a pharmaceutical formulation of the present invention is provided that includes a VEGF receptor fusion protein at a concentration of from about 80 mg/ml to about 275 mg/ml in a pharmaceutically acceptable buffer. In some cases, the stable pharmaceutical formulations can also include taurine or propane sulfonic acid. The formulations can be stable at a temperature of about 2^0C to about 8^0C for about 24-36 months. In some cases, the VEGF receptor fusion protein shows less than about a 5% increase in high molecular weight species, and more typically, less than about 4.5% increase in high molecular weight species, less than about 4.0% increase in high molecular weight species, less than about 3.5% increase in high molecular weight species, less than about 3.0% increase in high molecular weight species, less than about 2.5% increase in high molecular weight species, less than about 2.0 increase in high molecular weight species and/or less than about 1.5% increase in high molecular weight species, when stored at these temperatures and for this amount of time. In other cases, the VEGF receptor fusion protein shows less than a 2.0% to 3.0% increase in high molecular weight species when the formulations are stored at these temperatures and for this amount of time.

[0023] Embodiments herein can also include pharmaceutical formulations of the present invention that include aflibercept, a pH buffered solution, a sugar and a surfactant, where the aflibercept is at a concentration of 41 mg/ml to about 275 mg/ml. Alternatively, embodiments can be stable liquid pharmaceutical formulations that include conbercept, a pH buffered

solution, a sugar and a surfactant, where the conbercept is at a concentration of 41 mg/ml to about 275 mg/ml. Stable liquid pharmaceutical formulations can also include taurine or propane sulfonic acid. In some aspects, the aflibercept or conbercept is at a concentration of either 80 mg/ml or 150 mg/ml. In other aspects, the formulations do not include an inorganic salt, sodium chloride for example.

[0024] Some embodiments are directed to sterile syringes such that the syringes are pre-filled with an aqueous solution having 80 mg/ml aflibercept, conbercept, 10 mM histidine hydrochloride, histidine acetate or sodium phosphate, 5% (w/v) sucrose, mannitol, sorbitol or trehalose , 0.03% (w/v) polysorbate 20, polysorbate 80, poloxamer 188, or polyethylene glycol 3350, and 40 mM sodium chloride. Aspects herein include the further addition of about 25 mM to about 100 mM, and more typically about 50 mM to about 70 mM, of either taurine or propane sulfonic acid. In some aspects, the syringes are pre-filled with an aqueous solution having 80 mg/ml aflibercept, conbercept, 10 mM histidine HCl, histidine acetate or sodium phosphate, 8% (w/v) sucrose, mannitol, sorbitol or trehalose, and 0.03% (w/v) polysorbate 20, polysorbate 80, poloxamer 188, or polyethylene glycol 3350. Aspects herein include the further addition of about 25 mM to about 100 mM, and more typically about 50 mM to about 70 mM, of either taurine or propane sulfonic acid. In still other aspects, the syringes are pre-filled with an aqueous solution having 150 mg/ml aflibercept, conbercept, 10 mM histidine hydrochloride, histidine acetate, or sodium phosphate, 5% (w/v) sucrose, mannitol, sorbitol or trehalose, 0.03% (w/v) polysorbate 20, polysorbate 80, poloxamer 188, or polyethylene glycol 3350 and 40 mM sodium chloride, with a pH of about 6.2. Aspects herein can include the further addition of either taurine or propane sulfonic acid. In yet still other aspects, the syringes are pre-filled with an aqueous solution having 150 mg/ml aflibercept, conbercept, 10 mM histidine hydrochloride, histidine acetate, or sodium phosphate, 8% (w/v) sucrose, mannitol, sorbitol or trehalose, and 0.03% (w/v) polysorbate 20, polysorbate 80, poloxamer 188, or polyethylene glycol 3350, with a pH of about 6.2 and the aqueous solution does not include an inorganic salt. Aspects can further include about 25 mM to about 100 mM, and more typically, about 50 mM to about 70 mM, of either taurine or propane sulfonic acid. In yet still other aspects, the syringes are pre-filled with an aqueous solution having 150 mg/ml aflibercept or conbercept, 10 mM sodium acetate or acetic acid, 5% (w/v) glycerol and 0.03% (w/v) polysorbate 20, polysorbate 80, poloxamer 188, or polyethylene glycol 3350 and 40 mM sodium chloride, with a pH of about 6.2. In yet still other aspects, the syringes are pre-filled with an aqueous solution having 150 mg/ml aflibercept or conbercept, 10 mM sodium acetate or acetic acid, 8% (w/v) glycerol , and 0.03% (w/v) polysorbate 20, polysorbate 80, poloxamer 188, or polyethylene glycol 3350 and 40 mM sodium chloride, with a pH of about 6.2.

[0025] The present invention also provides a method for making any formulation set forth herein comprising the step of combining each component of the formulation into a single composition. Such a method may include the step of adding the resulting formulation into a vial or injection device. Any composition that is the product of such a method also forms part of the present invention. For example, embodiments herein also include methods for preparing a formulation by combining a histidine-based, citrate-based, acetate-based or phosphate-based buffer, with sucrose, polysorbate 20 and a VEGF receptor fusion protein; and, optionally, one or more additional components, *e.g.*, as discussed herein. In some cases, the formulation is prepared to also include taurine or propane sulfonic acid, but where it does not include taurine or propane sulfonic acid, it may also not include an inorganic salt. In aspects herein, the sucrose is at a weight per volume of from about 4% to about 10%, the polysorbate 20 has a weight per volume of about 0.02% to about 0.1%, and the receptor fusion protein is at a concentration of from about 41 mg/ml to about 275 mg/ml. Where the formulation includes taurine or propane sulfonic acid, it is present at, for example, about 25 mM to about 100 mM, but may also be included at 50 mM to 70 mM. The method can include loading a predetermined volume of prepared formulation into a sterile syringe, such that the volume has a 0.1 mg to 10 mg dose of the VEGF receptor fusion protein.

[0026] The present invention also provides a method for administering a formulation of the present invention to a subject (*e.g.*, a human) comprising intraocularly injecting (*e.g.*, intravitreal injection) the formulation into the eye of the subject. The present invention also provides a method for administering a formulation of the present invention to a subject (*e.g.*, a human) comprising implanting an intravitreal implant that contains a formulation of the present invention into the vitreous of the subject.

[0027] Embodiments herein also include methods for treating an angiogenic eye disorder, *e.g.*, age-related macular degeneration (wet), macular edema following retinal vein occlusion, retinal vein occlusion (RVO), central retinal vein occlusion (CRVO) branch retinal vein occlusion (BRVO), diabetic macular edema (DME), choroidal neovascularization (CNV), iris neovascularization, neovascular glaucoma, post-surgical fibrosis in glaucoma, proliferative vitreoretinopathy (PVR), optic disc neovascularization, corneal neovascularization, retinal neovascularization, vitreal neovascularization, pannus, pterygium, vascular retinopathy or diabetic retinopathy (*e.g.*, non-proliferative diabetic retinopathy and/or proliferative diabetic retinopathy) in a subject in need thereof by intraocularly injecting at least about 2 mg (*e.g.*, 4 mg, 6 mg or 8 mg) of VEGF receptor fusion protein (*e.g.*, aflibercept or conbercept) into the eye of a subject in need thereof, for example, any formulation set forth herein. In an embodiment of the invention, the injection volume is 100 microliters or less (*e.g.*, 100 microliters, 50 microliters or 57 microliters). The method comprises an intravitreal injection of a pre-mixed aqueous solution having a VEGF receptor

fusion protein at a concentration of from 41 mg/ml to about 275 mg/ml, a pharmaceutically acceptable sugar, a pharmaceutically acceptable buffer, and a pharmaceutically acceptable surfactant. Treatment methods using the pre-mixed aqueous solution do not require dilution. In aspects herein, the pre-mixed aqueous solution has a pH from about 6.0 and about 6.5, and has a viscosity of between about 10 cP and 13 cP.

[0028] In aspects of the treatment method, the VEGF receptor fusion protein comprises VEGFR1R2-FcΔC1(a) encoded by the nucleic acid sequence of SEQ ID NO:1, amino acids 27 to 129 of SEQ ID NO:2, a VEGFR2 component comprising amino acids 130 to 231 of SEQ ID NO:2, or a multimerization component comprising amino acids 232 to 457 of SEQ ID NO:2, or any combination thereof. In an embodiment of the invention, the VEGF receptor fusion protein is aflibercept or conbercept.

[0029] Other embodiments in accordance with the disclosure herein will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] The above and other aspects, features and advantages of embodiments disclosed herein will become more apparent to those of ordinary skill in the art with reference to the accompanying drawings.

[0031] **Figure 1A** is a graph determining VEGF receptor fusion protein (aflibercept) stability in four different formulations (B, H, D and G) over the course of 36 months at 2^0C to 8^0C. VEGF receptor fusion protein stability was tested using size-exclusion ultra-performance liquid chromatography (SE-UPLC) to identify formation of high molecular weight (HMW) species (a sign of protein degradation).

[0032] **Figure 1B** is also a graph determining VEGF receptor fusion protein (aflibercept) stability in four formulations (B, H, D and G) over the course of 36 months at 2^0C to 8^0C. VEGF receptor fusion protein stability was tested using SE-UPLC to identify the percent of the main species in each formulation.

[0033] **Figure 1C** is a bar graph showing formation of VEGF receptor fusion protein charge variants in four formulations (B, H, D and G) over the course of 36 months at 2^0C to 8^0C. The level of acidic species was tested at manufacture (0.0), 12 months, 24 months and 36 months using imaged capillary isoelectric focusing (iCIEF).

[0034] **Figure 1D** is a bar graph showing formation of VEGF receptor fusion protein charge variants in four formulations (B, H, D and G) over the course of 36 months at 2^0C to 8^0C. Identification of the main species was tested at manufacture (0.0), 12 months, 24 months and 36 months using iCIEF.

[0035] **Figure 2A** is a graph showing 150 mg/ml VEGF receptor fusion protein (aflibercept) stability in a 10 mM sodium phosphate, 8% (w/v) sucrose and 0.03% polysorbate 20 (w/v)

formulation pH6.2 with and without L-arginine monohydrochloride over the course of 28 days at 37°C. VEGF receptor fusion protein stability was tested using SE-UPLC to identify formation of HMW species.

[0036] **Figure 2B** is a graph showing 150 mg/ml VEGF receptor fusion protein stability in a 10 mM sodium phosphate, 8% sucrose (w/v) and 0.03% polysorbate 20 (w/v) formulation pH6.2 with and without L-arginine monohydrochloride over the course of 28 days at 37°C. VEGF receptor fusion protein stability was tested using SE-UPLC to identify the percent of the main species in each formulation.

[0037] **Figure 3A** is a bar graph showing viscosity of 10 mM sodium phosphate buffered formulations having 155 mg/ml VEGF receptor fusion protein and no salt, 100 mM arginine, 200 mM arginine, 50 mM lysine, 200 mM lysine, 50 mM sodium chloride or 100 mM sodium chloride. Viscosity was measured in cP at 20^0C.

[0038] **Figure 3B** is a bar graph showing viscosity of 10 mM histidine buffered formulations having 155 mg/ml VEGF receptor fusion protein and no salt, 100 mM arginine, 200 mM arginine, 50 mM lysine, 200 mM lysine, 50 mM sodium chloride or 100 mM sodium chloride. Viscosity was measured in cP at 20^0C.

[0039] **Figure 3C** is a graph showing viscosity of various concentrations of VEGF receptor fusion protein under different formulation conditions (with or without 50 mM arginine hydrochloride). Viscosity was measured in cP at 20^0C.

[0040] **Figure 4A** is a graph showing 150 mg/ml VEGF receptor fusion protein stability in 10 mM sodium phosphate, 8% (w/v) sucrose, and 0.03% (w/v) polysorbate 20, pH6.2 with and without 50 mM L-arginine monohydrochloride over 12 months at 5°C. The VEGF receptor fusion protein was at 150 mg/ml. VEGF receptor fusion protein stability was tested using SE-UPLC to identify formation of HMW species.

[0041] **Figure 4B** is a graph showing 150 mg/ml VEGF receptor fusion protein stability in 10 mM sodium phosphate, 8% (w/v) sucrose, and 0.03% (w/v) polysorbate 20, pH6.2 with and without 50 mM L-arginine monohydrochloride over 12 months at 5°C. The VEGF receptor fusion protein was at 150 mg/ml. VEGF receptor fusion protein stability was tested using SE-UPLC to identify the percent of the main species in each formulation.

[0042] **Figure 5** is a graph that shows viscosity of formulations under two buffer conditions at 20°C, 10 mM phosphate buffer or 10 mM histidine buffer. The VEGF receptor fusion protein concentration is varied from 10 mg/ml to 170 mg/ml. Viscosity was measured in mPa-S.

[0043] **Figure 6** shows a dynamic light scattering screen for a number of different formulation conditions, each having from 2 to 10 mg/ml protein. The diffusion interaction parameter indicated that taurine and PSA (propane sulfonic acid) improved Kd.

[0044] **Figure 7** is a graph monitoring the percentage of high molecular weight (HMW) species in formulations F1-F9 (formulations WW-EEE) over time as measured by size-exclusion ultra-performance liquid chromatography (SE-UPLC) after storage at 5°C for 6 months. Formulations F1-F9 are set forth in Table 7-1 herein.

[0045] **Figure 8 (A-B)** measures the percentage of high molecular weight species, by SE-UPLC, after storage at 5°C for 3 months (A) or incubation at 37°C for 28 days (B). Formulations F1-F4 (EEE, SSS, CCC (140 mg/ml) and TTT) are set forth in Table 8-1 below.

[0046] **Figure 9** shows the viscosity (cp) for Formulations F1-F12, at 20°C, at the initial time point. Formulations F1-F12 are set forth in Table A below.

Table A. Formulations F1-F12.

Formulation #	VEGF Trap (mg/mL)	Buffer	Other Excipients	Screening Excipients (mM)
F1 (GGG)	140	20 mM Histidine, pH 5.8	5% Sucrose, 0.03% Polysorbate 20	50mM Sodium sulfate
F2 (HHH)	140	20 mM Histidine, pH 5.8	5% Sucrose, 0.03% Polysorbate 20	50mM Sodium thiocyanate
F3 (III)	140	20 mM Histidine, pH 5.8	5% Sucrose, 0.03% Polysorbate 20	40mM Sodium citrate
F4 (JJJ)	140	20 mM Histidine, pH 5.8	5% Sucrose, 0.03% Polysorbate 20	50mM Glycine
F5 (KKK)	140	20 mM Histidine, pH 5.8	5% Sucrose, 0.03% Polysorbate 20	50mM Sodium chloride
F6 (LLL)	140	20 mM Histidine, pH 5.8	5% Sucrose, 0.03% Polysorbate 20	50mM Lysine
F7 (MMM)	140	20 mM Histidine, pH 5.8	5% Sucrose, 0.03% Polysorbate 20	50mM Sodium aspartate
F8 (NNN)	140	20 mM Histidine, pH 5.8	5% Sucrose, 0.03% Polysorbate 20	50mM Sodium glutamate
F9 (OOO)	140	20 mM Histidine, pH 5.8	5% Sucrose, 0.03% Polysorbate 20	50mM Sodium Citrate + 50mM Arginine hydrochloride
F10 (PPP)	140	20 mM Histidine, pH 5.8	5% Sucrose, 0.03% Polysorbate 20	50mM Glycine + 50mM Arginine hydrochloride
F11 (QQQ)	140	20 mM Histidine,	5% Sucrose, 0.03%	50mM Sodium aspartate +

		pH 5.8	Polysorbate 20	50mM Arginine hydrochloride
F12 (RRR)	140	20 mM Histidine, pH 5.8	5% Sucrose, 0.03% Polysorbate 20	50mM Sodium glutamate + 50mM Arginine hydrochloride

[0047] **Figure 10** shows the osmolality (mmol/Kg) of Formulations F1-F12 (set forth in Table A herein).

[0048] **Figure 11 (A-B)** measures the percentage of high molecular weight species in formulations F1-F6 (Formulations GGG, HHH, III, JJJ, LLL and KKK) over time, by SE-UPLC, after storage at 37°C for up to 28 days (A) or 5°C for up to 3 months (B) over time.

[0049] **Figure 12** shows the dynamic light scattering (diffusion coefficient (cm^2/second), radius (nm) and %Pd) at the initial time point of the formulations shown in Table 9-3 (GGG-RRR).

[0050] **Figure 13** shows the baseline FA images and OCT (30 degree lens) for two different rabbits (326-OS and 329-OD) before administration of aflibercept (OD=*oculus dexter* (right eye); OS=*oculus sinister* (left eye)). (A) FA image for rabbit 326, left eye; (B) OCT image for rabbit 326, left eye; (c) FA image for rabbit 329, right eye; (D) OCT image for rabbit 329, right eye.

[0051] **Figure 14 (A-F)** shows a time course of rabbit FA images at days 1 (A), 7 (B) and 14 (C) and the OCT at days 1 (D), 7 (E) and 14 (F) for one rabbit (326-OS) administered the histidine buffer formulation (30 degree lens) (7 mg/eye).

[0052] **Figure 15 (A-H)** shows a time course of rabbit FA images at 3 weeks (A), 4 weeks (B), 7 weeks (C) and 8 weeks (D); and the OCT at 3 weeks (E), 4 weeks (F), 7 weeks (G) and 8 weeks (H) for one rabbit (326-OS) administered the histidine buffer formulation (55 degree lens) (7 mg/eye).

