STABILIZED FORMULATIONS CONTAINING ANTI-ANG2 ANTIBODIES

FIELD

[0001] The present invention relates to the field of therapeutic antibody formulations. More specifically, the present invention relates to the field of pharmaceutical formulations comprising an antibody that specifically binds to angiopoietin-2 (Ang-2).

SEQUENCE LISTING

[0002] An ST.25 compliant computer readable text file of a sequence listing is filed concurrently with the present specification according to PCT Rule 5.2 and Administrative Instructions Section 802. The contents of the text file are herein incorporated by reference. A paper copy of the sequence listing, which is identical in content to the ST.25 compliant computer readable text file, is included as part of the present specification and is herein incorporated by reference.

BACKGROUND

[0003] Angiogenesis is the biological process whereby new blood vessels are formed. Aberrant angiogenesis is associated with several disease conditions including, e.g., proliferative retinopathies, rheumatoid arthritis, and psoriasis. In addition, it is well established that angiogenesis is critical for tumor growth and maintenance. Angiopoietin-2 (Ang-2) is a ligand for the Tie-2 receptor (Tie-2) and has been shown to play a role in angiogenesis. Ang-2 is also referred to in the art as Tie-2 ligand. (US 5,643,755; Yancopoulos et al., 2000, Nature 407:242-248).

[0004] Antibodies and other peptide inhibitors that bind to Ang-2 are described to some extent in, e.g., US 6,166,185; 7,521,053; 7,205,275; 2006/0018909 and 2006/0246071. There is a need in the art for novel Ang-2 modulating agents, including Ang-2 antibodies, that can be used to treat diseases and conditions caused by or exacerbated by angiogenesis.

[0005] Therapeutic antibodies must be formulated in a manner that not only makes the antibodies suitable for administration to patients, but also in a manner that maintains their stability during storage and subsequent use. For example, therapeutic antibodies in liquid solution are prone to degradation, aggregation, or undesired chemical modifications unless the solution is formulated properly. The stability of an antibody in liquid formulation depends not only on the kinds of excipients used in the formulation, but also on the amounts and proportions of the excipients relative to one another. Furthermore, other considerations aside from stability must be taken into account when preparing a liquid antibody formulation.

Examples of such additional considerations include the viscosity of the solution and the concentration of antibody that can be accommodated by a given formulation, and the visual quality or appeal of the formulation. Thus, when formulating a therapeutic antibody, great care must be taken to arrive at a formulation that remains stable, contains an adequate concentration of antibody, and possesses a suitable viscosity as well as other properties which enable the formulation to be conveniently administered to patients.

[0006] Antibodies to the angiopoietin-2 protein (Ang-2) are one example of a therapeutically relevant macromolecule that requires proper formulation. Although some anti-Ang-2 antibodies are known, there nonetheless remains a need in the art for novel pharmaceutical formulations comprising anti-Ang-2 antibodies that are sufficiently stable and suitable for administration to patients.

SUMMARY

[0007] The present invention satisfies the aforementioned need by providing pharmaceutical formulations comprising a human antibody that specifically binds to human angiopoietin-2 (Ang-2).

[0008] **In one aspect**, a liquid pharmaceutical formulation is provided, comprising: (i) an antibody that specifically binds to angiopoietin-2 (Ang-2); (ii) a buffer; (iii) an organic cosolvent; and (iv) a stabilizer.

[0009] In one embodiment, the antibody is provided at a concentration from about 5 ± 0.75 mg/mL to about 150 ± 22.5 mg/mL. In another embodiment, the antibody is provided at a concentration of about 5 mg/ml ± 0.75 mg/mL. In another embodiment, the antibody is provided at a concentration of about 25 mg/mL ± 3.75 mg/mL. In another embodiment, the antibody is provided at a concentration of about 50 mg/mL ± 7.5 mg/mL.

[0010] In some embodiments, exemplary anti-Ang-2 antibodies and Ang-2 antigen-binding fragments of the invention comprise HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 domains, respectively, selected from the group consisting of: (i) SEQ ID NO: 4, 6, 8, 12, 14 and 16 (e.g., H1H685); (ii) SEQ ID NO: 28, 30, 32, 36, 38 and 40 (e.g., H1H690); (iii) SEQ ID NO: 52, 54, 56, 60, 62 and 64 (e.g., H1H691); (iv) SEQ ID NO: 148, 150, 152, 156, 158 and 160 (e.g., H1H696); (v) SEQ ID NO: 196, 198, 200, 204, 206 and 208 (e.g., H1H706); (vi) SEQ ID NO: 268, 270, 272, 276, 278 and 280 (e.g., H1M724); and (vii) SEQ ID NO: 436, 438, 440, 444, 446 and 448 (e.g., H2M744).

[0011] In related embodiments, the invention comprises an anti-Ang-2 antibody or an antigen-binding fragment of an antibody which specifically binds Ang-2, wherein the antibody or antigen-binding fragment comprises the heavy and light chain CDR domains (i.e., CDR1, CDR2 and CDR3) contained within heavy and light chain variable domain sequences

selected from the group consisting of SEQ ID NO: 2/10, 18/20, 22/24, 26/34, 42/44, 46/48, 50/58, 66/68, 70/72, 74/82, 90/92, 94/96, 98/106, 114/116, 118/120, 122/130, 138/140, 142/144, 146/154, 162/164, 166/168, 170/178, 186/188, 190/192, 194/202, 210/212, 214/216, 218/226, 234/236, 238/240, 242/250, 258/260, 262/264, 266/274, 282/284, 286/288, 290/298, 306/308, 310/312, 314/322, 330/332, 334/336, 338/346, 354/356, 358/360, 362/370, 378/380, 382/384, 386/394, 402/404, 406/408, 410/418, 426/428, 430/432, 434/442, 450/452, 454/456, 458/466, 474/476, 478/480, 482/490, 498/500, and 502/504. In one embodiment, the antibody or fragment thereof comprises the CDR sequences contained within HCVR and LCVR selected from the amino acid sequence pairs of SEQ ID NO: 18/20, 42/44, 66/68, 162/164, 210/212, 266/274, and 434/442.

[0012] In one embodiment, the pH of the liquid formulation is about pH 6.0 ± 0.5, pH 6.0 ± 0.4, pH 6.0 ± 0.3, pH 6.0 ± 0.2, pH 6.0 ± 0.1, pH 6.0 ± 0.05, pH 6.0 ± 0.01, or pH 6.0. In a specific embodiment, the pH of the liquid formulation is about pH 6.0 ± 0.3. In one embodiment, the liquid pharmaceutical buffer comprises one or more buffers, which has or have an effective buffering range of about pH 5.5 to about pH 7.4, or a pKa of about 6.0.

[0013] In one embodiment, the buffer is histidine. In one embodiment, the histidine is at a concentration of 5 mM ± 0.75 mM to 50 mM ± 7.5 mM. In one embodiment, the histidine is at a concentration of 5 mM ± 0.75 mM or about 5 mM. In one embodiment, the histidine is at a concentration of 10 mM ± 1.5 mM or about 10 mM. In one embodiment, the histidine is at a concentration of 15 mM ± 2.25 mM or about 15 mM. In one embodiment, the histidine is at a concentration of 20 mM ± 3 mM or about 20 mM. In one embodiment, the histidine is at a concentration of 25 mM ± 3.75 mM or about 25 mM. In one embodiment, the histidine is at a concentration of 30 mM ± 4.5 mM or about 30 mM. In one embodiment, the histidine is at a concentration of 35 mM ± 5.25 mM or about 35 mM. In one embodiment, the histidine is at a concentration of 40 nM ± 6 mM or about 40 nM. In one embodiment, the histidine is at a concentration of 45 mM ± 6.75 mM or about 45 mM. In one embodiment, the histidine is at a concentration of 50 mM ± 7.5 mM or about 50 mM.

[0014] In one embodiment, the organic cosolvent is a nonionic polymer containing a polyoxyethylene moiety. In some embodiments, the organic cosolvent is any one or more of polysorbate 20, poloxamer 188 and polyethylene glycol 3350. In a specific embodiment, the organic cosolvent is polysorbate 20.

[0015] In one embodiment, the organic cosolvent is at a concentration of from about 0.005% ± 0.00075% to about 1% ± 0.15% “weight to volume” or “w/v”, wherein, *e.g.,* 0.1 g/ml = 10% and 0.01 g/ml = 1%. In one embodiment, the organic cosolvent is polysorbate 20, which is at a concentration of about 0.2% ± 0.03% w/v. In another embodiment, the

organic cosolvent is polysorbate 20, which is at a concentration of 0.01% ± 0.0015% w/v or about 0.01% w/v.

[0016] In one embodiment, the stabilizer is a sugar. In one embodiment, the sugar is selected from the group consisting of sucrose, mannitol and trehalose. In a specific embodiment, the stabilizer is sucrose.

[0017] In one embodiment, the stabilizer is at a concentration of from 1% ± 0.15% w/v to 20% ± 3% w/v. In a specific embodiment, the stabilizer is sucrose at a concentration of 5% ± 0.75% w/v or about 5% w/v. In another specific embodiment, the stabilizer is sucrose at a concentration of 7.5% ± 1.125% w/v or about 7.5% w/v. In another specific embodiment, the stabilizer is sucrose at a concentration of 10% ± 1.5% w/v or about 10% w/v. In another specific embodiment, the stabilizer is sucrose at a concentration of 12.5% ± 1.875% w/v or about 12.5% w/v. In another specific embodiment, the stabilizer is sucrose at a concentration of 15% ± 2.25% w/v or about 15% w/v. In another specific embodiment, the stabilizer is sucrose at a concentration of 20% ± 3% w/v or about 20% w/v.

[0018] In one embodiment, the viscosity of the formulation is about 1 cPoise to about 10 cPoise. In one embodiment, the viscosity of the formulation is 1.4 cPoise ± 0.21 cPoise, or about 1.4 cPoise.