[0053] **Figure 16 (A-F)** shows a time course of rabbit FA images at day 1 (A), week 1 (B) and week 2 (C) and the OCT at day 1 (D), week 1 (E) and week 2 (F) for one rabbit (329-OS) administered the phosphate buffer formulation (30 degree lens) (7 mg/eye).

[0054] **Figure 17 (A-H)** shows a time course of rabbit FA images at week 3 (A), week 4 (B), week 7 (C) and week 8 (D) and the OCT at week 3 (E), week 4 (F), week 7 (G) and week 8 (H) for one rabbit (329-OS) administered the phosphate buffer formulation (55 degree lens) (7 mg/eye).

[0055] **Figure 18 (A-B)** shows the purity (percentage of native species) (A) and percentage of high molecular weight (HMW) species (B) in formulations UUU-BBBB over time at 37°C (up to one month) (see Table 11-1) as analyzed by SE-UPLC.

[0056] **Figure 19 (A-E)** shows stability and purity analysis of a formulation having 114.3 mg/mL VEGF Trap (aflibercept) formulated in 10 mM Histidine, pH 5.8, 5% Sucrose, 0.03% Polysorbate 20, 50 mM Arginine Monohydrochloride by size exclusion chromatographic determination of the percentage of (i) high molecular weight species (HMW) after incubation at 5°C or 37°C for up to 2 months (A) and (ii) main species (Main peak) after incubation at 5°C or 37°C for up to 2 months (B); as well as by microflow imaging to determine the presence of subvisible particulate matter after incubation at 37°C for up to 28 days (C), by particle light obscuration analysis to determine the presence of subvisible particulate matter after incubation at 37°C for up to 28 days (D), and by microscopic determination of subvisible particulate matter after incubation at 37°C for up to 28 days (E).

[0057] **Figure 20** shows stability and purity analysis of formulation CCC, having 80, 100, 120 or 140 mg/ml aflibercept, by SE-UPLC determination of the percentage of high molecular weight species (HMW) after incubation at 2-8°C for up to 6 months (A) or 37°C for up to 28 days (B).

[0058] **Figure 21** shows the percentage of eyes in rabbits tested with complete leak suppression over time, when administered 500 micrograms or 2 mg aflibercept, over time (Gehan-Breslow-Wilcoxon test (P 0.0453)).

DETAILED DESCRIPTION

[0059] The present invention provides formulations having high concentrations of VEGF receptor fusion proteins (*e.g.*, aflibercept), showing excellent functional and storage properties which were developed in spite of significant technical hurdles. For example, a common method for identifying suitable excipients for a formulation that includes a polypeptide drug (such as a VEGF receptor fusion protein) is by assessment of polypeptide stability under accelerated stress conditions, such as high temperature (*e.g.*, 37°C). Excipients, which are not suitable under non-stress conditions (*e.g.*, a low temperature such as 5°C), will typically cause an undesired effect, within a short period of time, while under stress, *e.g.*, protein aggregation. This approach is common in the biotechnology and pharmaceutical industry insofar as it expedites the elimination of excipients unlikely to stabilize the drug product. See *e.g.*, Magari, *Assessing Shelf Life Using Real-Time and Accelerated Stability Tests*, Biopharm Intl. 16(11): 36-48 (2003). In some cases, a product may be released based on accelerated stability data, but this must be done in parallel with real-time shelf-life analysis (non-accelerated). Magari (2003) and FDA, *Guidelines for Submitting Documentation for the Stability of Human Drugs and Biologics*, Rockville, MD (1987). Here, however, at 5°C, the presence of arginine in histidine formulations was stabilizing, though arginine, it appeared, tended to decrease stability when under temperature stress (37°C). See Figure 8 (A-B). This property of the formulations set forth

herein would have presented a technical difficulty guiding a practitioner away from choosing arginine; thus, making it unlikely that a practitioner would have chosen arginine as an excipient. Nevertheless, the formulations set forth herein resulted from the overcoming of technical hurdles such as this to achieve a formulation with high stability. Formulation of VEGF trap in histidine buffer also led to a beneficial decrease in viscosity relative to that observed with a phosphate buffer formulation. Since small needle bores are preferred for performing intravitreal injections (due to decreased patient discomfort and eye trauma), a relative low viscosity is desirable. A lower viscosity formulation requires less force to push the formulation through the needle, thus making injection of the formulation through the needle easier for the treating physician. In addition, histidine and arginine containing formulation were well tolerated in rabbit eyes.

[0060] Reference will now be made in detail to representative embodiments. It should be understood that the following descriptions are not intended to limit the embodiments to one preferred embodiment. To the contrary, they are intended to cover alternatives, modifications, and equivalents as can be included within the spirit and scope of the described embodiments as defined in the appended claims.

[0061] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. So for example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (*e.g.*, 99.1%, 99.2%, 99.3%, 99.4%, *etc.*).

[0062] In an embodiment of the invention, a pharmaceutical formulation of the present invention is compliant with USP<789> for small volume injection (SVP) of ophthalmic solutions, *e.g.*, contains less than about 50 particles $\geq$10 µm in diameter per ml; contains less than about 5 particles $\geq$25 µm in diameter per ml; or contains less than about 2 particles $\geq$50 µm in diameter per ml.

[0063] Note that for purposes herein, "intravitreal injection" refers to injection into the vitreous of the eye (near the retina at the back of the eye). The expressions “suitable for intravitreal administration,” “suitable for intravitreal injection,” and the like mean that the formulation in question can be safely injected into the vitreous of a subject’s eye without causing any adverse reactions beyond those known to be associated with intravitreal injection of EYLEA.

[0064] The term "pharmaceutical formulation" herein refers to formulations including pharmaceutically acceptable carriers, *e.g.*, used to administer VEGF receptor fusion proteins (*e.g.*, aflibercept or conbercept) to a subject for a therapeutic/medicinal use.

INCORPORATED BY REFERENCE (RULE 20.6)

[0065] The term "pharmaceutically acceptable" refers to a formulation that within the scope of sound medical judgement is suitable for administration with the eye.

[0066] The term "subject" herein refers to any mammalian (*e.g.*, rabbit, mouse, rat or monkey) subject, particularly a human, *e.g.*, for whom diagnosis, prognosis or therapy is desired with the formulations as described herein.

[0067] The term “aqueous” formulation refers to a formulation which includes water.

[0068] The term "treat" or "treatment" refers to a therapeutic measure that reverses, stabilizes or eliminates an undesired disease or disorder (*e.g.*, an angiogenic eye disorder or cancer), for example, by causing the regression, stabilization or elimination of one or more symptoms or indicia of such disease or disorder by any clinically measurable degree, *e.g.*, with regard to an angiogenic eye disorder, by causing a reduction in or maintenance of diabetic retinopathy severity score (DRSS), by improving or maintaining vision (*e.g.*, in best corrected visual acuity *e.g.*, as measured by an increase in ETDRS letters), increasing or maintaining visual field and/or reducing or maintaining central retinal thickness and, with respect to cancer, stopping or reversing the growth, survival and/or metastasis of cancer cells in the subject. Typically, the therapeutic measure is administration of one or more doses of a therapeutically effective amount of VEGF receptor fusion protein to the subject with the disease or disorder.

[0069] “Prevent” or “prevention” refer to a prophylactic measure to cease the development of an undesired disease or disorder (*e.g.*, an angiogenic eye disorder).

[0070] SE-UPLC can be used in the present invention to quantitate the presence of high molecular weight species in a formulation. SE refers to size exclusion chromatography. UPLC refers to ultra-performance liquid chromatography. Suitable SE columns, which may be used in a UPLC system, to characterize such HMW species of VEGF receptor fusion proteins (*e.g.*, aflibercept or conbercept) in a formulation, are capable of resolving molecules in the molecular weight range of about 10,000–450,000 Daltons. See for example, the ACQUITY UPLC Protein BEH SEC 200Å Column. In an embodiment of the invention, two of such columns are connected in tandem when quantitating HMW species in a formulation. UPLC brings improvements over HPLC (high performance liquid chromatography) in sensitivity and resolution. UPLC uses instrumentation that operates at high pressures and uses finer particles (typically less than about 2.5µm) than that used in HPLC. Moreover, the UPLC mobile phases operate at high linear velocities than HPLC.

[0071] Embodiments herein include formulations that comprise a high concentration (*e.g.*, about 60 mg/ml, about 80 mg/ml, about 90 mg/ml, about 100 mg/ml, about 113.3 mg/ml, about 114.3 mg/ml, about 120 mg/ml, about 133.3 mg/ml, about 140 mg/ml, about 150 mg/ml, about 200 mg/ml or about 250 mg/ml) of VEGF receptor fusion protein (*e.g.*, aflibercept or conbercept). Suitable formulations included herein comprise a high

concentration of VEGF receptor fusion protein, a buffer, a thermal stabilizer and a surfactant. In some aspects, a suitable formulation further includes a viscosity reducing agent. In other aspects, the suitable formulation substantially excludes all viscosity reducing agents. Typical formulations have a pH of from about 5.0 to about 6.8 (*e.g.*, 5.8), but can include any pH useful for administration of the VEGF receptor fusion protein to the eye of a subject.

[0072] The present invention includes formulations that include a VEGF receptor fusion protein (*e.g.*, aflibercept or conbercept) in association with one or more further therapeutic agents (*e.g.*, an Ang-2 inhibitor (*e.g.*, an anti-ANG2 antibody or antigen-binding fragment thereof or nesvacumab), a Tie-2 receptor activator, an anti-PDGF, PDGF receptor or PDGF receptor beta antibody or antigen-binding fragment thereof and/or an additional VEGF antagonist such as bevacizumab, ranibizumab, pegaptanib or a soluble form of human vascular endothelial growth factor receptor-3 (VEGFR-3) comprising extracellular domains 1-3, expressed as an Fc-fusion protein) as well as methods of prevention or treatment comprising administering such formulations as discussed herein. In an embodiment of the invention, a formulation of the present invention includes a VEGF receptor fusion protein, such as aflibercept, but excludes any further therapeutic agent (*e.g.*, which is an antibody or antigen-binding fragment thereof).

[0073] The term "in association with" indicates that a formulation and a further therapeutic agent can be formulated into a single composition, *e.g.*, for simultaneous delivery, or formulated separately into two or more compositions (*e.g.*, a kit). The further therapeutic agent may, itself, be formulated in its own pharmaceutical formulation. Each can be administered to a subject at the same time as the other or at a different time than when the other is administered; for example, each administration may be given non-simultaneously (*e.g.*, separately or sequentially) at intervals over a given period of time. Moreover, the formulation and the further therapeutic agent may be administered to a subject by the same or by a different route.

[0074] In an embodiment of the invention, a formulation of the present invention includes any one or more of sodium sulfate (*e.g.*, 50 mM); sodium thiocyanate (*e.g.*, 50 mM); sodium citrate (*e.g.*, 40 mM); glycine (*e.g.*, 50 mM); sodium chloride (*e.g.*, 50 mM); lysine (*e.g.*, 50 mM); sodium aspartate (*e.g.*, 50 mM); and/or sodium glutamate (*e.g.*, 50 mM). For example, in an embodiment the formulation comprises a combination of sodium citrate (*e.g.*, 50 mM) and arginine hydrochloride (*e.g.*, 50 mM); glycine (*e.g.*, 50 mM) and arginine hydrochloride (*e.g.*, 50 mM); sodium aspartate (*e.g.*, 50 mM) and arginine hydrochloride (*e.g.*, 50 mM); or sodium glutamate (*e.g.*, 50 mM) and arginine hydrochloride (*e.g.*, 50 mM).

VEGF Receptor Fusion Proteins and Other VEGF inhibitors

[0075] For purposes herein, a "VEGF receptor fusion protein" refers to a molecule that comprises one or more VEGF receptors or domains thereof, fused to another polypeptide, which interferes with the interaction between VEGF and a natural VEGF receptor, *e.g.*, wherein two of such fusion polypeptides are associated thereby forming a homodimer or other multimer. Such VEGF receptor fusion proteins may be referred to as a "VEGF-Trap" or "VEGF Trap". VEGF receptor fusion proteins within the context of the present disclosure that fall within this definition include, chimeric polypeptides which comprise two or more immunoglobulin (Ig)-like domains of a VEGF receptor such as VEGFR1 (also known as Flt1) and/or VEGFR2 (also known as Flk1 or KDR), and may also contain a multimerizing domain (for example, an Fc domain).

[0076] An exemplary VEGF receptor fusion protein is a molecule referred to as VEGF1R2-FcΔC1(a) which is encoded by the nucleic acid sequence of SEQ ID NO:1 or nucleotides 79-1374 or 79-1371 thereof.

[0077] VEGF1R2-FcΔC1(a) comprises three components:

(1) a VEGFR1 component comprising amino acids 27 to 129 of SEQ ID NO:2;

(2) a VEGFR2 component comprising amino acids 130 to 231 of SEQ ID NO:2; and

(3) a multimerization component ("FcΔC1(a)") comprising amino acids 232 to 457 of SEQ ID NO:2 (the C-terminal amino acids of SEQ ID NO:2, *i.e.*, K458, may or may not be included in the VEGF receptor fusion proteins, see U.S. Patent No. 7,396,664 or 7,354,579, incorporated herein for all purposes). Note that amino acids 1 to 26 of SEQ ID NO:2 are the signal sequence.

In an embodiment of the invention, the VEGF receptor fusion protein comprises amino acids 27-458 or 27-457 of SEQ ID NO: 2.

[0078] In an embodiment of the invention, the VEGF receptor fusion protein comprises

(1) an immunoglobin-like (Ig) domain 2 of a first VEGF receptor (*e.g.*, VEGFR1), and

(2) an Ig domain 3 of a second VEGF receptor (*e.g.*, VEGFR2),

(3) and, optionally, further including an Ig domain 4 of the second VEGF receptor (*e.g.*, VEGFR2) and

(4) a multimerizing component (*e.g.*, Fc domain of IgG).

For example, in an embodiment of the invention, the VEGF receptor fusion protein has the following arrangement of said domains:

- [VEGFR1 Ig domain 2]-[VEGFR2 Ig domain 3]-[MC] (*e.g.*, a homodimer thereof) or
- [VEGFR1 Ig domain 2]-[VEGFR2 Ig domain 3]-[VEGFR2 Ig domain 4]-[MC] (*e.g.*, a homodimer thereof).

[0079] In an embodiment of the invention, the VEGF receptor fusion protein is a VEGF mini-trap which is a VEGF trap molecule with a truncated multimerizing component (*e.g.*, Fc), *e.g.*, wherein the mini-trap still includes an Fc hinge region. See *e.g.*, WO2005/00895 or U.S. Patent No. 7,396,664.

[0080] Note that the present disclosure also includes, within its scope, high concentration formulations including, instead of a VEGF receptor fusion proteins, VEGF binding molecules and anti-VEGF antibodies and antigen-binding fragments thereof,

- bevacizumab (*e.g.*, at a concentration of about 80-90 or 88 mg/ml),
- ranibizumab (*e.g.*, at a concentration of about 20-40 mg/ml, *e.g.*, 21-35, 21 or 35 mg/ml),
- an anti-VEGF aptamer such as pegaptanib (*e.g.*, pegaptanib sodium),
- a single chain (*e.g.*, V_L-V_H) anti-VEGF antibody such as brolucizumab (*e.g.*, at a-*--*--------- concentration of about 200-400 or 200, 210, 400 or 420 mg/ml),
- an anti-VEGF DARPin such as the Abicipar Pegol DARPin (*e.g.*, at a concentration of about 70-140, 70 or 140 mg/ml), or
- a bispecific anti-VEGF antibody, *e.g.*, which also binds to ANG2, such as RG7716 (*e.g.*, at a concentration of about 100-400, 100, 105, 400 or 420 mg/ml).

In order to minimize the repetitiveness of the embodiments discussed herein, it is contemplated that the scope of the present invention includes embodiments wherein any of the formulations discussed herein include, in place of a VEGF receptor fusion protein, an anti-VEGF antibody or antibody fragment or other VEGF binding molecule as discussed herein (*e.g.*, substituted with an anti-VEGF DARPin) at any of the concentrations discussed herein. For example, the present invention includes a formulation having 35 or 80 mg/ml ranibizumab, a buffer, a thermal stabilizer, a viscosity reducing agent and a surfactant.

[0081] DARPins are Designed Ankyrin Repeat Proteins. DARPins generally contain three to four tightly packed repeats of approximately 33 amino acid residues, with each repeat containing a β-turn and two anti-parallel α-helices. This rigid framework provides protein stability whilst enabling the presentation of variable regions, normally comprising six amino acid residues per repeat, for target recognition.

[0082] An “anti-VEGF” antibody or antigen-binding fragment of an antibody refers to an antibody or fragment that specifically binds to VEGF.

[0083] Illustrative VEGF receptor fusion proteins include aflibercept (EYLEA®, Regeneron Pharmaceuticals, Inc.) or conbercept (sold commercially by Chengdu Kanghong Biotechnology Co., Ltd.). See International patent application publication no. WO2005/121176 or WO2007/112675. The terms “aflibercept” and “conbercept” include biosimilar versions thereof. A biosimilar version of a reference product (*e.g.*, aflibercept)

generally refers to a product comprising the identical amino acid sequence, but includes products which are biosimilar under the U.S. Biologics Price Competition and Innovation Act.

[0084] Pharmaceutical formulations of the present invention are “high concentration”. High concentration pharmaceutical formulations of the present invention include VEGF receptor fusion protein at a concentration of at least 41 mg/ml, of at least 80 mg/ml, of at least 100 mg/ml, of at least 125 mg/ml, of at least 140 mg/ml, of at least 150 mg/ml, of at least 175 mg/ml, of at least 200 mg/ml, of at least 225 mg/ml, of at least 250 mg/ml, or of at least 275 mg/ml. Alternatively, "high concentration” can refer to formulations that include a concentration of VEGF receptor fusion protein of from about 140 mg/ml to about 160 mg/ml, at least about 140 mg/ml but less than 160 mg/ml, from about 41 mg/ml to about 275 mg/ml, from about 70 mg/ml to about 75 mg/ml or from about 80 mg/ml to about 250 mg/ml. In some aspects, the VEGF receptor fusion protein concentration in the formulation is about any of the following concentrations: 41 mg/ml; 42 mg/ml; 43 mg/ml; 44 mg/ml; 45 mg/ml; 46 mg/ml; 47 mg/ml; 48 mg/ml; 49 mg/ml; 50mg/ml; 51 mg/ml; 52 mg/ml; 53 mg/ml; 54 mg/ml; 55 mg/ml; 56 mg/ml; 57 mg/ml; 58 mg/ml; 59 mg/ml; 60 mg/ml; 61 mg/ml; 62 mg/ml; 63 mg/ml; 64 mg/ml; 65 mg/ml; 66 mg/ml; 67 mg/ml; 68 mg/ml; 69 mg/ml; 70 mg/ml; 71 mg/ml; 72 mg/ml; 73 mg/ml; 74 mg/ml; 75 mg/ml; 76 mg/ml; 77 mg/ml; 78 mg/ml; 79 mg/ml; 80 mg/ml; 81 mg/ml; 82mg/ml; 83 mg/ml; 84 mg/ml; 85 mg/ml; 86 mg/ml; 87mg/ml; 88 mg/ml; 89 mg/ml; 90 mg/ml; 91 mg/ml; 92 mg/ml; 93 mg/ml; 94 mg/ml; 95mg/ml; 96 mg/ml; 97 mg/ml; 98 mg/ml; 99 mg/ml; 100 mg/ml; 101 mg/ml; 102 mg/ml; 103 mg/ml; 104 mg/ml; 105 mg/ml; 106mg/ml; 107 mg/ml; 108 mg/ml; 109 mg/ml; 110 mg/ml; 111 mg/ml; 112 mg/ml; 113 mg/ml; 113.3 mg/ml; 114 mg/ml; 114.1 mg/ml; 114.2 mg/ml; 114.3 mg/ml; 114.4 mg/ml; 114.5 mg/ml; 114.6 mg/ml, 114.7 mg/ml, 114.8 mg/ml; 114.9 mg/ml; 115 mg/ml; 116 mg/ml; 117 mg/ml; 118 mg/ml; 119 mg/ml; 120 mg/ml; 121 mg/ml; 122 mg/ml; 123 mg/ml; 124 mg/ml; 125 mg/ml; 126 mg/ml; 127mg/ml; 128 mg/ml; 129 mg/ml; 130 mg/ml; 131 mg/ml; 132 mg/ml; 133 mg/ml; 133.3 mg/ml; 133.4 mg/ml, 134 mg/ml; 135 mg/ml; 136 mg/ml; 137 mg/ml; 138 mg/ml; 139 mg/ml; 140 mg/ml; 141 mg/ml; 142 mg/ml; 143 mg/ml; 144 mg/ml; 145 mg/ml; 146 mg/ml; 147 mg/ml; 148 mg/ml; 149 mg/ml; 150 mg/ml; 151 mg/ml; 152 mg/ml; 153 mg/ml; 154mg/ml; 155 mg/ml; 156 mg/ml; 157mg/ml; 158 mg/ml; 159 mg/ml; 160 mg/ml; 161 mg/ml; 162 mg/ml; 163 mg/ml; 164 mg/ml; 165 mg/ml; 166 mg/ml; 167 mg/ml; 168 mg/ml; 169 mg/ml; 170 mg/ml; 171 mg/ml; 172 mg/ml; 173 mg/ml; 174 mg/ml; 175 mg/ml; 176 mg/ml; 177 mg/ml; 178 mg/ml; 179 mg/ml; 180 mg/ml; 181 mg/ml; 182 mg/ml; 183 mg/ml; 184 mg/ml; 185 mg/ml; 186 mg/ml; 187 mg/ml; 188 mg/ml; 189 mg/ml; 190 mg/ml; 191 mg/ml; 192 mg/ml; 193 mg/ml; 194 mg/ml; 195 mg/ml; 196 mg/ml; 197 mg/ml; 198 mg/ml; 199 mg/ml; 200 mg/ml; 201 mg/ml; 202 mg/ml; 203 mg/ml; 204 mg/ml; 205 mg/ml; 206 mg/ml; 207 mg/ml; 208 mg/ml; 209 mg/ml; 210

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[0085] In an embodiment of the invention, a pharmaceutical formulation of the present invention is of such a concentration as to contain about 4, 6, 8, 10, 12, 14, 16, 18 or 20 mg VEGF receptor fusion protein (*e.g.*, aflibercept), or the amount of such protein in any of the acceptable doses thereof which are discussed herein, in about 100 µl or less, about 75 µl or less or about 70 µl or less, *e.g.*, about 50 µl; 51 µl; 52 µl; 53 µl; 54 µl; 55 µl; 56 µl; 57 µl; 58 µl; 59 µl; 60 µl; 61 µl; 62 µl; 63 µl; 64 µl; 65 µl; 66 µl; 67 µl; 68 µl; 69 µl; 70 µl; 71 µl; 72 µl; 73 µl; 74 µl; 75 µl; 76 µl; 77 µl; 78 µl; 79 µl; 80 µl; 81 µl; 82 µl; 83 µl; 84 µl; 85 µl; 86 µl; 87 µl; 88 µl; 89 µl; 90 µl; 91 µl; 92 µl; 93 µl; 94 µl; 95 µl; 96 µl; 97 µl; 98 µl; 99 µl; or 100 µl.

[0086] The present invention includes any of the formulations set forth under "*Illustrative Formulations*" herein, but wherein the concentration of the VEGF receptor fusion protein (*e.g.*, aflibercept) is substituted with a concentration which is set forth in this section ("VEGF Receptor Fusion Proteins and Other VEGF inhibitors").

Buffers

[0087] Buffers for use herein refer to solutions that resist pH change by use of acid-base conjugates. Buffers are capable of maintaining pH in the range of from about 5.0 to about 6.8, and more typically, from about 5.8 to about 6.5, and most typically, from about 6.0 to about 6.5. In some cases, the pH of the formulation of the present invention is about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, or about 6.8. Example buffers for inclusion in formulations herein include histidine-based buffers, for example, histidine, histidine hydrochloride, and histidine acetate. Buffers for inclusion in formulations herein can alternatively be phosphate-based buffers, for example, sodium phosphate, acetate-based buffers, for example, sodium acetate or acetic acid, or can

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be citrate-based, for example, sodium citrate or citric acid. It is also recognized that buffers can be a mix of the above, as long as the buffer functions to buffer the formulations in the above described pH ranges. In some cases, the buffer is from about 5 mM to about 25 mM, or more typically, about 5 mM to about 15 mM. Buffers can be about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, about 20 mM, about 21 mM, about 22 mM, about 23 mM, about 24 mM, or about 25 mM.

[0088] In an embodiment of the invention, a histidine-based buffer is prepared using histidine and histidine monohydrochloride.

Surfactants

[0089] Surfactant for use herein refers to ingredients that protect the higher concentration of VEGF receptor fusion protein from various surface and interfacial induced stresses. As such, surfactants can be used to limit or minimize VEGF receptor fusion protein aggregation, and promote protein solubility. Suitable surfactants herein have been shown to be non-ionic, and can include surfactants that have a polyoxyethylene moiety. Illustrative surfactants in this category include: polysorbate 20, polysorbate 80, poloxamer 188, polyethylene glycol 3350, and mixtures thereof. Surfactants in the formulations can be present at from about 0.02% to about 0.1% weight per volume (w/v), and more typically, about 0.02% to about 0.04% (w/v). In some cases, the surfactant is about 0.02% (w/v), about 0.03% (w/v), about 0.04% (w/v), about 0.05% (w/v), about 0.06% (w/v), about 0.07% (w/v), about 0.08% (w/v), about 0.09% (w/v), or about 0.1% (w/v).

Thermal Stabilizers

[0090] Thermal stabilizers for use herein refers to ingredients that provide thermal stability against thermal denaturation of the VEGF receptor fusion protein, as well as protect against loss of VEGF receptor fusion protein potency or activity. Suitable thermal stabilizers include sugars, and can be sucrose, trehalose, sorbitol or mannitol, or can be amino acids, for example L-proline, L-arginine (*e.g.*, L-arginine monohydrochloride), or taurine. Additionally, thermal stabilizers may also include substituted acrylamides or propane sulfonic acid, or may be compounds like glycerol.

[0091] In some cases, the formulations herein include both a sugar and taurine, a sugar and an amino acid, a sugar and propane sulfonic acid, a sugar and taurine, glycerol and taurine, glycerol and propane sulfonic acid, an amino acid and taurine, or an amino acid and propane sulfonic acid. In addition, formulations can include a sugar, taurine and propane sulfonic acid, glycerol, taurine and propane sulfonic acid, as well as L-proline, taurine and propane sulfonic acid.

[0092] Embodiments herein typically have thermal stabilizers present alone, each independently present at a concentration of, or present in combination at a total concentration of, from about 2% (w/v) to about 10% (w/v) or 4% (w/v) to about 10% (w/v), or about 4% (w/v) to about 9% (w/v), or about 5% (w/v) to about 8% (w/v). Thermal stabilizers in the formulation can be at a concentration of about 2% (w/v), about 2.5% (w/v), about 3% (w/v), about 4% (w/v), about 5% (w/v), about 6% (w/v), about 7% (w/v), about 8% (w/v), about 9% (w/v), about 10% (w/v) or about 20% (w/v).

[0093] With respect to taurine and propane sulfonic acid, in an embodiment of the invention, these thermal stabilizers can be present in the formulations at about from 25 mM to about 100 mM, and more typically from about 50 mM to about 75 mM (as compared to the other thermal stabilizers).

Viscosity Reducing Agents

[0094] Viscosity reducing agents typically are used to reduce or prevent protein aggregation. Viscosity reducing agents for inclusion herein include: sodium chloride, magnesium chloride, D- or L-arginine (*e.g.*, L-arginine monohydrochloride), lysine, or mixtures thereof. When present herein, viscosity reducing agents can be present at from about 10 mM to about 100 mM, and more typically from about 30 mM to about 75 mM, and even more typically from about 40 mM to about 70 mM. In some cases the viscosity reducing agent is present at about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM or about 100 mM.

Formulation Viscosity

[0095] Formulations in accordance with embodiments herein can also have a pharmaceutically acceptable viscosity for ocular administration, for example, intravitreal injection. Viscosity generally refers to the measure of resistance of a fluid which is being deformed by either shear stress or tensile stress (typically measured by techniques known in the art, viscometer or rheometer, for example). Typical viscosities of formulations in accordance with embodiments herein are from about 5.0 cP (centipoise) to about 15 cP, from about 11 cP to about 14 cP, from about 12 cP to about 15 cP or from about 11 cP to about 12 cP. As such, formulation viscosity herein can be about 5.0 cP, about 6.0, about 7.1 cP, about 7.2 cP, about 7.3 cP, about 7.4 cP, about 7.5 cP, about 7.6 cP, about 10 cP, about 10.5 cP, about 11.0 cP, about 11.5 cP, about 12.0 cP, about 12.5 cP, about 13.0 cP, about 13.5 cP, about 14.0 cP, about 14.5 cP, or about 15.0 cP (*e.g.*, when measured at 20°C).

[0096] Various embodiments herein do not require inclusion of an inorganic salt, or other viscosity reducing agent, to maintain these highly useful viscosities. Typically, high concentration protein solutions require viscosity reducing agents to avoid protein aggregation and higher viscosity, making the formulations difficult for intravitreal injection and reducing the potency of the VEGF receptor fusion protein. As such, embodiments herein include formulations that have had substantially no, or no added, sodium chloride (NaCl), magnesium chloride ($MgCl_2$), D- or L-arginine hydrochloride, lysine or other viscosity reducing agent.

Formulation osmolality

[0097] Osmolality is a critical attribute for injectable formulations. It is desirable to have products match physiological osmotic conditions. Furthermore, osmolality provides confirmation of soluble content in solution. In an embodiment of the invention, the osmolality of a formulation of the present invention is less than or equal to about 506 mmol/Kg or from about 250 to about 506 mmol/Kg., *e.g.*, about 250, 260, 270, 280, 290, 299, 300, 310, 314, 315, 316, 324, 343, 346, 349, 369, 384, 403, 426, 430 or 506 mmol/Kg. In an embodiment of the invention, the osmolality is lower than about 250 mmol/Kg.

Purity and Stability of Formulations

[0098] High concentration VEGF receptor fusion protein containing formulations described herein are stable during manufacture and storage. The term "stable" herein refers to formulations that include VEGF receptor proteins that retain both chemical and physical stability over the period of formulation manufacturing and storage, *e.g.*, that maintains integrity and has minimal degradation, denaturation, or unfolding. VEGF receptor protein stability can be determined using analytical techniques available in the art at different temperatures and over different periods of time. In particular, VEGF receptor chemical stability (potency) can be determined using various bioassays (for example, a BAF/3 VEGFR1/EPOR cell line used to determine $VEGF_{165}$ binding by the VEGF receptor fusion proteins herein), and physical stability, by size exclusion (SE) chromatographic analysis, UPLC (ultra-performance liquid chromatography) size exclusion (SE) chromatography, visual appearance, OD, pH, charge variant formation, and rate of high molecular weight (HMW) species formation. A stable VEGF receptor fusion protein is one that shows limited change in its OD, pH, formation of charge variants, and formation of HMW species.

[0099] A “high molecular weight” (HMW) species as used herein, with reference to a formulation containing a given VEGF Trap (*e.g.*, aflibercept), refers to any species of polypeptide or polypeptide complex in the formulation which elutes from a size exclusion column (*e.g.*, SE-UPLC) ahead of (*e.g.*, with a higher molecular weight than) VEGF Trap

polypeptide and/or a homodimer thereof. The percentage of HMW species refers to the percentage of such species relative to the overall quantity of polypeptides in the formulation, *e.g.*, by SE-UPLC analysis.

[00100] In an embodiment of the invention, as discussed more fully in the Examples below, stable formulations show significant VEGF receptor fusion protein potency and physical stability for a period of up to 12 months, up to 24 months, and/or up to 36 months when stored at about 2°C to about 8°C.

[00101] In an embodiment of the invention, formulation of the present invention is one that:

- After about 28 days at about 37°C has about a 3, 5, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 30, 35 or 37% (or 10-15% or 15-20% or 10-20%) increase in high molecular weight species (*e.g.*, as measured by SE-UPLC or SEC)
- After about 28 days at about 37°C has about a 5, 6, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22% (or 5-20% or 5-10% or 10-15% or 15-20%) decrease in main species (*e.g.*, as measured by SE-UPLC or SEC)
- After about 28 days at about 37°C, has about 80, 81, 82, 83, 84, 85, 86 or 87% (or about 80-85%) or more protein as main species (*e.g.*, as measured by SE-UPLC or SEC)
- After about 28 days at about 37°C has about a 1 or 1.5 or 2% (or 1-2%) increase in low molecular weight species (*e.g.*, as measured by SE-UPLC or SEC)
- After about one month at about 37°C has about a 16% increase in high molecular weight species; and/or about a 17% decrease in main species and/or about a 0.5% or <1% increase in low molecular weight species; and/or after about two months at about 5°C, has about a 1% decrease in main species, and/or about a 1% increase in high molecular weight species and/or no significant or detectable amounts low molecular weight (LMW) species; and/or after about 2 months at about 5°C, has about 97% main species (*e.g.*, as measured by SE-UPLC or SEC)
- After about 12 months at about 2-8°C has about a 3-3.5% increase in high molecular weight species (*e.g.*, as measured by SE-UPLC or SEC)
- After about 12 months at about 2-8°C has less than about a 1% or a 1, 2, 3 or 4% (*e.g.*, 1-4% or 3-4%) decrease in main species (*e.g.*, as measured by SE-UPLC or SEC)
- After about 12 months at about 2-8°C, has about 94 or 95% or more protein as main species (*e.g.*, as measured by SE-UPLC or SEC)
- After about 3 months at about 2-8°C has about a 1 or 2% increase in high molecular weight species (*e.g.*, as measured by SE-UPLC or SEC)

- After about 6 months at about 2-8°C has about a <1, 1 or 2% increase in high molecular weight species (*e.g.*, as measured by SE-UPLC or SEC)
- After about $5^1/_2$ or 6 months at about 5°C has about 2.5, 3.0 or 3.5 (or about 2.5-3.5%) total high molecular weight species (*e.g.*, as measured by SE-UPLC or SEC).
- After about 6 months at about 2-8°C has less than about a 1% or 1 or 2% (or about 0.5-2% or 1-2%) decrease in main species (*e.g.*, as measured by SE-UPLC or SEC)
- After about 6 months at about 2-8°C, has about 96 or 97 or 98% (or 96-98%) or more protein as main species (*e.g.*, as measured by SE-UPLC or SEC)
- After about 24 months or 36 months at about 2-8°C, had less than about 5 or 6 or 7% (*e.g.*, about 1.5, 2, 3, 4 or 5%) (or 1.5-5% or 1.5-2.5%) increase in high molecular weight species; and/or has about 3.0, 3.25, 3.5, 4.0, 4.5 or 5% total high molecular weight species; and/or a decrease of about 2 or 3% (or 2-3%) main species; and/or a total amount of main species of about 95 or 96% or more (or 95-96%) (*e.g.*, as measured by SE-UPLC or SEC)
- After about 1 month at about 37°C, has about 97, 98, 99 or 100% (or 97-100%) of aflibercept in the formulation which is recoverable by RP-HPLC
- Immediately after manufacture and purification, has less than about 1.5, 2, 2.5, 3.0 or 3.5% high molecular weight species (*e.g.*, as measured by SE-UPLC or SEC)
- After about 6 months at about 37°C, has at least about 70% or 75% (*e.g.*, 70-75%) of aflibercept as main species/main peak (*e.g.*, as measured by capillary isoelectric focusing (cIEF) or imaged capillary isoelectric focusing) (non-acidic and non-basic species)
- After about 36 months at about 2-8°C, has about a 1 or 2% (or 1-2%) increase in acidic species (*e.g.*, as measured by capillary isoelectric focusing (cIEF) or imaged capillary isoelectric focusing)
- After about 36 months at about 2-8°C, has about a 1% or less decrease in main species/main peak (*e.g.*, as measured by capillary isoelectric focusing (cIEF) or imaged capillary isoelectric focusing)
- After about 36 months at about 2-8°C, has about 78-79% main species/main peak (*e.g.*, as measured by capillary isoelectric focusing (cIEF) or imaged capillary isoelectric focusing)

and/or

- When administered intravitreally to a mammal, such as a human or rabbit or mouse (*e.g.*, with an angiogenic eye disorder such as wet AMD), does not cause any adverse events that are clinically different from those observed for EYLEA (*e.g.*, when EYLEA is dosed intravitreally at 0.5 or 2.0 mg); or does not cause clinically

significant inflammation in the eye, prolonged intraocular pressure (IOP) increases, prolonged increases in blood pressure or decreases and/or retinal detachment.