[0019] In one embodiment, the osmolality of the formulation is within a physiological range. In one embodiment, the formulation has an osmolality of about 300 milli-Osmoles per kilogram (mOsm) to about 400 mOsm. In one embodiment, the osmolality of the formulation is 363 mOsm ± 54 mOsm, or about 363 mOsm.

[0020] In one embodiment, at least 96% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after six months of storage at -80ºC is non-aggregated and un-degraded, as determined by size exclusion chromatography. In one embodiment, at least 55% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after six months of storage at -80ºC is of the non-basic and non-acidic form (*i.e.,* main peak or main charge form or "region 2 peak"), as determined by ion exchange chromatography.

[0021] In one embodiment, at least 96% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after six months of storage at -30ºC is non-aggregated and un-degraded, as determined by size exclusion chromatography. In one embodiment, at least 55% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after six months of storage at -30ºC is of the main charge form, as determined by ion exchange chromatography.

[0022] In one embodiment, at least 96% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after six months of storage at -20ºC is non-

aggregated and un-degraded, as determined by size exclusion chromatography. In one embodiment, at least 55% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after six months of storage at -20°C is of the main charge form, as determined by ion exchange chromatography.

[0023] In one embodiment, at least 96% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after nine months of storage at 5°C is of the non-aggregated and un-degraded form, as determined by size exclusion chromatography. In one embodiment, at least 56% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after nine months of storage at 5°C is of the main charge form, as determined by ion exchange chromatography.

[0024] In one embodiment, at least 98% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after three months of storage at 25°C is of the non-aggregated and un-degraded form, as determined by size exclusion chromatography. In one embodiment, at least 54% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after three months of storage at 25°C is of the main charge form, as determined by ion exchange chromatography.

[0025] In one embodiment, at least 97% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after one month of storage at 37°C is of the non-aggregated and un-degraded form, as determined by size exclusion chromatography. In one embodiment, at least 47% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after one month of storage at 37°C is of the main charge form form, as determined by ion exchange chromatography.

[0026] In one embodiment, at least 95% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after 28 days of storage at 45°C is of the non-aggregated and un-degraded form, as determined by size exclusion chromatography. In one embodiment, at least 32% of the anti-Ang-2 antibody recovered from the liquid pharmaceutical formulation after 28 days of storage at 45°C is of the main charge form, as determined by ion exchange chromatography.

[0027] In one aspect, a liquid pharmaceutical formulation is provided, comprising: (i) from 5 ± 0.75 mg/ml to 150 ± 22.5 mg/ml of a human antibody that specifically binds to human Ang-2; (ii) from 5 mM ± 0.75 mM to 50 mM ± 7.5 mM histidine; (iii) from 0.005% ± .000075% to 1% ± 0.15% (w/v) polysorbate 20; and (iv) from 1% ± 0.15% to 20% ± 3% (w/v) sucrose, at a pH of from about 5.5 to about 6.5. The anti-Ang-2 antibody of this aspect comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR) such that the HCVR / LCVR combination comprises heavy and light chain complementarity determining regions (HCDR1-HCDR2-HCDR3 / LCDR1-LCDR2-LCDR3), which comprise the amino acid

sequences of SEQ ID NOs:4 – 6 – 8 / SEQ ID NOs:12 – 14 – 16, respectively. In a particular embodiment, the anti-Ang-2 antibody comprises a heavy chain variable region (HCVR) and light chain variable region (LCVR) comprising an amino acid sequence of SEQ ID NO: 18 and SEQ ID NO: 20, respectively (antibody HIH685P of U.S. Pub. Pat. App. No. 20110027286, which is specifically incorporated herein by reference in its entirety).

[0028] In another embodiment, the liquid formulation comprises (i) 50 ± 7.5 mg/mL of HIH685P; (ii) 10 ± 1.5 mM histidine; (iii) 0.2% ± 0.03% (w/v) polysorbate 20; and (iv) 10% ± 1.5% (w/v) sucrose, at a pH of 6.0 ± 0.5.

[0029] In one embodiment of this aspect, the liquid formulation comprises (i) 25 ± 3.75 mg/mL of HIH685P; (ii) 10 ± 1.5 mM histidine; (iii) 0.2% ± 0.03% (w/v) polysorbate 20; and (iv) 10% ± 1.5% (w/v) sucrose, at a pH of 6.0 ± 0.5. In one embodiment of this particular formulation, after storage of the formulation at 45° for 28 days, ≥ 95% of the antibody is native and ≥ 32% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at 37° for one month, ≥ 97% of the antibody is native and ≥ 47% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at 25° for three months, ≥ 98% of the antibody is native and ≥ 54% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at 5° for nine months, ≥ 96% of the antibody is native and ≥ 56% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at -20° for three months, ≥ 9% of the antibody is native and ≥ 55% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at -30° for six months, ≥ 96% of the antibody is native and ≥ 55% of the antibody is of the main charge form. In one embodiment of this particular formulation, after storage of the formulation at -80° for six months, ≥ 96% of the antibody is native and ≥ 55% of the antibody is of the main charge form.