[00102] Further, in an embodiment of the invention, high concentration VEGF receptor fusion proteins are stable as they show little or no formation of acidic charge variants over the course of manufacture and storage, for example, little or no formation of charge variants as tested by Imaged Capillary Isoelectric Focusing, for example.

[00103] In an embodiment of the invention, a formulation of the present invention exhibits less than or equal to about 8% low molecular weight (LMW) species.

[00104] In an embodiment of the invention, a formulation of the present invention has less than about 0.2, 0.4 or 0.5 EU (endotoxin units)/ml of endotoxin;

[00105] In an embodiment of the invention, a formulation of the present invention is essentially free of particulate matter or particulate matter of about 1, 2, 5, 10, 25 or 50 micrometers (or more) in size.

[00106] In an embodiment of the invention, when a formulation of the present invention is analyzed by non-reduced CE-SDS (SDS capillary gel electrophoresis), at least 97% of the total peak area is the main peak.

[00107] In an embodiment of the invention, when a formulation of the present invention is analyzed by size-exclusion UPLC (SE-UPLC), at least 93, 94 or 95% of the total peak area is the main peak and less than or equal to 3.5, 4, 5 or 6% is aggregate.

[00108] In an embodiment of the invention, a formulation of the present invention is at about 2°C, 3°C, 4°C, 5°C, 6°C, 7°C or 8°C, 2-8°C (*e.g.*, an average temperature of 5°C), 23°C, 25°C, 30°C or 37°C.

Illustrative Formulations

[00109] Illustrative high concentration VEGF receptor fusion protein containing formulations include the following:

[00110] Formulation A: 80 mg/ml aflibercept, 10 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03% (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00111] Formulation B: 80 mg/ml aflibercept, 10 mM phosphate-based buffer, 5 % (w/v) sucrose, 0.03% (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00112] Formulation C: 80 mg/ml aflibercept, 10 mM citrate-based buffer, 5 % (w/v) sucrose, 0.03% (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00113] Formulation D: 80 mg/ml aflibercept, 10 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 6.2.

[00114] Formulation E: 80 mg/ml aflibercept, 10 mM phosphate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00115] Formulation F: 80 mg/ml aflibercept, 10 mM citrate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00116] Formulation G: 80 mg/ml aflibercept, 10 mM histidine-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00117] Formulation H: 80 mg/ml aflibercept, 10 mM phosphate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00118] Formulation I: 80 mg/ml aflibercept, 10 mM citrate-based buffer, 8 % (w/v) sucrose, and 0.0 3% (w/v) polysorbate 20, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00119] Formulation J: 80 mg/ml aflibercept, 10 mM histidine-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00120] Formulation K: 80 mg/ml aflibercept, 10 mM phosphate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00121] Formulation L: 80 mg/ml aflibercept, 10 mM citrate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00122] Formulation M: 150 mg/ml aflibercept, 10 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00123] Formulation N: 150 mg/ml aflibercept, 10 mM phosphate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00124] Formulation O: 150 mg/ml aflibercept, 10 mM citrate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00125] Formulation P: 150 mg/ml aflibercept, 10 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 6.2.

[00126] Formulation Q: 150 mg/ml aflibercept, 10 mM phosphate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00127] Formulation R: 150 mg/ml aflibercept, 10 mM citrate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00128] Formulation S: 150 mg/ml aflibercept, 10 mM histidine-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00129] Formulation T: 150 mg/ml aflibercept, 10 mM phosphate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20, with a pH of 5.8 to 6.2 (*e.g.*, 6.2), and, optionally, specifically excluding a viscosity reducing agent.

[00130] Formulation U: 150 mg/ml aflibercept, 10 mM citrate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00131] Formulation V: 150 mg/ml aflibercept, 10 mM histidine-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00132] Formulation W: 150 mg/ml aflibercept, 10 mM phosphate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00133] Formulation X: 150 mg/ml aflibercept, 10 mM citrate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00134] Formulation Y: 80 mg/ml conbercept, 10 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00135] Formulation Z: 80 mg/ml conbercept, 10 mM phosphate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00136] Formulation AA: 80 mg/ml conbercept, 10 mM citrate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00137] Formulation BB: 80 mg/ml conbercept, 10 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 6.2.

[00138] Formulation CC: 80 mg/ml conbercept, 10 mM phosphate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00139] Formulation DD: 80 mg/ml conbercept, 10 mM citrate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00140] Formulation EE: 80 mg/ml conbercept, 10 mM histidine-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00141] Formulation FF: 80 mg/ml conbercept, 10 mM phosphate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00142] Formulation GG: 80 mg/ml conbercept, 10 mM citrate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00143] Formulation HH: 80 mg/ml conbercept, 10 mM histidine-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00144] Formulation II: 80 mg/ml conbercept, 10 mM phosphate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00145] Formulation JJ: 80 mg/ml conbercept, 10 mM citrate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00146] Formulation KK: 150 mg/ml conbercept, 10 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00147] Formulation LL: 150 mg/ml conbercept, 10 mM phosphate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00148] Formulation MM: 150 mg/ml conbercept, 10 mM citrate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00149] Formulation NN: 150 mg/ml conbercept, 10 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 6.2.

[00150] Formulation OO: 150 mg/ml conbercept, 10 mM phosphate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00151] Formulation PP: 150 mg/ml conbercept, 10 mM citrate-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 80, and 40 mM sodium chloride, with a pH of 5.8 to 6.2.

[00152] Formulation QQ: 150 mg/ml conbercept, 10 mM histidine-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00153] Formulation RR: 150 mg/ml conbercept, 10 mM phosphate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00154] Formulation SS: 150 mg/ml conbercept, 10 mM citrate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00155] Formulation TT: 150 mg/ml conbercept, 10 mM histidine-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00156] Formulation UU: 150 mg/ml conbercept, 10 mM phosphate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00157] Formulation VV: 150 mg/ml conbercept, 10 mM citrate-based buffer, 8 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 80, with a pH of 5.8 to 6.2, and, optionally, specifically excluding a viscosity reducing agent.

[00158] Formulation WW: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 10 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 50 mM taurine, with a pH of 5.8.

[00159] Formulation XX: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 20 mM histidine-based buffer, 4 % (w/v) proline, 0.03 % (w/v) polysorbate 20, and 50 mM arginine hydrochloride, with a pH of 5.8.

[00160] Formulation YY: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 20 mM histidine-based buffer, 2.5 % (w/v) sucrose, 2.0 % (w/v) proline, 0.03 % (w/v) polysorbate 20, and 50 mM taurine, with a pH of 5.8.

[00161] Formulation ZZ: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 10 mM histidine-based buffer, 2.5 % (w/v) sucrose, 2.0 % (w/v) proline, 0.03 % (w/v) polysorbate 20, and 50 mM arginine hydrochloride, with a pH of 5.8.

[00162] Formulation AAA: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 20 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03% (w/v) polysorbate 20, and 50 mM PSA, with a pH of 5.8.

[00163] Formulation BBB: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 20 mM histidine-based buffer, 2.5 % (w/v) sucrose, 2.0 % (w/v) proline, 0.03 % (w/v) polysorbate 20, and 50 mM PSA, with a pH of 5.8.

[00164] Formulation CCC: 80, 100, 120 or 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 20 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 50 mM arginine hydrochloride, with a pH of 5.8.

[00165] Formulation DDD: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 10 mM histidine-based buffer, 4 % (w/v) proline, 0.03 % (w/v) polysorbate 20, and 50 mM PSA, with a pH of 5.8.

[00166] Formulation EEE: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 20 mM histidine-based buffer, 5 % (w/v) sucrose, and 0.03 % (w/v) polysorbate 20 and, optionally, no thermal stabilizer, with a pH of 5.8.

[00167] Formulation FFF: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 10mM sodium phosphate, 5 % (w/v) sucrose and 0.03 % polysorbate 20 with a pH of 6.2.

[00168] Formulation GGG: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5% sucrose; 0.03 % polysorbate 20; 50 mM sodium sulfate

[00169] Formulation HHH: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5% sucrose; 0.03 % polysorbate 20; 50 mM sodium thiocyanate

[00170] Formulation III: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5 % sucrose, 0.03 % polysorbate 20; 40 mM sodium citrate

[00171] Formulation JJJ: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5% Sucrose, 0.03 % polysorbate 20; 50 mM glycine

[00172] Formulation KKK: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5 % sucrose, 0.03 % polysorbate 20; 50 mM sodium chloride

[00173] Formulation LLL: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5 % sucrose; 0.03 % polysorbate 20; 50 mM lysine

[00174] Formulation MMM: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5 % sucrose; 0.03% polysorbate 20; 50 mM sodium aspartate

[00175] Formulation NNN: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5 % sucrose; 0.03 % polysorbate 20; 50 mM sodium glutamate

[00176] Formulation OOO: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5 % sucrose; 0.03% polysorbate 20; 50 mM sodium citrate; 50 mM arginine hydrochloride

[00177] Formulation PPP: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5 % sucrose; 0.03% polysorbate 20; 50 mM glycine; 50 mM arginine hydrochloride

[00178] Formulation QQQ: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5 % sucrose; 0.03% polysorbate 20; 50 mM sodium aspartate; 50 mM arginine hydrochloride

[00179] Formulation RRR: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM histidine, pH 5.8; 5 % sucrose; 0.03% polysorbate 20; 50 mM sodium glutamate; 50 mM arginine hydrochloride

[00180] Formulation SSS: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM His, pH 5.8; 5 % sucrose; 0.03 % polysorbate 20; 10 mM L-arginine hydrochloride

[00181] Formulation TTT: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept); 20 mM His, pH 5.8; 5 % sucrose; 0.03 % polysorbate 20; 100 mM L-arginine hydrochloride

[00182] Formulation UUU: 30 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 10 % sucrose, 10 mM phosphate, 0.03 % polysorbate 20, pH 6.2

[00183] Formulation VVV: 30 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 20 % sucrose, 10 mM phosphate, 0.03 % polysorbate 20, pH 6.2

[00184] Formulation WWW: 60 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 10 % sucrose, 10 mM phosphate, 0.03 % polysorbate 20, pH 6.2

[00185] Formulation XXX: 60 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 20 % sucrose, 10 mM phosphate, 0.03 % polysorbate 20, pH 6.2

[00186] Formulation YYY: 120 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 10 % sucrose, 10 mM phosphate, 0.03 % polysorbate 20, pH 6.2

[00187] Formulation ZZZ: 120 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 20 % sucrose, 10 mM phosphate, 0.03 % polysorbate 20, pH 6.2

[00188] Formulation AAAA: 120 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 10 % sucrose, 10 mM phosphate, 0.03 % polysorbate 20, 50 mM NaCl, pH 6.2

[00189] Formulation BBBB: 120 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 20 % sucrose, 10 mM phosphate, 0.03 % polysorbate 20, 50 mM NaCl, pH 6.2

[00190] Formulation CCCC: 140 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 10 mM sodium phosphate, 5 % sucrose, 40 mM sodium chloride, 0.03 % PS20, pH 6.2

[00191] Formulation DDDD: 80 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept), 20 mM histidine-based buffer, 5 % (w/v) sucrose, 0.03 % (w/v) polysorbate 20, and 50 mM L-arginine monohydrochloride, with a pH of 5.8.

[00192] Formulation EEEE: 120.0 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept) (*e.g.*, $\pm$ 12 mg/ml), 20 mM histidine-based buffer (*e.g.*, $\pm$ 2 mM), 5 % (w/v) sucrose (*e.g.*, $\pm$ 0.5%), 0.03 % (w/v) polysorbate 20 (*e.g.*, 0.02-0.04%), and 50 mM L-arginine monohydrochloride (*e.g.*, $\pm$ 5 mM), with a pH of 5.8 (*e.g.*, 5.6-6.0 or 5.5-6.1).

[00193] Formulation FFFF: 113.3 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept) (*e.g.*, 102-125 mg/ml), 20 mM histidine-based buffer (*e.g.*, $\pm$ 2 mM), 5 % (w/v) sucrose (*e.g.*, $\pm$ 0.5%), 0.03 % (w/v) polysorbate 20 (*e.g.*, 0.02-0.04%), and 50 mM L-arginine monohydrochloride (*e.g.*, $\pm$ 5 mM), with a pH of 5.8 (*e.g.*, 5.6-6.0 or 5.5-6.1).

[00194] Formulation GGGG: 114.3 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept) (*e.g.*, 103-126 mg/ml), 10 mM histidine-based buffer (*e.g.*, ± 1 mM), 5 % (w/v) sucrose (*e.g.*, ± 0.5%), 0.03 % (w/v) polysorbate 20 (*e.g.*, 0.02-0.04%), and 50 mM L-arginine monohydrochloride (*e.g.*, ± 5 mM), with a pH of 5.8 (*e.g.*, 5.6-6.0 or 5.5-6.1).

[00195] Formulation HHHH: 100.0 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept) (*e.g.*, ± 10 mg/ml), 20 mM histidine-based buffer (*e.g.*, ± 2 mM), 5 % (w/v) sucrose (*e.g.*, ± 0.5%), 0.03 % (w/v) polysorbate 20 (*e.g.*, 0.02-0.04%), and 50 mM L-arginine monohydrochloride (*e.g.*, ± 5 mM), with a pH of 5.8 (*e.g.*, 5.6-6.0 or 5.5-6.1).

[00196] Formulation IIII: 133.3 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept) (*e.g.*, ± 13 mg/ml), 20 mM histidine-based buffer (*e.g.*, ± 2 mM), 5 % (w/v) sucrose (*e.g.*, ± 0.5%), 0.03 % (w/v) polysorbate 20 (*e.g.*, 0.02-0.04%), and 50 mM L-arginine monohydrochloride (*e.g.*, ± 5 mM), with a pH of 5.8 (*e.g.*, 5.6-6.0 or 5.5-6.1).

[00197] Formulation JJJJ: 150 mg/ml aflibercept (*e.g.*, aflibercept) (*e.g.*, ± 15 mg/ml), 10 mM sodium phosphate, 8% (w/v) sucrose (*e.g.*, ± 0.8%), 0.03% (w/v) polysorbate 20 (*e.g.*, 0.02-0.04%) and 50 mM L-arginine hydrochloride, pH 6.2 (*e.g.*, 6.0-6.4 or 5.9-6.5).

[00198] Formulation KKKK: 114.3 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept) (*e.g.*, ± 14 mg/ml), 20 mM histidine-based buffer (*e.g.*, ± 2 mM), 5% (w/v) sucrose (*e.g.*, ± 0.5%), 0.03% (w/v) polysorbate 20 (*e.g.*, 0.02-0.04%), and 50 mM L-arginine monohydrochloride (*e.g.*, ± 5 mM), with a pH of 5.8 (*e.g.*, 5.6-6.0 or 5.5-6.1);

[00199] or any formulation which is set forth herein.

[00200] In an embodiment of the invention, the concentration of any formulation constituent (*e.g.*, all constituents) listed above (*e.g.*, any one of formulations A-KKKK) or which is discussed herein is ± about 3%, 5% or about 10% of the concentration specifically mentioned.

Methods of Manufacture

[00201] Embodiments herein include methods of manufacturing a pharmaceutical formulation of the present invention including a VEGF receptor fusion protein (*e.g.*, any of formulations A-KKKK as set forth herein) comprising combining the components of the formulation into a single composition, and, optionally, introducing the formulation into a vessel or device *e.g.*, a vial, or delivery device, *e.g.*, a pre-filled syringe. The formulations, vials or devices that are the product of such methods are part of the present invention.

[00202] In an embodiment of the invention, the method of manufacturing a VEGF receptor fusion protein containing pharmaceutical formulation of the present invention (*e.g.*, any of formulations A-KKKK as set forth herein) comprises the steps of culturing a host cell (*e.g.*, Chinese hamster ovary cell) comprising one or more polynucleotides encoding a VEGF receptor fusion protein (*e.g.*, aflibercept) in a culture medium and under conditions

whereby the protein is expressed; and, purifying the protein from the host cell and/or culture medium and combining a portion of the protein with excipients of a pharmaceutical formulation as set forth herein. Again, the formulations, vials or devices that are the product of such methods are part of the present invention.

[00203] In an embodiment of the invention, a determination is made as to the amount and type of VEGF receptor fusion protein needed in the formulation to make it a high concentration and for its end-use. The same determination is made regarding the amount and type of buffer, the amount and type of surfactant, the amount and type of thermal stabilizer, and the inclusion, or specific exclusion, of a viscosity reducing agent. These components are combined and mixed, making sure that the formulation pH is at the desired value, *e.g.*, between about 5.0 and about 6.8 (*e.g.*, 5.8), and/or that the viscosity is at a desired value, *e.g.*, about 6.0, 7.3, 11.5 or 12.0 cP at 20°C. In an embodiment of the invention, high concentration VEGF receptor fusion protein containing formulations can be sterilized and stored in a stable condition, *e.g.*, for up to 24 or 36 months at 2°C to 8°C (*e.g.*, 5°C).

[00204] See, *e.g.*, Hardman, *et al.* (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y.; Gennaro (2000) Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y.; Avis, *et al.* (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY; Lieberman, *et al.* (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY; Weiner and Kotkoskie (2000) Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y.

Angiogenic Eye Disorders and Cancer

[00205] The pharmaceutical formulations of the present invention comprising VEGF receptor fusion proteins (*e.g.*, any of pharmaceutical formulations A-KKKK) can be used in the treatment or prevention of any angiogenic eye disorder by administration of a therapeutically effective amount of VEGF receptor fusion protein in a formulation of the present invention to a subject in need thereof, *e.g.*, by intravitreal injection. Angiogenic eye disorders herein refer to any disease of the eye which is caused by or associated with the growth or proliferation of blood vessels and/or by blood vessel leakage. Non-limiting examples of angiogenic eye disorders that are treatable or preventable using the formulations and methods herein, include:

- age-related macular degeneration (wet),
- macular edema,
- macular edema following retinal vein occlusion,

- retinal vein occlusion (RVO),
- central retinal vein occlusion (CRVO),
- branch retinal vein occlusion (BRVO),
- diabetic macular edema (DME),
- choroidal neovascularization (CNV),
- iris neovascularization,
- neovascular glaucoma,
- post-surgical fibrosis in glaucoma,
- proliferative vitreoretinopathy (PVR),
- optic disc neovascularization,
- corneal neovascularization,
- retinal neovascularization,
- vitreal neovascularization,
- pannus,
- pterygium,
- vascular retinopathy,
- diabetic retinopathies (*e.g.*, non-proliferative diabetic retinopathy (*e.g.*, characterized by a Diabetic Retinopathy Severity Scale (DRSS) level of about 47 or 53) or proliferative diabetic retinopathy; *e.g.*, in an subject that does not suffer from DME) and
- Diabetic retinopathy in a patient who has diabetic macular edema (DME).

[00206] The pharmaceutical formulations of the present invention comprising VEGF receptor fusion proteins (*e.g.*, any of pharmaceutical formulations A-KKKK) can be used in the treatment or prevention of any cancer by administration of a therapeutically effective amount of VEGF receptor fusion protein in a formulation of the present invention to a subject in need thereof, *e.g.*, by intramuscular, intratumoral, subcutaneous or intravenous injection. Cancers include those whose growth, proliferation, survival and/or metastasis is dependent, to a degree, on angiogenesis. In an embodiment of the invention, the cancer is colorectal cancer, lung cancer, skin cancer, breast cancer, brain cancer, stomach cancer, renal cancer, prostate cancer, liver cancer or pancreatic cancer.

[00207] Thus, the present invention provides methods for treating or preventing an angiogenic eye disorder in a subject in need thereof comprising administering a therapeutically effective amount of VEGF receptor fusion protein (*e.g.*, aflibercept) (*e.g.*, about 4, 6, or 8.0, 8.1, 8.4 or 8.5 mg), *e.g.*, in a pharmaceutical formulation according to the present invention, intraocularly, *e.g.*, into the vitreous, of an eye of the subject. In an embodiment of the invention, both eyes are administered the VEGF receptor fusion protein.

In an embodiment of the invention, therapeutically effective doses of the VEGF receptor fusion protein are administered about every 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 weeks, 16 weeks, 17 weeks 18 weeks, 19 weeks, 20 weeks, 21, weeks, 22 weeks, 23 weeks or 24 weeks. In an embodiment of the invention, such methods of treatment or prevention are done in the absence of significant increases in blood pressure (systolic and/or diastolic) and/or the development of hypertension (*e.g.*, Grade 1, Grade 2 or Grade 3) and/or abnormally high intraocular pressure in the subject. In an embodiment of the invention, the method includes the step of, following said administration, monitoring the subject for significant increases in blood pressure (systolic and/or diastolic) and/or the development of hypertension (*e.g.*, Grade 1, Grade 2 or Grade 3) and/or abnormally high intraocular pressure.

Modes of Administration

[00208] The pharmaceutical formulations of the present invention which include a VEGF receptor fusion protein may be administered in accordance with known medically approved delivery systems. In embodiments herein, these delivery systems may include administering the formulations to the patient by ocular, intraocular, intrachoroidal, intravitreal or subconjunctival injection. Alternatively, pharmaceutical formulations of the present invention can also be administered to the patient by topical routes, *e.g.*, eye drops, eye gels, eye ointments, and the like. Other possible routes of delivery for the formulations herein include intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral.

[00209] In an embodiment of the invention, intravitreal injection of a pharmaceutical formulation of the present invention includes the step of piercing the eye with a syringe and needle (*e.g.*, 30-gauge injection needle) containing the formulation and injecting the formulation (*e.g.*, less than or equal to about 100 microliters; about 40, 50, 55, 56, 57, 57.1, 58, 60, 70 or 75 microliters) into the vitreous of the eye (*e.g.*, with a sufficient volume as to deliver a therapeutically effective amount of VEGF receptor fusion protein, *e.g.*, of about 4, 5, 6, 7, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8 or 8.9, 9, 10, 12, 14, 16, 18 or 20 mg VEGF receptor fusion protein). Optionally, the method includes the steps of administering a local anesthetic (*e.g.*, proparacaine, lidocaine or tetracaine), an antibiotic (*e.g.*, a fluoroquinolone), antiseptic (*e.g.*, povidone-iodine) and/or a pupil dilating agent to the eye being injected. In an embodiment of the invention, a sterile field around the eye to be injected is established before the injection. In an embodiment of the invention, following intravitreal injection, the subject is monitored for elevations in intraocular pressure and/or blood pressure. In an embodiment of the invention, the other eye is also injected by the same procedure.

Amounts of VEGF Receptor Fusion Protein to be Administered

[00210] Each dose of high concentration VEGF receptor fusion protein to a subject over the course of a treatment may contain the same or substantially the same amount of fusion protein. Alternatively, the quantity of any one dose may be different or variable over a treatment course.

[00211] An effective or therapeutically effective amount of VEGF receptor fusion protein for treating or preventing cancer (*e.g.*, which is mediated, at least in part, by angiogenesis) or an angiogenic eye disorder refers to the amount of the VEGF receptor fusion protein sufficient to cause the regression, stabilization or elimination of the cancer or angiogenic eye disorder, *e.g.*, by regressing, stabilizing or eliminating one or more symptoms or indicia of the cancer or angiogenic eye disorder by any clinically measurable degree, *e.g.*, with regard to an angiogenic eye disorder, by causing a reduction in or maintenance of diabetic retinopathy severity score (DRSS), by improving or maintaining vision (*e.g.*, in best corrected visual acuity *e.g.*, as measured by an increase in ETDRS letters), increasing or maintaining visual field and/or reducing or maintaining central retinal thickness and, with respect to cancer, stopping or reversing the growth, survival and/or metastasis of cancer cells in the subject. In an embodiment of the invention, an effective or therapeutically effective amount of VEGF receptor fusion protein for treating or preventing an angiogenic eye disorder is about 0.5 mg to about 10 mg or 0.5 mg to about 20 mg per dose, including: about 0.5 mg or more; or about 2 mg or more, *e.g.*, about 2.1 mg; 2.2 mg; 2.3 mg; 2.4 mg; 2.5 mg; 2.6 mg; 2.7 mg; 2.8 mg; 2.9 mg; 3.0 mg; 3.1 mg; 3.2 mg; 3.3 mg; 3.4 mg; 3.5 mg; 3.6 mg; 3.7 mg; 3.8 mg; 3.9 mg; 4.0 mg; 4.1 mg; 4.2 mg; 4.3 mg; 4.4 mg; 4.5 mg; 4.6 mg; 4.7 mg; 4.8 mg; 4.9 mg; 5.0 mg; 5.1 mg; 5.2 mg; 5.3 mg; 5.4 mg; 5.5 mg; 5.6 mg; 5.7 mg; 5.8 mg; 5.9 mg; 6.0 mg; 6.1 mg; 6.2 mg; 6.3 mg; 6.4 mg; 6.5 mg; 6.6 mg; 6.7 mg; 6.8 mg; 6.9 mg; 7.0 mg; 7.1 mg; 7.2 mg; 7.3 mg; 7.4 mg; 7.5 mg; 7.6 mg; 7.7 mg; 7.8 mg; 7.9 mg; 8.0 mg; 8.1 mg; 8.2 mg; 8.3 mg; 8.4 mg; 8.5 mg; 8.6 mg; 8.7 mg; 8.8 mg; 8.9 mg; 9 mg; 9.1 mg; 9.2 mg; 9.3 mg; 9.4 mg; 9.5 mg; 9.6 mg; 9.7 mg; 9.8 mg; 9.9 mg, 10.0 mg, 10.1 mg; 10.2 mg; 10.3 mg; 10.4 mg; 10.5 mg; 10.6 mg; 10.7 mg; 10.8 mg; 10.9 mg; 11 mg; 11.1 mg; 11.2 mg; 11.3 mg; 11.4 mg; 11.5 mg; 11.6 mg; 11.7 mg; 11.8 mg; 11.9 mg; 12 mg; 12.1 mg; 12.2 mg; 12.3 mg; 12.4 mg; 12.5 mg; 12.6 mg; 12.7 mg; 12.8 mg; 12.9 mg; 13 mg; 13.1 mg; 13.2 mg; 13.3 mg; 13.4 mg; 13.5 mg; 13.6 mg; 13.7 mg; 13.8 mg; 13.9 mg; 14 mg; 14.1 mg; 14.2 mg; 14.3 mg; 14.4 mg; 14.5 mg; 14.6 mg; 14.7 mg; 14.8 mg; 14.9 mg; 15 mg; 15.1 mg; 15.2 mg; 15.3 mg; 15.4 mg; 15.5 mg; 15.6 mg; 15.7 mg; 15.8 mg; 15.9 mg; 16 mg; 16.1 mg; 16.2 mg; 16.3 mg; 16.4 mg; 16.5 mg; 16.6 mg; 16.7 mg; 16.8 mg; 16.9 mg; 17 mg; 17.1 mg; 17.2 mg; 17.3 mg; 17.4 mg; 17.5 mg; 17.6 mg; 17.7 mg; 17.8 mg; 17.9 mg; 18 mg; 18.1 mg; 18.2 mg; 18.3 mg; 18.4 mg; 18.5 mg; 18.6 mg; 18.7 mg; 18.8 mg; 18.9 mg; 19 mg; 19.1

mg; 19.2 mg; 19.3 mg; 19.4 mg; 19.5 mg; 19.6 mg; 19.7 mg; 19.8 mg; 19.9 mg; or 20 mg. In an embodiment of the invention, an effective or therapeutically effective amount of VEGF receptor fusion protein for treating or preventing cancer is about 4 mg/kg (*e.g.*, intravenously). This dose may be administered, for example, every 2 weeks.

[00212] In an embodiment of the invention, a VEGF receptor fusion protein is administered in a volume sufficient to deliver the desired dose of fusion protein, *e.g.*, as discussed above. In an embodiment of the invention, the volume delivered (*e.g.*, for treating or preventing an angiogenic eye disorder, *e.g.*, by intravitreal injection) is less than or equal to about 100 microliters (*e.g.*, about any of the following volumes: 25 microliters; 26 microliters; 27 microliters; 28 microliters; 29 microliters; 30 microliters; 31 microliters; 32 microliters; 33 microliters; 34 microliters; 35 microliters; 36 microliters; 37 microliters; 38 microliters; 39 microliters; 40 microliters; 41 microliters; 42 microliters; 43 microliters; 44 microliters; 45 microliters; 46 microliters; 47 microliters; 48 microliters; 49 microliters; 50 microliters; 51 microliters; 52 microliters; 53 microliters; 54 microliters; 55 microliters; 56 microliters; 57 microliters; 58 microliters; 59 microliters; 60 microliters; 61 microliters; 62 microliters; 63 microliters; 64 microliters; 65 microliters; 66 microliters; 67 microliters; 68 microliters; 69 microliters; 70 microliters; 71 microliters; 72 microliters; 73 microliters; 74 microliters; 75 microliters; 76 microliters; 77 microliters; 78 microliters; 79 microliters; 80 microliters; 81 microliters; 82 microliters; 83 microliters; 84 microliters; 85 microliters; 86 microliters; 87 microliters; 88 microliters; 89 microliters; 90 microliters; 91 microliters; 92 microliters; 93 microliters; 94 microliters; 95 microliters; 96 microliters; 97 microliters; 98 microliters or 99 microliters).

[00213] In an embodiment of the invention, the formulation is administered (*e.g.*, for treating or preventing an angiogenic eye disorder, *e.g.*, by intravitreal injection) in a volume which is about 60 microliters or less, about 70 microliters or less or about 75 microliters or less or about 100 microliters or less. For example, in an embodiment of the invention, about 2, 4, 6, 8.0, 8.1, 8.0-8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8 or 8.9, 9.0 or 10 mg of VEGF receptor fusion protein is administered in about 50, 60, 70 or 75 microliters.

[00214] The scope of the present invention also encompasses methods for delivering 0.5 or 2 mg of VEGF receptor fusion protein (*e.g.*, for treating or preventing an angiogenic eye disorder, *e.g.*, by intravitreal injection) in pharmaceutical formulation of the present invention in a low volume such as less than 50 µl (*e.g.*, about 1, 5, 10, 17, 17.5, 18, 18.5, 20, 30, 40 or 45 µl).

[00215] The present invention also includes a composition including or consisting or consisting essentially of a “single dosage volume” of pharmaceutical formulation of the present invention, *i.e.*, a volume containing a single dose of VEGF receptor fusion protein (*e.g.*, aflibercept) in a pharmaceutical formulation of the present invention (*e.g.*, about 50 µl

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or 60 µl, 70 µl or 75 µl or whatever volume contains about 4, 6, 8 or 10 mg of VEGF receptor fusion protein). As discussed below, a container (*e.g.*, vial or injection device) comprising a single dosage volume, optionally including a small overfill volume of formulation, is also part of the present invention.

Containers and Injection Devices

[00216] High concentration VEGF receptor fusion protein formulations in accordance with embodiments herein (*e.g.*, any of formulations A-KKKK) can be pre-packaged or pre-loaded in various useful containers and injection devices. Thus, the present invention includes containers and injection devices containing such formulations. In one embodiment herein, the container is a vial which may be sterile. In another embodiment herein, the container is a test tube which may be sterile. In an embodiment of the invention, an injection device (which may be sterile) is a syringe (*e.g.*, a pre-filled syringe or autoinjector). In an embodiment of the invention, the injection device is an intravitreal implant, *e.g.*, a refillable intravitreal implant.

[00217] A “pre-filled” syringe is a syringe which is filled with a formulation of the present invention prior to sale or use by the physician or patient.

[00218] "Sterile" herein refers to aseptic or free from substantially all, or all, living microorganisms and their spores.

[00219] Syringes as used herein include barrels made, for example, of glass or polymer, for example, cycloolefin as described in U.S. Patent Publication No. 2017/0232199, incorporated herein for all purposes, a plunger and a needle.

[00220] Containers and injection devices may be coated with silicone (*e.g.*, silicone oil or baked-silicone (*e.g.*, ≤40 µg or ≤100 µg)).

[00221] In an embodiment of the invention, a container or injection device is substantially metal-free or substantially tungsten-free or low-tungsten.

[00222] In an embodiment of the invention, the syringe contains one or more dose line graduations and/or is a dose metering system.

[00223] Containers in accordance with embodiments herein can hold the high concentration VEGF receptor fusion protein formulations. In some aspects, the container or injection device can include a label stating indications of use. In some instances, the container or injection device herein can include package inserts with instructions for use as described throughout this specification.