[0030] **In one aspect**, a liquid pharmaceutical formulation of any of the preceding aspects is provided in a container. In one embodiment, the container is a polycarbonate vial. In another embodiment, the container is a glass vial. In one embodiment, the glass vial is a type 1 borosilicate glass vial with a fluorocarbon-coated butyl rubber stopper. In another embodiment, the container is a microinfuser. In another embodiment, the container is a syringe. In a specific embodiment, the syringe comprises a fluorocarbon-coated plunger. In one specific embodiment, the syringe is a 1 mL long glass syringe containing less than about 500 parts per billion of tungsten equipped with a 27-G needle, a fluorocarbon–coated butyl rubber stopper, and a latex-free, non-cytotoxic rubber tip cap. In a more specific embodiment, the syringe is a NUOVA OMPI 1 mL long glass syringe equipped with a 27-G

thin wall needle, a FLUROTEC–coated 4023/50 rubber stopper, and a FM 27 rubber tip cap. In another specific embodiment, the syringe is a l mL or 3 mL plastic syringe fitted with a 27-G needle. In a more specific embodiment, the plastic syringe is distributed by BECTON DICKINSON. In one embodiment, the container is a polyvinyl chloride IV bag. In another embodiment, the container is a polyolefin IV bag.

[0031] In one aspect, a pharmaceutical formulation comprising (a) 50 mg/mL ± 7.5 mg/mL of an anti-Ang-2 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.5, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose is provided, wherein (a) the antibody comprises an HCVD of SEQ ID NO: 18 and an LCVD of SEQ ID NO: 20, (b) over 96% of the antibodies in the formulation have a molecular weight of about 150.9 kDa ± 1 kDa, (c) at least 53% of the antibodies in the formulation have an isoelectric point of about 8.13 ± 0.01, (d) from about 90% to about 92% of the antibodies in the formulation are fucosylated, and (e) about 2.5% of the heavy chains of the antibodies lack a C-terminal lysine.

[0032] In one embodiment, the pharmaceutical formulation consists of (a) 50 mg/mL ± 7.5 mg/mL of an anti-Ang-2 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.5, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose is provided, wherein (a) the antibody comprises an HCVD of SEQ ID NO: 18 and an LCVD of SEQ ID NO: 20, (b) over 96% of the antibodies in the formulation have a molecular weight of about 150.9 kDa ± 1 kDa, (c) at least 53% of the antibodies in the formulation have an isoelectric point of about 8.13 ± 0.01, (d) from about 90% to about 92% of the antibodies in the formulation are fucosylated, and (e) about 2.5% of the heavy chains of the antibodies lack a C-terminal lysine.

[0033] In one aspect, a pharmaceutical formulation comprising (a) 25 mg/mL ± 3.75 mg/mL of an anti-Ang-2 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.5, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose is provided, wherein (a) the antibody comprises an HCVD of SEQ ID NO: 18 and an LCVD of SEQ ID NO: 20, (b) over 96% of the antibodies in the formulation have a molecular weight of about 150.9 kDa ± 1 kDa, (c) at least 53% of the antibodies in the formulation have an isoelectric point of about 8.13 ± 0.01, (d) from about 90% to about 92% of the antibodies in the formulation are fucosylated, and (e) about 2.5% of the heavy chains of the antibodies lack a C-terminal lysine.

[0034] In one embodiment, the pharmaceutical formulation consists of (a) 25 mg/mL ± 3.75 mg/mL of an anti-Ang-2 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.5, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose is provided, wherein (a) the antibody comprises an HCVD of SEQ ID NO: 18 and an LCVD of SEQ ID NO: 20, (b) over 96% of the antibodies in the formulation have a molecular weight of about 150.9 kDa ± 1

kDa, (c) at least 53% of the antibodies in the formulation have an isoelectric point of about 8.13 ± 0.01, (d) from about 90% to about 92% of the antibodies in the formulation are fucosylated, and (e) about 2.5% of the heavy chains of the antibodies lack a C-terminal lysine.

[0035] In one aspect, a kit comprising a pharmaceutical composition of any one of the preceding aspects, a container, and instructions is provided. In one embodiment, the container is a prefilled syringe. In one embodiment, the container is a borosilicate vial fitted with a FLUROTEC–coated 4023/50 rubber stopper.

[0036] Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

DETAILED DESCRIPTION

[0037] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0038] 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 this invention belongs. As used herein, the term "about", when used in reference to a particular recited numerical value or range of values, means that the value may vary from the recited value by no more than 2%. For example, as used herein, the expression "about 100" includes 98 and 102 and all values in between (*e.g.*, 98.00, 98.01, 98.02, 98.03, 98.04, …, 101.96, 101.97, 101.98, 101.99, 102.00).

[0039] Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to describe in their entirety.

PHARMACEUTICAL FORMULATIONS

[0040] As used herein, the expression "pharmaceutical formulation" means a combination of at least one active ingredient (*e.g.*, a small molecule, macromolecule, compound, etc. which is capable of exerting a biological effect in a human or non-human animal), and at least one inactive ingredient which, when combined with the active ingredient or one or more additional inactive ingredients, is suitable for therapeutic administration to a human or non-human animal. The term "formulation", as used herein, means "pharmaceutical formulation" unless specifically indicated otherwise. The present invention provides pharmaceutical

formulations comprising at least one therapeutic polypeptide. According to certain embodiments of the present invention, the therapeutic polypeptide is an antibody, or an antigen-binding fragment thereof, which binds specifically to human angiopoietin-2 (Ang-2) protein. More specifically, the present invention includes pharmaceutical formulations that comprise: (i) a human antibody that specifically binds to human Ang-2 (ii) a histidine buffer; (iii) an organic cosolvent that is a non-ionic surfactant; and (iv) a thermal stabilizer that is a carbohydrate. Specific exemplary components and formulations included within the present invention are described in detail below.

ANTIBODIES THAT BIND SPECIFICALLY TO ANG-2

[0041] The pharmaceutical formulations of the present invention may comprise a human antibody, or an antigen-binding fragment thereof, that binds specifically to human Ang-2. As used herein, the term "Ang-2" or "ANG2" means a human angiopoietin-2, which is generally known as an autocrine antagonist of Tie2 activation. Ang-2 is generally known in the art to "prime" the vascular endothelium to receive the effects of cytokines. Ang-2 is strongly expressed in tumor vasculature, and is generally thought to act synergistically with other cytokines (*i.e.,* vascular endothelial growth factor) to promote angiogenesis and tumor progression. An exemplary human Ang-2 amino acid sequence is described in SEQ ID NO: 518. Antibodies to human Ang-2 are described in patent application publications US 2010/0166768, US 2011/0065902, WO 2010/077854, and US 2011/0027286, which are herein incorporated by reference.

[0042] The term "antibody", as used herein, is generally intended to refer to immunoglobulin molecules comprising four polypeptide chains: two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, as well as multimers thereof (*e.g.*, IgM); however, immunoglobulin molecules consisting of only heavy chains (*i.e.*, lacking light chains) are also encompassed within the definition of the term "antibody". Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region comprises three domains, CH1, CH2 and CH3. Each light chain comprises a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region comprises one domain (CL1). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementary determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0043] Unless specifically indicated otherwise, the term "antibody", as used herein, shall be understood to encompass complete antibody molecules as well as antigen-binding

fragments thereof. The term "antigen-binding portion" or "antigen-binding fragment" of an antibody (or simply "antibody portion" or "antibody fragment"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to human Ang-2 or an epitope thereof.

[0044] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.,* an isolated antibody that specifically binds human Ang-2 is substantially free of antibodies that specifically bind antigens other than human Ang-2).

[0045] The term "specifically binds", or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by a dissociation constant of at least about $1x10^{-6}$ M or greater. Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. An isolated antibody that specifically binds human Ang-2 may, however, have cross-reactivity to other antigens, such as Ang-2 molecules from other species (orthologs). In the context of the present invention, multispecific (*e.g.*, bispecific) antibodies that bind to human Ang-2 as well as one or more additional antigens are deemed to "specifically bind" human Ang-2. Moreover, an isolated antibody may be substantially free of other cellular material or chemicals.

[0046] Exemplary anti-human Ang-2 antibodies that may be included in the pharmaceutical formulations of the present invention are set forth in patent application publications US 2010/0166768, US 2011/0065902, US 2011/0027286 and WO 2010/077854, the disclosures of which are incorporated herein by reference in their entirety.

[0047] According to certain embodiments of the present invention, the anti-human Ang-2 HIH685P antibody is a human IgG1 comprising a heavy chain variable region that is of the IGHV3-13.01 subtype and a light chain variable region that is of the IGKV3-20.01 subtype (see Barbie and Lefranc, The Human Immunoglobulin Kappa Variable (IGKV) Genes and Joining (IGKJ) Segments, Exp. Clin. Immunogenet. 1998; 15:171-183; and Scaviner, D. et al., Protein Displays of the Human Immunoglobulin Heavy, Kappa and Lambda Variable and Joining Regions, Exp. Clin. Immunogenet., 1999; 16:234-240). The germline IGHV3-13 and IGKV3-20 sequences, and the amino acid position assignment numbers presented herein comport with the international Immunogenetics (IMGT) information system, as described in Lefranc, M.-P., et al., IMGT®, the international ImMunoGeneTics information system®, Nucl. Acids Res, 37, D1006-D1012 (2009).

[0048] In some embodiments, the anti-human Ang-2 HIH685P comprises at least one amino acid substitution relative to the canonical heavy chain variable region, which results in a change in the angle of rotation of the peptide chain within a CDR, which is reasonably

expected to alter the exposed surface of the antibody relative to the germline IGHV3-13 sequence. In some embodiments, the amino acid substitution comprises the sustitution of proline for the isoleucine at position 39 within CDR2 of IGHV3-13.