[00224] In other embodiments, a volume containing a single dose or multiple doses (*e.g.*, 2 or more than 2) of a high concentration VEGF receptor fusion protein (*e.g.*, wherein the dose is 2 mg, 4 mg, 6 mg, 8 mg or 10 mg of VEGF receptor fusion protein), as described above, can be pre-packaged into a container or injection device, *e.g.*, a sterile syringe, for

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example, for storage until use. In one example, the volume in the container includes a single dose of VEGF receptor fusion protein optionally further including a small amount of overfill volume. Sterile, pre-loaded syringes can be maintained under storage conditions (*e.g.*, 2°C-8°C), for example, for up to 12 months, 24 months or 36 months. Overfill is an excess volume that is meant to be sufficient to permit withdrawal and/or administration of the proper volume. In an embodiment of the invention, the container has a single dosage volume or multiple dosage volumes and about 5%-10% overfill volume.

[00225] Syringe sizes can be, for example, 0.3 cc, 0.5 cc or 1 cc, for example. Sterile syringes typically include a needle useful for ocular injection, typically about ½ inch, or 12.5 mm to 16 mm in length, and can be 29 gauge, 30 gauge, 31 gauge, 32 gauge or 33 gauge based on the patient and health care specialist preference. Other needle lengths and gauges can be used, as long as the needle is effective at accomplishing intravitreal injection.

[00226] While the invention has been particularly shown and described with reference to a number of embodiments, it would be understood by those skilled in the art that changes in the form and details may be made to the various embodiments disclosed herein without departing from the spirit and scope of the invention and that the various embodiments disclosed herein are not intended to act as limitations on the scope of the claims.

Additional formulations

[00227] The present invention includes the following formulations comprising greater than 40 mg/ml VEGF receptor fusion protein (*e.g.*, aflibercept or conbercept) and:

[00228] (a) a buffer including a histidine salt (*e.g.*, histidine-HCl or histidine-acetate, for example, 10 mM to 50 mM) and having pH ranging from 5.7 to 6.2; a sugar (*e.g.*, more than 6%, but not more than 10%) such as sucrose, trehalose, mannitol or glucose; a surfactant selected from the group consisting of polysorbate 20 and polysorbate 80 (*e.g.*, 0% to 0.1%);

[00229] (b) a histidine containing buffer such as L-histidine/histidine hydrochloride (*e.g.*, 10 mM); a non-ionic surfactant such as polysorbate 20 (*e.g.*, 0.03%), an inorganic salt such as NaCl (*e.g.*, 40 mM), and a carbohydrate such as sucrose (*e.g.*, 5%), *e.g.* pH 6.0-6.5 (*e.g.*, 6.2 or 6.5);

[00230] (c) citric acid (*e.g.*, 5 mM, 10 mM, 15 mM, 20 mM, 25 mM or 30 mM), sucrose (*e.g.*, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or 10%), arginine (*e.g.*, 5 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM or 50 mM or 100 mM), and polysorbate 20 (*e.g.*, 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.10%);

[00231] (d) a buffer such as phosphate, histidine, acetate, succinate, citrate, glutamate, and/or lactate (*e.g.*, at 5-20 or 5-50 mM); a non-ionic surfactant such as a

polysorbate (*e.g.*, PS20 or PS80), a polyethylene glycol dodecyl ether, a poloxamer, 4-(I,I,3,3-Tetramethylbutyl)phenyl-polyethylene glycol, an alkylsaccharide or an alkylglycoside, a tonicifying agent such as a polyol or an amino acid, for example, sucrose, trehalose, sorbitol, mannitol, glycerol, proline, arginine, methionine, glycine, or lysine, wherein the formulation has a final osmolality of about 300 mOsm/kg, and wherein the concentration of chloride anion is less than about 10 mM; pH 5.0-6.5; or

[00232] (e) 10 mM sodium phosphate, 40 mM sodium chloride, 0.03% polysorbate 20, and 5% sucrose, pH 6.2.

EXAMPLES

[00233] The following examples are provided for illustrative purposes only and are not intended to limit the scope of the invention. Efforts have been made to ensure accuracy with respect to numbers used, but some experimental errors and deviations should be accounted for. Any formulations set forth in these examples are part of the present invention.

[00234] In these examples, when an experiment is conducted at 2-8°C, the temperature is targeted to 5°C with a tolerance of ±3°C variation.

Example 1: 80 mg/ml VEGF Receptor Fusion Protein Formulations Maintain Potency, Physical Stability and Charge Over A 36 Month Period.

[00235] A series of four different formulations having 80 mg/ml VEGF receptor fusion protein (aflibercept) were tested for long term potency, physical stability and development of charge variants. The ingredient listing of each of the four formulations is shown in Table 1-1. Each formulation was evaluated for VEGF receptor fusion protein HMW species formation over the course of storage at 2°C to 8°C for 36 months, as well as for the percent main species by SE-UPLC. Each VEGF receptor fusion protein containing formulation was also evaluated using Imaged Capillary Isoelectric Focusing over the same course of time and temperature. Finally, each formulation's potency was tested by bioassay over the same course of time and temperature.

Table 1-1. VEGF Receptor Fusion Protein Containing Formulations

Formulation	VEGFT (mg/mL)	Sodium phosphate (mM)	Histidine (mM)	Sucrose (w/v)	Polysorbate 20 (w/v)	Sodium Chloride (mM)	pH
F1 (B)	80	10	NA	5	0.03	40	6.2
F2 (H)	80	10	NA	8	0.03	0	6.2
F3 (D)	80	NA	10	5	0.03	40	6.2
F4 (G)	80	NA	10	8	0.03	0	6.2

Table 1-2. %HMW Species

	Formulation B	Formulation H	Formulation D	Formulation G
	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Months at 2-8C				
0.0	1.7	1.6	1.6	1.3
1	2.1	2.1	1.6	1.7
2.0	2.3	2.2	1.8	1.8
3.0	2.4	2.5	2.0	2.1
6.0	2.9	2.8	2.0	2.3
9.0	3.1	3.0	2.2	2.4
12.0	2.8	3.0		2.4
18.0	3.2	3.2	2.6	2.5
24.0	3.6	3.6	3.1	2.8
36.0	4.2	4.2	3.2	3.2

Table 1-3. %Main Peak

	Formulation B	Formulation H	Formulation D	Formulation G
	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Months at 2-8C				
0.0	98.4	98.4	98.4	98.8
1	97.9	98.0	98.4	98.3
2.0	97.7	97.8	98.2	98.2
3.0	97.7	97.5	98.0	98.0
6.0	97.1	97.2	97.9	97.7
9.0	96.9	97.0	97.8	97.6
12.0	97.2	97.0		97.4
18.0	96.8	96.8	97.4	97.5
24.0	96.5	96.4	96.8	97.2
36.0	95.7	95.7	96.8	96.8

Table 1-4. %Acidic Species

	Formulation B	Formulation H	Formulation D	Formulation G
	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Months at 2-8C				
0.0	15.8	15.1	16.0	15.2
12	15.6	15.9	NP	16.0
24.0	16.0	17.1	16.2	16.9
36.0	16.9	17.4	17.2	17.6

Table 1-5. %Main Species

	Formulation B	Formulation H	Formulation D	Formulation G
	Formulation 1	Formulation 2	Formulation 3	Formulation 4
Months at 2-8C				
0.0	78.9	79.1	79.2	79.9
12	79.3	79.3	NP	79.7
24.0	79.3	78.5	79.5	79.3
36.0	78.6	78.1	78.7	78.6

[00236] Data is shown in Figures 1A, 1B, 1C and 1D. Figure 1A shows the percent of VEGF receptor fusion protein HMW species formed for each of the four formulations. VEGF TRAP shows a slightly slower rate of HMW species formation when the formulation is histidine-based, as compared to sodium phosphate. As shown in Figure 1A, the %HMW species after storage at 2^0 to 8^0C for 36 months increased by 2.6% in sodium phosphate formulations (Formulations 1 and 2), as compared to the 1.6 to 1.9% increase in histidine buffers (Formulations 3 and 4). The data was confirmed, as shown in Figure 1B, where SE-UPLC was used to identify the percent of the VEGF receptor fusion protein main species present over the same time frame and temperature range. As a comparison point, assays were run on the current EYLEA® formulation (40 mg/ml) having 10 mM sodium phosphate, which showed a 1.2% increase in the %HMW species after storage at 2^0 to 8^0C for 36 months (not shown). In addition, each of the four 80 mg/ml VEGF TRAP formulations was tested for potency and compared to EYLEA® activity (not shown). Potency was maintained by bioassay for all four formulations as well as EYLEA®. These data show that formulations herein are able to maintain VEGF receptor fusion protein stability comparable to EYLEA®.

[00237] Referring to Figures 1C and 1D, the percent of VEGF TRAP charge variants formed after storage at 2^0 to 8^0C for 36 months was also tested. Figure 1C shows that the

VEGF TRAP in Formulations 1 - 4 showed no meaningful change in percent acidic species over the course of 36 months, and Figure 1D shows the same finding for percent main species over the course of 36 months. The finding that the VEGF TRAP maintained charge variant species during storage shows that the quality of the VEGF TRAP was maintained, and processes like deamination, N-terminal pyroglutamate formation, isomerization, aggregation, *etc.* did not occur over the course of the storage parameters.

[00238] The data in Example 1 shows that formulations having double the VEGF TRAP concentration found in EYLEA®, can maintain physical stability, quality and potency over the course of 36 months at 2^0 to 8^0C. Further, although all four formulations showed similar potency over the period, histidine containing formulations resulted in a slightly smaller increase in HMW species formation (sign of protein degradation), showing that under some circumstances, histidine may be a buffer of choice. However, the findings for both histidine and phosphate based buffers showed excellent stability over the course of the study.

Example 2: 150 mg/ml VEGF TRAP Stability in Sodium Phosphate Buffer/Sucrose Formulations.

[00239] Two sodium phosphate formulations were tested for physical stability of 150 mg/ml VEGF Trap (aflibercept) at 37^0C for 28 days. The ingredient listing of each of the two formulations is shown in Table 2-1. Each formulation was evaluated for HMW species formation over the course of storage at 37^0C for the 28 days as well as for the percent main species by SE-UPLC.

Table 2-1. VEGF Receptor Fusion Protein (aflibercept) Containing Formulations for Example 2

Formulation	VEGFT (mg/mL)	Sodium phosphate (mM)	Sucrose (w/v)	Polysorbate 20 (w/v)	Arginine-HCl (mM)	pH
F1 (T)	150	10	8%	0.03	N/A	6.2
F2 (JJJJ)	150	10	8%	0.03	50	6.2

Table 2-2. %HMW Species

	10 mM Sodium Phosphate, 8% Sucrose, 0.03% Polysorbate 20, pH 6.2	10 mM Sodium Phosphate, 8% Sucrose, 0.03% Polysorbate 20, 50 mM L-Arginine Monohydrochloride, pH 6.2
	Phosphate	**Phosphate-Arginine**
Days at 37C		
0.0	2.4	1.9
7	6.4	6.1
14.0	8.4	8.3
21.0	10.4	10.5
28.0	12.2	12.5

Table 2-3. %Main Peak

	10 mM Sodium Phosphate, 8% Sucrose, 0.03% Polysorbate 20, pH 6.2	10 mM Sodium Phosphate, 8% Sucrose, 0.03% Polysorbate 20, 50 mM L-Arginine Monohydrochloride, pH 6.2
	Phosphate	**Phosphate-Arginine**
Days at 37C		
0.0	97.6	98.1
7	93.6	93.9
14.0	91.0	91.1
21.0	88.9	89.0
28.0	87.1	86.9

[00240] Figure 2A shows that there was no meaningful difference of %HMW species formed between the two formulations during storage at 37^0C for 28 days, which as confirmed by the percent of main species by SE-UPLC (Figure 2B). There was no change in the appearance, turbidity or pH of the two formulations over the course of the 28 days (not shown).

[00241] The results were then compared to formulations that included 40 mg/ml EYLEA®, and Formulation 1 from Example 1 (80 mg/ml VEGF TRAP in sodium phosphate buffer). As expected, the 150 mg/ml VEGF TRAP formulations showed a higher percent of HMW species formation as compared to the EYLEA® formulation, and a somewhat higher percent of HMW species formation as compared to Formulation 1 of Example 1.

[00242] Further testing was performed on high concentration VEGF TRAP containing formulations, and histidine containing formulations showed better protection of these molecules than did formulations based in sodium phosphate. Further, inclusion of sodium chloride into each of the tested formulations actually caused destabilization of the VEGF

TRAP at 37^{0}C. The data in Example 2 shows that even at high concentrations of 150 mg/ml VEGF receptor fusion protein, and fairly extreme temperature storage, the formulations herein provide excellent protection for physical stability.

Example 3: Pharmaceutically Acceptable Formulation Viscosity Can be Attained for 150 mg/ml VEGF TRAP.

[00243] A number of VEGF Trap (aflibercept) containing formulations were tested for their viscosity behavior. Formulations having from between 10 mg/ml to 160 mg/ml VEGF Trap were tested in the presence and absence of various viscosity reducing agents.

[00244] Figure 3A shows formulations having 155 mg/ml VEGF Trap in 10 mM sodium phosphate buffer, 5% sucrose and a pH of 6.2 in combination with no inorganic salt, arginine, lysine, sodium chloride and magnesium chloride showed little difference in viscosity. In each case viscosity was measured in cP at 20^{0}C. A range of viscosity between about 17 cP (no inorganic salt) and 15 cP (100 mM magnesium chloride) was observed, with little variation between which viscosity reducing agent was used, or with the presence of a viscosity reducing agent at all. Figure 3B shows a similar series of formulations, except that the base buffer is 10 mM histidine and pH 5.8. Here, the lack of any inorganic salt provided a formulation with a viscosity of 11.5 cP , and 50 mM lysine a viscosity of 14 cP. The histidine containing buffer provided excellent viscosity, one in line with other low-concentration biologic injectables.

[00245] Figure 3C shows viscosity of VEGF Trap between the concentrations of 10 mg/ml and 160 mg/ml, with 10 mM sodium phosphate, 5% sucrose at a pH of 6.2 in the absence of arginine, and in 50 mM of arginine hydrochloride. Inclusion of the viscosity reducing agent resulted in similar viscosities for all VEGF Trap concentrations tested. As shown in Figures 3A and 3B, the viscosity reducing agent had little positive effect on the formulation viscosity, even at higher protein concentrations. This is a surprising result, as arginine and other viscosity reducing agents have previously been shown to have beneficial properties on high concentration protein viscosity.

Table 3-1. Viscosity-Phosphate formulations (cP at 20°C)

Buffer Composition	10 mM Sodium Phosphate, 5% Sucrose, pH 6.2
No Viscosity Reducer	17.3
100 mM Arginine	16.2
200 mM Arginine	15.8
50 mM Lysine	16.2
100 mM Sodium Chloride	16.5
50 mM Magnesium Chloride	16.3
100 mM Magnesium Chloride	15.3

Table 3-2. Viscosity-Histidine formulations (cP at 20°C)

Buffer Composition	10 mM Histidine, pH 5.8
No Viscosity Reducer	11.5
100 mM Arginine	12.0
200 mM Arginine	12.7
50 mM Lysine	14.0
200 mM Lysine	14.1
50 mM Sodium Chloride	13.1
100 mM Sodium Chloride	12.1
50 mM Magnesium Chloride	12.6
100 mM Magnesium Chloride	11.4

Example 4: Arginine Hydrochloride Improves Stability of 150 mg/ml VEGF TRAP In Sodium Phosphate Buffer/Sucrose Formulations.

[00246] Two sodium phosphate formulations were tested for physical stability of 150 mg/ml VEGF Trap (aflibercept) at 2-8°C for 12 months. The ingredient listing of each of the two formulations is shown in Table 4-1. Formulation 2 further includes 50 mM arginine hydrochloride. Each formulation was evaluated for HMW species formation over the course of storage at 37°C for the 12 months as well as for the percent main species by SE-UPLC.

Table 4-1. VEGF Receptor Fusion Protein (aflibercept) Containing Formulations for Example 3

Formulation	VEGFT (mg/mL)	Sodium phosphate (mM)	Sucrose (w/v)	Polysorbate 20 (w/v)	pH	Arginine Hydrochloride (mM)
F1 (T)	150	10	8%	0.03	6.2	0
F2 (JJJJ)	150	10	8%	0.03	6.2	50

[00247] Figure 4A shows that inclusion of 50 mM arginine hydrochloride to the formulation improved the VEGF TRAP physical stability. This stabilizing attribute on the VEGF TRAP was confirmed in Figure 4B, where the percent main species remains higher for the VEGF TRAP in the presence of 50 mM arginine hydrochloride. This data shows that there are situations under which addition of arginine hydrochloride provides a stabilizing effect to the stored VEGF TRAP. Tabulated size-exclusion chromatography data are set forth in Table 4-2 and 4-3.

Table 4-2. %HMW Species

	10 mM Sodium Phosphate, 8% Sucrose, 0.03% Polysorbate 20, pH 6.2	**10 mM Sodium Phosphate, 8% Sucrose, 0.03% Polysorbate 20, 50 mM L-Arginine Monohydrochloride, pH 6.2**
Months at 2-8C		
0.0	2.4	1.9
1	3.3	2.8
2.0	3.8	3.2
3.0	4.1	3.5
6.0	4.9	4.1
9.0	5.5	4.7
12.0	5.9	5.0

Table 4-3. %Main Peak

	10 mM Sodium Phosphate, 8% Sucrose, 0.03% Polysorbate 20, pH 6.2	**10 mM Sodium Phosphate, 8% Sucrose, 0.03% Polysorbate 20, 50 mM L-Arginine Monohydrochloride, pH 6.2**
Months at 2-8C		
0.0	97.6	98.1
1	96.8	97.2
2.0	96.2	96.8
3.0	95.9	96.5
6.0	95.1	95.8
9.0	94.5	95.3
12.0	94.1	94.5

Example 5: Viscosity of 10 mg/ml to 170 mg/ml VEGF TRAP in Histidine and Phosphate Buffers

[00248] Figure 5 showed that 10 mM phosphate buffer and 10 mM histidine buffer both had highly useful viscosities for a range of VEGF Trap (aflibercept) concentrations. At higher VEGF Trap concentrations, the histidine buffer showed an improvement in viscosity (relative to phosphate). Viscosity was measured at 20°C.