[0049] In some embodiments, the anti-human Ang-2 HIH685P antibody comprises at least one amino acid substitution, which creates a charge change within the third CDR of the germline IGKV3-20. In some embodiments, the amino acid substitution or substitutions are selected from the group consisting of (a) a basic amino acid substituted for an uncharged polar amino acid within CDR3 (*e.g.,* at position 106) of IGHV3-20, and (b) an acidic amino acid substituted for an uncharged polar amino acid within CDR3 (*e.g.,* at position 108) of IGKV3-20. Changes in the charge display at the CDR surface is expected to affect the antibody's interface with the solvent, and thus create unpredictable conditions for maintaining or advancing the stability of the antibody in solution.

[0050] According to certain embodiments of the present invention, the anti-human Ang-2 antibody, or antigen-binding fragment thereof, comprises a heavy chain complementary determining region (HCDR) 1 of SEQ ID NO: 4, an HCDR2 of SEQ ID NO: 6, and an HCDR3 of SEQ ID NO: 8. In certain embodiments, the anti-human Ang-2 antibody, or antigen-binding fragment thereof, comprises an HCVD of SEQ ID NO: 18.

[0051] According to certain embodiments of the present invention, the anti-human Ang-2, or antigen-binding fragment thereof, comprises a light (kappa) chain complementary determining region (LCDR) 1 of SEQ ID NO: 12, an LCDR2 of SEQ ID NO: 14, and an LCDR3 of SEQ ID NO: 16. In certain embodiments, the anti-human Ang-2 antibody, or antigen-binding fragment thereof, comprises an LCVD of SEQ ID NO: 20.

[0052] The non-limiting, exemplary antibody used in the Examples herein is referred to as "HIH685P", as in US 2011/0027286. This antibody comprises an HCVR/LCVR amino acid sequence pair having SEQ ID NOs: 18/20, and HCDR1-HCDR2-HCDR3 / LCDR1-LCDR2-LCDR3 domains represented by SEQ ID NOs: 4 – 6 – 8 / SEQ ID NOs: 12 – 14 – 16.

[0053] The amount of antibody, or antigen-binding fragment thereof, contained within the pharmaceutical formulations of the present invention may vary depending on the specific properties desired of the formulations, as well as the particular circumstances and purposes for which the formulations are intended to be used. In certain embodiments, the pharmaceutical formulations are liquid formulations that may contain 5 ± 0.75 mg/mL to 150 ± 22.5 mg/mL of antibody; 7.5 ± 1.125 mg/mL to 140 ± 21 mg/mL of antibody; 10 ± 1.5 mg/mL to 130 ± 19.5 mg/mL of antibody; 12.5 ± 1.875 mg/mL to 120 ± 18 mg/mL of antibody; 15 ± 2.25 mg/mL to 110 ± 16.5 mg/mL of antibody; 17.5 ± 2.625 mg/mL to 100 ± 15 mg/mL of antibody; 20 ± 3 mg/mL to 90 ± 13.5 mg/mL of antibody; 22.5 ± 3.375 mg/mL to 80 ± 12 mg/mL of antibody; 25 ± 3.75 mg/mL to 70 ± 10.5 mg/mL of antibody; 27.5 ± 4.125

mg/mL to 60 ± 9 mg/mL of antibody; 30 ± 4.5 mg/mL to 50 ± 7.5 mg/mL of antibody; 25 ± 3.75 mg/mL of antibody; or 50 ± 7.5 mg/ml. For example, the formulations of the present invention may comprise about 20 mg/mL; about 25 mg/mL; about 30 mg/mL; about 35 mg/mL; about 40 mg/mL; about 45 mg/mL; about 50 mg/mL; about 55 mg/mL; or about 60 mg/mL of an antibody or an antigen-binding fragment thereof, that binds specifically to human Ang-2.

EXCIPIENTS AND PH

[0054] The pharmaceutical formulations of the present invention comprise one or more excipients. The term "excipient", as used herein, means any non-therapeutic agent added to the formulation to provide a desired consistency, viscosity or stabilizing effect.

[0055] In certain embodiments, the pharmaceutical formulation of the invention comprises at least one organic cosolvent in a type and in an amount that stabilizes the human Ang-2 antibody under conditions of rough handling or agitation, such as, *e.g.,* vortexing. In some embodiments, what is meant by "stabilizes" is the prevention of the formation of more than 4% aggregated antibody of the total amount of antibody (on a molar basis) over the course of rough handling. In some embodiments, rough handling is vortexing a solution containing the antibody and the organic cosolvent for about 60 minutes or about 120 minutes.

[0056] In certain embodiments, the organic cosolvent is a non-ionic surfactant, such as an alkyl poly(ethylene oxide). Specific non-ionic surfactants that can be included in the formulations of the present invention include, *e.g.*, polysorbates such as polysorbate 20, polysorbate 28, polysorbate 40, polysorbate 60, polysorbate 65, polysorbate 80, polysorbate 81, and polysorbate 85; poloxamers such as poloxamer 181, poloxamer 188, poloxamer 407; or polyethylene glycol (PEG). Polysorbate 20 is also known as TWEEN 20, sorbitan monolaurate and polyoxyethylenesorbitan monolaurate. Poloxamer 188 is also known as PLURONIC F68.

[0057] The amount of non-ionic surfactant contained within the pharmaceutical formulations of the present invention may vary depending on the specific properties desired of the formulations, as well as the particular circumstances and purposes for which the formulations are intended to be used. In certain embodiments, the formulations may contain 0.01% ± 0.0015% to 1% ± 0.15% surfactant. For example, the formulations of the present invention may comprise about 0.0085%; about 0.01%; about 0.02%; about 0.03%; about 0.04%; about 0.05%; about 0.06%; about 0.07%; about 0.08%; about 0.09%; about 0.1%; about 0.11%; about 0.12%; about 0.13%; about 0.14%; about 0.15%; about 0.16%; about 0.17%; about 0.18%; about 0.19%; about 0.20%; about 0.21%; about 0.22%; about 0.23%; about 0.24%; about 0.25%; about 0.3%; about 0.4%; about 0.5%; about 0.6%; about 0.7%;

about 0.8%; about 0.9%; about 1%; about 1.1%; about 1.15%; or about 1.2% polysorbate 20 or poloxamer 188.

[0058] The pharmaceutical formulations of the present invention may also comprise one or more stabilizers in a type and in an amount that stabilizes the human Ang-2 antibody under conditions of thermal stress. In some embodiments, what is meant by "stabilizes" is maintaining greater than about 93% of the antibody in a native conformation when the solution containing the antibody and the thermal stabilizer is kept at about 45°C for up to about 28 days. In some embodiments, what is meant by "stabilizes" is wherein less than about 4% of the antibody is aggregated when the solution containing the antibody and the thermal stabilizer is kept at about 45°C for up to about 28 days. In some embodiments, what is meant by "stabilizes" is maintaining greater than about 96% of the antibody in a native conformation when the solution containing the antibody and the thermal stabilizer is kept at about 37°C for up to about 28 days. In some embodiments, what is meant by "stabilizes" is wherein less than about 2% of the antibody is aggregated when the solution containing the antibody and the thermal stabilizer is kept at about 37°C for up to about 28 days. As used herein, "native" means the major form of the antibody by size exclusion, which is generally an intact monomer of the antibody.

[0059] In certain embodiments, the thermal stabilizer is a sugar or sugar alcohol selected from sucrose, sorbitol, glycerol, trehalose and mannitol, or any combination thereof, the amount of which contained within the formulation can vary depending on the specific circumstances and intended purposes for which the formulation is used. In certain embodiments, the formulations may contain about 3% to about 20% sugar or sugar alcohol; about 4% to about 19% sugar or sugar alcohol; about 5 % to about 18% sugar or sugar alcohol; about 6% to about 17% sugar or sugar alcohol; about 7% to about 16% sugar or sugar alcohol; about 8% to about 15% sugar or sugar alcohol; about 9% to about 16% sugar or sugar alcohol; about 7% to about 13% sugar or sugar alcohol; about 8% to about 12% sugar or sugar alcohol; about 9% to about 11% sugar or sugar alcohol; or about 10% sugar or sugar alcohol. For example, the pharmaceutical formulations of the present invention may comprise 4%± 0.6%; 5% ± 0.75%; 6% ± 0.9%; 7% ± 1.05%; 8% ± 1.2%; 9% ± 1.35%; 10% ± 1.5%; 11% ± 1.65%; 12% ± 1.8%; 13% ± 1.95%; or about 14% ± 2.1% sugar or sugar alcohol (*e.g.,* sucrose, trehalose or mannitol).

[0060] The pharmaceutical formulations of the present invention may also comprise a buffer or buffer system, which serves to maintain a stable pH and to help stabilize the human Ang-2 antibody. In some embodiments, what is meant by "stabilizes" is wherein less than 5% ± 0.5% or no more than about 4.3% of the antibody is aggregated when the solution containing the antibody and the buffer is kept at about 45°C for up to about 28 days. In some

embodiments, what is meant by "stabilizes" is wherein at least 92% ± 0.5% of the antibody is in its native conformation as determined by size exclusion chromatography when the solution containing the antibody and the buffer is kept at about 45°C for up to about 28 days. By "native" or "native conformation", what is meant is the antibody fraction that is not aggregated or degraded. This is generally determined by an assay that measures the relative size of the antibody entity, such as a size exclusion chromatographic assay. The non-aggregated and non-degraded antibody elutes at a fraction that equates to the native antibody, and is generally the main elution fraction. Aggregated antibody elutes at a fraction that indicates a size greater than the native antibody. Degraded antibody elutes at a fraction that indicates a size less than the native antibody.

[0061] In some embodiments, what is meant by "stabilizes" is wherein at least 52% ± 0.5% of the antibody is in its main charge form as determined by cation exchange chromatography when the solution containing the antibody and the buffer is kept at about 45°C for up to about 28 days. By "main charge" or "main charge form", what is meant is the fraction of antibody that elutes from an ion exchange resin in the main peak, which is generally flanked by more "basic" peaks on one side and more "acidic" peaks on the other side.