Example 6: Taurine and Propane Sulfonic Acid Provide Improved Stability to High Concentration VEGF Trap (aflibercept) Formulations.

[00249] An experiment was conducted to determine the effect on protein stability with a number of formulation combinations. Figure 6 shows a dynamic light scattering plot for formulations having anywhere from 2 mg/ml protein to 10 mg/ml protein (aflibercept). Formulations that included 70 mM taurine or 70 mM PSA showed reduced self-interaction at higher concentrations. These data indicate that taurine and PSA can be useful inclusions in high concentration protein formulations, like the ones at issue in this case.

Example 7: Long Term Study of Stability of Various Histidine Containing Formulations.

[00250] A total of 9 different aflibercept formulations were tested with varying buffer concentration, thermal stabilizers and hydrophobic salts. The 9 formulations, F1-F9, are summarized below in Table 7-1.

Table 7-1. Formulations F1-F9.

Formulation	VEGFT (mg/mL)	Buffer Type	Buffer Concentration (mM)	Sucrose (%w/v)	Proline (%w/v)	Polysorbate 20 (w/v)	Stabilizer (mM)	Stabilizer Concentration (mM)	pH
F1 (WW)	140	His	10	5	0	0.03	Taurine	50	5.8
F2 (XX)	140	His	20	0	4	0.03	Arginine HCl	50	5.8
F3 (YY)	140	His	20	2.5	2	0.03	Taurine	50	5.8
F4 (ZZ)	140	His	10	2.5	2	0.03	Arginine HCl	50	5.8
F5 (AAA)	140	His	20	5	0	0.03	PSA	50	5.8
F6 (BBB)	140	His	20	2.5	2	0.03	PSA	50	5.8

F7 (CCC)	140	His	20	5	0	0.03	Arginine HCl	50	5.8
F8 (DDD)	140	His	10	0	4	0.03	PSA	50	5.8
F9 (EEE)	140	His	20	5	0	0.03	NA	NA	5.8

VEGFT=VEGF Trap (aflibercept)

[00251] Fourteen (14) mL of each formulation was compounded in 15 mL Falcon tubes. The formulation was then sterile filtered using a 0.22 µM syringe filter and filled in sterile 2 mL Type 1 glass vials. Eight (8) vials were filled for each formulation with 0.4 mL volume in a laminar flow hood.

[00252] SE-UPLC (molecular weight species) was performed on all samples to determine the chemical stability of each formulation.

[00253] The percentage of HMW species over time for each of formulations F1-F9 are shown in Figure 7 (see also Table 7-2). Formulations F4 and F7 exhibited the lowest percentage of HMW species after 4 months at 5°C. The results showed that addition of excipients like arginine and proline significantly reduced the rate of %HMW species formation in VEGF Trap formulations.

Table 7-2. SE-UPLC Analysis (2-8°C)

	F1 (WW)		F2 (XX)		F3 (YY)	
Months at 2-8C	%HMW	%Main Peak	%HMW	%Main Peak	%HMW	%Main Peak
0.0	1.53	98.46	1.48	98.52	1.39	98.61
0.5	1.87	98.13	1.87	98.13	1.92	98.08
1.0	2.19	97.81	2.16	97.84	2.19	96.15
2.0	2.64	97.36	2.63	97.37	2.71	97.29
3.0	2.83	97.17	2.76	97.24	2.87	97.13
4.0	3.07	96.93	3.00	97.00	3.11	96.89
5.0	3.24	96.76	3.11	96.89	3.28	96.72
6.0	3.35	96.65	3.24	96.76	3.38	96.62

F4 (ZZ)		F5 (AAA)		F6 (BBB)	
%HMW	%Main Peak	%HMW	%Main Peak	%HMW	%Main Peak
1.31	98.69	1.55	98.45	1.45	98.55
1.69	98.31	1.79	98.21	1.96	98.04
1.95	98.05	2.31	97.69	2.26	97.74
2.37	97.63	2.80	97.20	2.72	97.28
2.61	97.39	2.99	97.01	2.91	97.09
2.84	97.16	3.26	96.74	3.17	96.83
3.03	96.97	3.44	96.56	3.35	96.65
3.13	96.87	3.52	96.48	3.41	96.59

F7 (CCC)		F8 (DDD)		F9 (EEE)	
%HMW	%Main Peak	%HMW	%Main Peak	%HMW	%Main Peak
1.20	98.80	1.56	98.44	1.53	98.47
1.74	98.26	1.87	98.13	2.05	97.95
2.08	97.92	2.16	97.84	2.39	97.61
2.52	97.48	2.59	97.41	2.89	97.11
2.76	97.24	2.80	97.20	3.07	96.93
2.97	97.03	3.06	96.94	3.34	96.66
3.14	96.86	3.21	96.79	3.51	96.49
3.26	96.74	3.28	96.72	3.69	96.31

Example 8: Stability Study of 140 mg/mL VEGF Trap with Varying Arginine Concentrations.

[00254] The impact of varying the concentration of arginine-HCl on the stability of four 140 mg/mL VEGF Trap (aflibercept) eye drug product formulations was studied. The evaluation of stability was performed at the storage condition of 2-8°C. The drug product (DP) was also incubated under stress (37°C) conditions. The four formulations being evaluated in this stability study (F1-F4) are described in Table 8-1 set forth below.

Table 8-1. Formulations F1-F4

Formulation Number	Composition					
	VEGFT (mg/mL)	Histidine (mM)	Sucrose (w/v)	Polysorbate 20	L-Arginine-	pH
F1	140	20	5	0.03	0	5.8
F2					10	
F3					50	
F4					100	

[00255] Approximately 145 mL of 187 mg/mL VEGF Trap histidine formulations were thawed. Thirty six (36) mL of each formulation, with the exception of F3, was compounded; 39 mL of bulk F3 was compounded. Each formulation was filter sterilized within a LFH (laminar flow hood) using a 0.22 µm Durapore PVDF sterilizing filter prior to filling. Clean depyrogenated 2 mL type I Schott glass vials stoppered with 13 mm serum stoppers (S2-F451 4432/50B2-40) were used to perform stability studies.

[00256] SE-UPLC analysis of the formulations was performed as set forth above. The %HMW measurements, using SE-UPLC, in each formulation over time are set forth in Figure 8 (A-B). See also Tables 8-2 and 8-3.

[00257] When stored at 5°C, these formulations exhibited a reduction in the rate of formation of %HMW species that was proportional to the concentration of arginine-HCl. Under stress, however, this effect was reversed. The formulations, at 37°C, exhibited an apparent increase in %HMW species proportional to the concentration of arginine-HCl. This property made the discovery of arginine's beneficial effects on aflibercept in the presence of histidine buffer highly unlikely. Typically, for formulation development in the biotechnology industry, the effect of various excipients on a drug is initially screened under stress (*e.g.*, at elevated temperature such as 37°C) for a short period of time. The purpose of this approach it to rapidly eliminate excipients which will not likely perform well in the absence of stress (*e.g.*, at lower temperature such as 5°C) over a longer period of time. Here, arginine was identified as a helpful excipient in spite of its effect on stability at 37°C. Since formulated drug product is typically stored for months at 4-5°C after manufacture, aflibercept in arginine

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and histidine buffer would be a highly valuable formulation for long term, stable drug product storage.

Table 8-2. SE-UPLC Analysis (2-8°C)

	F1 (EEE)		F2 (SSS)	
Months at 2-8C	%Main Peak	%HMW	%Main Peak	%HMW
0.0	97.79	2.21	97.78	2.22
0.5	97.56	2.43	97.59	2.41
1.0	97.30	2.70	97.34	2.66
1.5	97.21	2.79	97.26	2.74
2.0	97.05	2.95	97.08	2.92
2.5	96.96	3.04	97.02	2.98
3.0	96.82	3.18	96.88	3.12
6.0	96.35	3.65	96.40	3.60

F3 (CCC)		F4 (TTT)	
%Main Peak	%HMW	%Main Peak	%HMW
97.85	2.15	97.81	2.19
97.67	2.33	97.73	2.27
97.47	2.53	97.59	2.41
97.37	2.63	97.50	2.50
97.22	2.78	97.36	2.64
97.14	2.86	97.31	2.69
97.04	2.96	97.16	2.84
96.60	3.40	96.77	3.23

Table 8-3. SE-UPLC Analysis (37°C)

	F1 (EEE)		F2 (SSS)	
Days at 37C	%Main Peak	%HMW	%Main Peak	%HMW
0.0	97.79	2.21	97.78	2.22
7	93.66	6.34	93.32	6.68
14.0	89.48	10.35	88.79	10.98
21.0	86.64	13.08	85.69	13.82
28.0	83.90	15.85	82.66	16.90

F3 (CCC)		F4 (TTT)	
%Main Peak	%HMW	%Main Peak	%HMW
97.85	2.15	97.81	2.19
92.38	7.61	91.32	8.68
86.90	12.91	84.61	15.21
83.11	16.65	80.11	19.47
79.44	20.29	75.80	23.91

Example 9: VEGF Trap Stability in the presence of counterions from the Hofmeister Series and other Excipients.

[00258] The effect of various counterions and other excipients on stability of several formulations was determined.

A. Counterion Screening

[00259] Counter ions (*e.g.*, sulfate, thiocyanate and citrate) were tested in the form of sodium salts with high concentration VEGF Trap (aflibercept) formulations (140 mg/ml). Additionally, other amino add excipients (*e.g.*, glycine and lysine) were also tested.

[00260] Approximately 42 ml of 187 mg/ml VEGF Trap eye drug substance were thawed. 50 ml of intermediate formulated drug substance (155.56 mg/ml), having a 110% concentration of the 140 mg/ml formulated drug substance and target excipient concentration (excluding the counterions, glycine and lysine), were prepared.

[00261] The intermediate formulated drug substance was further diluted with the 0.5 M stock excipient solutions (counterions, glycine or lysine) to produce the 140 mg/ml formulated drug substance formulations listed in Table 9-1 below. Each final formulation was filter sterilized with a 0.22 µm PVDF syringe filter within a laminar flow hood prior to filling into clean depyrogenated 2mL type I Schott glass vials stoppered with 13 mm serum stoppers S2-F451 4432/50 82-40 (ELN item# 19700004 Wash# 0 000078949). Sixty (60) vials were filled with 0.5 ml of each formulation. Six (6) vials were filled with 1.5 ml.

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Table 9-1. Formulations for Counterion Screening

Excipient (mM)	Formulation	VEGF Trap (mg/ml)
Sodium Sulfate 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM Sodium Sulfate	140
Sodium thiocyanate 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM Sodium thiocyanate	140
Sodium Citrate 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM Sodium Citrate	140
Glycine 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM Glycine	140
Sodium chloride 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM sodium chloride	140
Lysine 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM Lysine	140

B. Glutamate And Aspartate Screening

[00262] The stability of VEGF Trap (aflibercept) high concentration formulations (140 mg/ml) in the presence of organic counter ions in combination with arginine hydrochloride was tested. In addition, two new counter ions (glutamate and asparate) were tested for compatibility with high concentration VEGF Trap (in combination and without arginine hydrochloride). The formulations tested are summarized below in Table 9-2.

[00263] Approximately 50 ml of 187 mg/ml VEGF Trap drug substance were thawed. 60 ml of intermediate formulated drug substance (155.56 mg/ml) with a 110% of the 140 mg/ml formulated drug substance and target excipient concentration (excluding the counterions, citrate,
glycine, glutamate and aspartate) was prepared. The intermediate formulated drug substance was further diluted with the 1M stock excipient solutions (arginine hydrochloride, sodium citrate, glycine, sodium glutamate and sodium aspartate) to produce the 140 mg/ml formulated drug substance formulations listed in the table below. Each final formulation was filter sterilized with a 0.22 µm PVDF syringe filter within a laminar flow hood prior to filling into clean depyrogenated 2 ml type I Schott glass vials stoppered with 13 mm serum stoppers S2-F451 4432/50 B2-40 (ELN item# 19700004 Wash# 0000078949). Sixty (60) vials were filled with 0.5 ml of each formulation. Six (6) vials were filled with 1.5 ml.

Table 9-2. Formulations for Glutamate And Aspartate Screening

Excipient (mM)	Formulation	VEGF Trap (mg/ml)
Sodium Citrate 50mM+ Arginine Hydrochloride 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM Sodium Citrate/50mM Arginine hydrochloride	140
Glycine 50mM+Arginine hydrochloride 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM Glycine/50mM Arginine hydrochloride	140
Sodium aspartate 50mM+Arginine hydrochloride 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM Sodium Aspartate/50mM Arginine hydrochloride	140
Sodium glutamate 50mM+Arginine hydrochloride 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM Sodium Glutamate/50mM Arginine hydrochloride	140
Sodium aspartate 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM sodium aspartate	140
Sodium glutamate 50mM	20mM Histidine/0.03%PS20/5% Sucrose/50mM Sodium glutmate	140

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[00264] Viscosity testing was performed using a RHEOSENSE m-VROC® viscometer. Approximately, 0.5 mL of undiluted sample was loaded onto a glass syringe. The sample was equilibrated to the desired temperature and injected into a chip or measuring cell. Viscosity was calculated by measuring the pressure drop from inlet to the outlet which is correlated to the shear-stress at the wall of the chip. Results are expressed as mPas-1 or cp. See Figure 9 and Table 9-3.

[00265] Osmolality testing was performed using a VAPRO vapor pressure osmometer. Approximately, 10 µL of undiluted sample was inoculated into a paper disc. The dew point temperature depression, a function of the solution vapor pressure, was measured through a sensitive thermocouple and reported as osmolality of the solution. Results are expressed as mmol/kg or mOsm. See Figure 10 and Table 9-3.

[00266] High molecular weight species in certain formulations over time, after storage at 37°C or 5°C, was also evaluated by SE-UPLC. See Figure 11 (A-B) and Tables 9-4 and 9-5.

[00267] Dynamic light scattering experiments were performed using a DynaPro® Plate Reader II. Approximately, 100 µL of undiluted sample was loaded onto a 96-well plate. Thirty five acquisitions were obtained for each well at 25°C. Analysis of autocorrelation function using regularization was performed on the DYNAMICS v7.1 software. Radius (nm) vs %intensity plots and % mass vs radius (nm) plots were generated to derive the average molecular radius and % polydispersity values (%Pd) for each sample. See Figure 12.

Table 9-3. Viscosity and Osmolality of Various Formulations

Parameter tested	50mM Sodium Sulfate (GGG)	50mM Sodium thiocyanate (HHH)	40mM Sodium citrate (III)	50mM Glycine (JJJ)	50mM Sodium Chloride (KKK)	50mM Lysine (LLL)
viscosity (cp)	11.9	12.4	14.8	12.2	11.6	11.8
Osmolality(mOsmol/Kg)	384	324	403	299	349	349

50mM Sodium Aspartate (MMM)	50mM Sodium Glutamate (NNN)	50mM Sodium citrate+50mM Arginine HCl (OOO)	50mM Glycine+ 50mM Arginine HCl (PPP)	50mM Sodium Aspartate+ 50mM Arginine HCl (QQQ)	50mM Sodium Glutamate + 50mM Arginine HCl (RRR)
13.0	13.1	14.8	12.5	13.1	13.3
343	346	506	369	426	430

Table 9-4. SE-UPLC Analysis; %HMW (37°C)

%HMW (Days at 37C)	50mM Sodium Sulfate (GGG)	50mM Sodium thiocyanate (HHH)	40mM Sodium citrate (III)	50mM Glycine (JJJ)	50mM Sodium Chloride (KKK)	50mM Lysine (LLL)
0	2.3	2.3	2.3	2.3	2.3	2.2
7	8.6	15.5	8.2	6.3	8.1	7.7
14	13.5	24.8	12.1	9.5	12.4	11.7
28	22.0	39.2	19.1	15.3	20.4	19.3

Table 9-5. SE-UPLC Analysis; %HMW (5°C)

%HMW (Months at 5C)	50mM Sodium Sulfate (GGG)	50mM Sodium thiocyanate (HHH)	40mM Sodium citrate (III)	50mM Glycine (JJJ)	50mM Sodium Chloride (KKK)	50mM Lysine (LLL)
0	2.3	2.3	2.3	2.3	2.3	2.2
0.25	2.3	2.4	2.4	2.3	2.3	2.3
0.5	2.4	2.5	2.5	2.4	2.4	2.3
1	2.5	2.8	2.7	2.6	2.6	2.5
2	2.8	2.9	2.9	2.8	2.8	2.8
3	3.1	3.3	3.2	3.1	3.2	3.0

Example 10: Tolerability of IVT delivery of High Dose VEGF Trap (140mg/ml) in Normal Rabbits.

[00268] Anti-VEGF therapeutics, administered via intravitreal injection, are currently the standard of care for treatment of neovascular age-related macular degeneration, diabetic macular edema, and retinal vascular occlusive disease. However, the monthly or bi-monthly intravitreal injections represent a significant treatment burden on patients, caregivers, and physicians. There is a pressing need for more effective and durable therapies in clinical practice. This study is to investigate the tolerability of stably formulated high dose VEGF Trap (140 mg/ml, equivalently 14 folds of the clinical dose) in normal New Zealand white rabbit eyes.

[00269] Two formulations of high dose VEGF-Trap (aflibercept) in histidine buffer or in phosphate buffer were tested. Three rabbits per cohort received 7mg of VEGF Trap in 50 uL by single bilateral intravitreal injection of each formulation. Ocular inflammation signs were monitored by slit lamp, optical coherence tomography (OCT) and fundus angiograph at day 1, day 4, week 1 then weekly until 12 weeks post-intravitreal administration. Intraocular pressure was measured with Tonopen before and 10 minutes and 30 minutes post intravitreal injection, then at every follow up time point. Animals were euthanized at week 12.

[00270] The rabbit eyes were dosed with either:

(1) 140 mg/ml aflibercept, 20 mM Histidine, 5% Sucrose, 50 mM Arginine HCl, 0.03% PS20, pH 5.8;

or

(2) 140 mg/ml aflibercept, 10 mM Sodium phosphate, 5% Sucrose, 40 mM Sodium Chloride, 0.03% PS20, pH 6.2.

[00271] Two of six eyes from the histidine buffer group showed dark shadows in the vitreous until 8 weeks post IVT, which were confirmed by slit-lamp examination as local cataract due to posterior lens injury, procedure related. One of 6 eyes the from phosphate buffer group showed dark shadows in the vitreous until 8 weeks post IVT, which were confirmed by slit-lamp examination as local cataract due to posterior lens injury (procedure related).

[00272] The high dose VEGF Trap (140 mg/ml) formulations tested were well tolerated in normal New Zealand White rabbit eyes within 12 weeks post-single intravitreal administration. After a single intravitreal injection of 7 mg of VEGF Trap, no signs of abnormalities or inflammation were seen in the fundus. The vitreous was clear and the retinal vascular pattern appeared normal. No retinal detachment, hemorrhage or optic nerve head changes were seen. There was no intraocular pressure change compared to baseline. The baseline fluorescein angiography (FA) and OCT images of rabbit eyes are represented in Figure 13(A-D). The time course FA and OCT images of rabbit eyes in each treatment group over 8 weeks is represented in figures 14, 15, 16 and 17. Data from the left eye (OS) or right eye (OD) of rabbits 326 and 329 are shown.

Example 11: Evaluation of Stability of Formulations UUU-BBBB

[00273] This study examined the stability of 60 mg/ml and 120 mg/ml VEGF Trap (aflibercept) in 10 mM phosphate, 10% or 20% sucrose (Suc.), 0 or 50 mM NaCl, 0.03% polysorbate 20, pH 6.2 when incubated at 37°C for up to 6 months. The formulations for this stability study are set forth below in Table 11-1.

Table 11-1. Formulations UUU-BBBB

Formulation Designation	[VEGF Trap] mg/mL	% Sucrose	mM NaCl
F1 (UUU)	30	10	-
F2 (VVV)	30	20	-
F3 (WWW)	60	10	-
F4 (XXX)	60	20	-
F5 (YYY)	120	10	-
F6 (ZZZ)	120	20	-
F7 (AAAA)	120	10	50
F8 (BBBB)	120	20	50

Base formulation: 10 mM phosphate, 0.03% polysorbate 20, pH 6.2

VEGF Trap=aflibercept

[00274] The formulation was filter sterilized with a PVDF 0.2 µm filter in a laminar flow hood prior to dispensing.

[00275] Vials containing each formulation were stored at 37°C for 1 month.

Table 11-2. Reverse-phase High Performance Liquid Chromatography (RP-HPLC) Results

Formulation	Storage Condition	Actual Incubation (months)	% VEGFT Recovered	[VGFT] mg/ml
30 mg/mL 10% sucrose (UUU)	37ºC	0.0	100.0	29.99
		1.0	97.9	29.37
30 mg/mL 20% sucrose (VVV)	37ºC	0.0	100.0	29.97
		1.0	98.2	29.44
60 mg/mL 10% sucrose (WWW)	37ºC	0.0	100.0	58.90
		1.0	98.3	57.89
60 mg/mL 20% sucrose (XXX)	37ºC	0.0	100.0	58.80
		1.0	98.9	58.18
120 mg/mL 10% sucrose (YYY)	37ºC	0.0	100.0	120.80
		1.0	97.4	117.63
120 mg/mL 20% sucrose (ZZZ)	37ºC	0.0	100.0	117.85
		1.0	98.9	116.51
120 mg/mL 10% sucrose 50 mM NaCl (AAAA)	37ºC	0.0	100.0	120.14
		1.0	97.9	117.64
120 mg/mL 20% sucrose 50 mM NaCl (BBBB)	37ºC	0.0	100.0	120.55
		1.0	97.0	116.94

VGFT=VEGF Trap, aflibercept

Table 11-3. Size-exclusion Ultra Performance Liquid Chromatography (SE-UPLC) Results

Formulation	Storage Condition	Actual Incubation (months)	Total % HMW	%Native	Total % LMW
30 mg/mL VGFT 10% sucrose (UUU)	37ºC	0	0.8	99.0	0.1
		1.0	4.1	94.0	1.9
30 mg/mL VGFT 20% sucrose (VVV)	37ºC	0	0.8	99.0	0.2
		1.0	2.4	95.6	2.0
60 mg/mL VGFT 10% sucrose (WWW)	37ºC	0	1.0	98.8	0.2
		1.0	7.8	89.5	2.7
60 mg/mL VGFT 20% sucrose (XXX)	37ºC	0	1.0	98.8	0.2
		1.0	4.4	92.8	2.7
120 mg/mL VGFT 10% sucrose (YYY)	37ºC	0	1.3	98.5	0.2
		1.0	18.3	79.5	2.2
120 mg/mL VGFT 20% sucrose (ZZZ)	37ºC	0	1.2	98.5	0.2
		1.0	10.7	87.1	2.2
120 mg/mL VGFT 10% sucrose 50 mM NaCl (AAAA)	37ºC	0	1.3	98.5	0.2
		1.0	20.8	77.1	2.2
120 mg/mL VGFT 20% sucrose 50 mM NaCl (BBBB)	37ºC	0	1.2	98.5	0.3
		1.0	13.0	84.8	2.2

VGFT=VEGF Trap, aflibercept

[00276] All of the formulations were essentially particle-free by visual inspection and optical density measurement. There was no significant protein loss in recovery by RP-HPLC after 1 month at 37□C (Table 11-2) and this trend continued out to 6 months.

[00277] The main degradation pathway for VEGF Trap was aggregation under these conditions (Figure 18 (A-B); Table 11-3). The stability of VEGF Trap was dependent on the protein and sucrose concentrations. Formulations with lower VEGF Trap concentrations and higher sucrose were more stable. Maintaining the same protein:sucrose ratio did not result in the same degradation rate. For example, F1 and F4 have the same protein:sucrose ratio of 3:1 but F4 was less stable than F1 due to its higher protein concentration. Similar trends were observed out to 6 months.

Example 12: Repeat-Dose Intravitreal Toxicology Study in Monkeys with Different Aflibercept Formulations.

[00278] The safety of various formulations in cynomolgus monkeys is assessed in this example. A total of 7 intravitreal doses are administered to both eyes approximately Q4W to Groups 1-3; a total of 3 intravitreal doses are administered to both eyes approximately Q4W to groups 4-9. A terminal necropsy is performed approximately one week after last dose (N=3/sex/group) with recovery necropsy (2/sex/group) approximately 12-week after last dose

[00279] The following assessments are performed:

- Safety assessment based on clinical signs, body weights, vital signs, electrocardiographic data (pre-dose end of treatment and end of recovery), blood pressure measurements (via tail cuff), clinical and anatomic pathology
- Comprehensive ocular exams at regular intervals during the dosing and recovery intervals including slit lamp microscopy, indirect ophthalmoscopy and intraocular pressure measurements
- Fundus photography, fluorescein angiography and electroretinography at pre-treatment, during study week 9 (all groups), week 26 (Groups 1-3 only) and end of recovery period
- Blood and vitreous samples (at termination) for bioanalytical, ADA analysis and toxicokinetic evaluation

Table 13. Study group summary*

Group	Test Material	IVT Dose Volume (µL/dose/eye)	mg aflibercept/eye	N/sex	N/sex for terminal necropsy	N/sex for Recovery
1	20 mM Histidine, **50 mM Arginine**, 5% Sucrose, 0.03% PS20	50	0 (control)	5	3	2
2	**80 mg/mL Aflibercept** in 20 mM Histidine, **50mM Arginine**, 5% Sucrose, 0.03% PS20	50	4	5	3	2
3	**~140 mg/mL Aflibercept** in 20 mM Histidine, **50mM Arginine**, 5% Sucrose, 0.03% PS20	50	~7	5	3	2
4	20 mM Histidine, 5% Sucrose, 0.03% PS20	50	0 (control)	5	3	2
5	**80 mg/mL Aflibercept** in 20 mM Histidine, 5% Sucrose, 0.03% PS20	50	4	5	3	2
6	**~140 mg/mL Aflibercept** in 20 mM Histidine, 5% Sucrose, 0.03% PS20	50	~7	5	3	2
7	20 mM Histidine, **50 mM Glycine**, 5% Sucrose, 0.03% PS20	50	0 (control)	5	3	2
8	**80 mg/mL Aflibercept** in 20 mM Histidine, **50 mM Glycine**, 5% Sucrose, 0.03% PS20	50	4	5	3	2
9	**~140 mg/mL Aflibercept** in 20 mM Histidine, **50 mM Glycine**, 5% Sucrose, 0.03% PS20	50	~7	5	3	2

*each of said formulations are part of the present invention

[00280] It is expected that the formulations tested in any one or more of Groups 1, 2, 3, 4, 5, 6, 7, 8 and/or 9 will be determined to have a safety profile comparable to EYLEA in cynomolgous monkeys as measured in the assessments discussed above.

Example 13: Stability Study with VEGF Trap Product.

[00282] A formulation including 114.3 mg/mL VEGF Trap (aflibercept) and 10 mM Histidine, pH 5.8, 5% Sucrose, 0.03% Polysorbate 20, 50 mM Arginine Monohydrochloride was prepared within a LFH (laminar flow hood) using a 0.22 µm Durapore PVDF sterilizing filter prior to filling. Clean depyrogenated 3 mL type I Schott glass vials were filled with 0.3 mL of Drug Product and stoppered with 13 mm serum stoppers (S2-F451 4432/50B2-40). Samples were analyzed at various timepoints after storage at 37°C or 5°C for purity by size-exclusion chromatography (SEC) to determine the presence (%) of high molecular weight species (HMW), low molecular weight species (LMW) and the main peak (main); as well as microflow imaging, HIAC liquid particle counting (by light obscuration) and microscopically to determine the presence of particles of various sizes.

[00283] Stability and purity data after storage at 37°C, 25°C and 2-8°C are shown in Figure 19 (A-E). See also Tables 13-1 – 13-4.

Table 13-1. Size-Exclusion Chromatography (SEC) Purity Analysis

37°C			
Months	**%Main**	**%HMW**	**%LMW**
0	98.23	1.77	0
0.229	92.88	7.07	0.05
0.459	88.59	11.18	0.23
0.688	84.67	15.03	0.3
0.918	81.84	17.59	0.57
5°C			
Months	**%Main**	**%HMW**	**%LMW**
0	98.23	1.77	0
1	97.73	2.27	0
2	97.4	2.59	0

Table 13-2. Microflow Imaging/Subvisible Particles

37°C					
Months	**≥1µm**	**≥2µm**	**≥5µm**	**≥10µm**	**≥25µm**
T=0	1125	234	42	9	0
28 days	1128	184	17	0	0

Table 13-3. Subvisible Particulate Matter as Measured by Light Obscuration (HIAC)/Subvisible Particles

37°C			
Months	**≥10µm**	**≥25µm**	**≥50µm**
T=0	15	0	0
28 days	23	1	1

Table 13-4. Microscopic Analysis of Subvisible Particles

37°C			
Months	**≥10µm**	**≥25µm**	**≥50µm**
T=0	8	1	1
28 days	6	2	1

Example 14: Stability Study with Varying VEGF Trap Concentrations.

[00284] The impact of varying the concentration of VEGF Trap on the stability of four VEGF Trap (aflibercept) eye drug product formulations was studied. The concentrations ranged between 80 and 140 mg/mL. The evaluation of stability was performed at the storage condition of 2-8°C. The drug product (DP) was also incubated under stress (37°C) conditions. The four formulations (each of which is named "CCC" herein) being evaluated in this stability study are described in Table 14-1 set forth below.

[00285] Approximately 27 mL of each formulation was prepared within a LFH (laminar flow hood) using a 0.22 µm Durapore PVDF sterilizing filter prior to filling. Clean depyrogenated 2 mL type I Schott glass vials were filled and stoppered with 13 mm serum stoppers (S2-F451 4432/50B2-40). Samples were analyzed at various timepoints after storage at 37°C or 2-8°C for purity by size-exclusion-ultraperformance liquid chromatography (SE-UPLC) to determine the presence (%) of high molecular weight species (HMW) and the main peak (main).

[00286] Data showing the percentage HMW after incubation at 2-8°C or 37°C for up to 6 months are set forth in Figure 20 (A-B). See also Tables 14-2 and 14-3. When stored at 37°C and 5°C, these formulations exhibited a positive correlation of the rate of HMW

formation to the concentration of VEGF Trap. The 140 mg/mL formulation exhibited highest rate of %HMW species formation.

Table 14-1. Formulations tested.

Formulation Number	composition					
	L-Arginine mM	Histidine (mM)	Sucrose (w/v)	Polysorbate 20 (w/v)	VEGFT (mg/mL)	pH
F1 (CCC)	50	20	5	0.03	80	5.8
F2 (CCC)					100	
F3 (CCC)					120	
F4 (CCC)					140	

Table 14-2. SE-UPLC Analysis (2-8°C)

	F1 (CCC): 80 mg/mL		F2 (CCC): 100 mg/mL		F4 (CCC): 120 mg/mL		F3 (CCC): 140 mg/mL	
Months at 2-8C	%Main Peak	%HMW	%Main Peak	%HMW	%Main Peak	%HMW	%Main Peak	%HMW
0.0	97.90	2.10	97.86	2.14	97.83	2.17	97.85	2.15
1	97.91	2.09	97.79	2.21	97.65	2.35	97.47	2.53
2.0	97.78	2.22	97.56	2.44	97.38	2.62	97.22	2.78
3.0	97.61	2.39	97.38	2.62	97.17	2.83	97.04	2.96
5.5	97.39	2.61	97.10	2.90	96.79	3.21		
6.0	97.36	2.64	97.04	2.96	96.74	3.26	96.60	3.40

Table 14-3. SE-UPLC Analysis (37°C)

	F1 (CCC): 80 mg/mL		F2 (CCC): 100 mg/mL		F4 (CCC): 120 mg/mL		F3 (CCC): 140 mg/mL	
Days at 37C	**%Main Peak**	**%HMW**	**%Main Peak**	**%HMW**	**%Main Peak**	**%HMW**	**%Main Peak**	**%HMW**
0.0	**97.90**	**2.10**	**97.86**	**2.14**	**97.83**	**2.17**	**97.85**	**2.15**
7	**94.83**	**5.15**	**93.82**	**6.17**	**92.81**	**7.18**	**92.38**	**7.61**
14.0	**92.00**	**7.77**	**90.31**	**9.39**	**88.67**	**11.13**	**86.90**	**12.91**
21.0	**89.47**	**10.31**	**87.00**	**12.58**	**85.04**	**14.85**	**83.11**	**16.65**
28.0	**86.76**	**12.71**	**83.98**	**15.55**	**81.19**	**18.46**	**79.44**	**20.29**

Example 15: High dose Eylea has Prolonged Duration of Action in the D,L-AAA Model of Sustained Neovascular Leak.

[00287] The effect of a four-fold increase in dose on duration of action of aflibercept to inhibit chronic retinal vascular leak in the D,L-AAA model was determined in this Example.

[00288] Animals used were New Zealand White rabbits 3 months post-DL-AAA (DL-α-aminoadipic acid) disease induction. The treatment groups were:

- Placebo (formulation buffer) 50 mcl/eye; n= 6 eyes
- Aflibercept 500 mcg/eye in 50 mcl; n= 7 eyes
- Aflibercept 2 mg/eye in 50 mcl; n= 8 eyes

[00289] The formulation used in each treatment group was: 10 mM Histidine, 8% sucrose, 0.03% PS20, pH 5.8. Ophthalmic exams, which were performed at baseline and at weeks 1, 2, 4, 6, 7, 9, 10, 11, 13 and 18, were intra-ocular pressure (IOP), red-free (RF) imaging (to determine blood vessel morphology), fluorescein angiography (FA; to determine vascular leak), optical coherence tomography (OCT; to identify vitreal inflammation). Serum (ADA) and plasma (drug levels) were collected at baseline and at weeks 1, 2, 4, 6 and 9.

[00290] Data from FA exams of each group are set forth in Figure 21. These data demonstrated that vascular leak inhibition in the retinas of animals receiving higher doses of aflibercept lasted longer than that of animals receiving lower doses. The number of treated eyes in which vascular permeability was completely inhibited was significantly greater in the 2 mg dose group at all sampling times up to 18 weeks post dosing.