[0062] The pharmaceutical formulations of the present invention may have a pH of from about 5.5 to about 6.5. For example, the formulations of the present invention may have a pH of 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; or about 6.5. In some embodiments, the pH is 6.0 ± 0.4; 6.0 ± 0.3; 6.0 ±0.2; 6.0± 0.1; about 6.0; or 6.0.

[0063] In some embodiments, the buffer or buffer system comprises at least one buffer that has a buffering range that overlaps fully or in part the range of pH 5.5 – 7.4. In one embodiment, the buffer has a pKa of about 6.0 ± 0.5. In certain embodiments, the buffer comprises a histidine buffer. In certain embodiments, the histidine is present at a concentration of 5 mM ± 0.75 mM to 15 mM ± 2.25 mM; 6 mM ± 0.9 mM to 14 mM ± 2.1 mM; 7 mM ± 1.05 mM to 13 mM ± 1.95 mM; 8 mM ± 1.2 mM to 12 mM ± 1.8 mM; 9 mM ± 1.35 mM to 11 mM ± 1.65 mM; 10 mM ± 1.5 mM; or about 10 mM. In certain embodiments, the buffer system comprises histidine at 10 mM ± 1.5 mM, at a pH of 6.0 ± 0.3 or 6.4 ± 0.3.

EXEMPLARY FORMULATIONS

[0064] According to one aspect of the present invention, the pharmaceutical formulation is a low viscosity, *i.e,* having a viscosity of under 10 cPoise or about 1.4 ± 0.21 cPoise, generally physiologically isotonic, *i.e.,* between 300 and 400 mOsm or about 363 ± 54 mOsm, liquid formulation, which comprises: (i) a human antibody that specifically binds to human Ang-2 (*e.g.*, HIH685P), at a concentration of 25 mg/mL ± 3.75 mg/mL, or 50 mg/mL ±

7.5 mg/mL; (ii) a buffer system that provides sufficient buffering at about pH 6.0 ± 0.3; (iii) a sugar which serves as a thermal stabilizer; and (iv) an organic cosolvent, which protects the structural integrity if the antibody.

[0065] According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human Ang-2 and which comprises a substituted IGHV3-13.01 type heavy chain variable region and a substituted IGKV3-20.01 type light chain variable region (*e.g.*, HIH685P) at a concentration from 20 ± 3 mg/mL to about 60 ± 9 mg/mL; (ii) a buffer system comprising histidine, which buffers effectively at about pH 6.0 ± 0.3; (iii) sucrose; and (iv) a non-ionic detergent, such as a polysorbate.

[0066] According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human Ang-2, and which comprises an HCDR1 of SEQ ID NO: 4, an HCDR2 of SEQ ID NO: 6, an HCDR3 of SEQ ID NO: 8, an LCDR1 of SEQ ID NO: 14, an LCDR2 of SEQ ID NO: 14, and an LCDR3 of SEQ ID NO: 16, at a concentration of 25 mg/ml ± 3.75 mg/mL; (ii) histidine at 10 mM ± 1.5 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 10% w/v ± 1.5% w/v; and (iv) polysorbate 20 at 0.2% w/v ± 0.03% w/v.

[0067] According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human Ang-2, and which comprises an HCDR1 of SEQ ID NO: 4, an HCDR2 of SEQ ID NO: 6, an HCDR3 of SEQ ID NO: 8, an LCDR1 of SEQ ID NO: 14, an LCDR2 of SEQ ID NO: 14, and an LCDR3 of SEQ ID NO: 16, at a concentration of about 50 mg/ml ± 7.5 mg/mL; (ii) histidine at 10 mM ± 1.5 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 10% w/v ± 1.5% w/v; and (iv) polysorbate 20 at 0.2% w/v ± 0.03% w/v.

[0068] According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human Ang-2, and which comprises a heavy chain variable domain of SEQ ID NO: 18, and a light chain variable domain of SEQ ID NO: 20, at a concentration of 25 mg/ml ± 3.75 mg/mL; (ii) histidine at 10 mM ± 1.5 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 10% w/v ± 1.5% w/v; and (iv) polysorbate 20 at 0.2% w/v ± 0.03% w/v.

[0069] According to one embodiment, the pharmaceutical formulation comprises: (i) a human IgG1 antibody that specifically binds to human Ang-2, and which comprises a heavy chain variable domain of SEQ ID NO: 18, and a light chain variable domain of SEQ ID NO: 20, at a concentration of about 50 mg/ml ± 7.5 mg/mL; (ii) histidine at 10 mM ± 1.5 mM, which buffers at pH 6.0 ± 0.3; (iii) sucrose at 10% w/v ± 1.5% w/v; and (iv) polysorbate 20 at 0.2% w/v ± 0.03%.

[0070] Additional non-limiting examples of pharmaceutical formulations encompassed by the present invention are set forth elsewhere herein, including the working Examples presented below.

STABILITY AND VISCOSITY OF THE PHARMACEUTICAL FORMULATIONS

[0071] The pharmaceutical formulations of the present invention typically exhibit high levels of stability. The term "stable", as used herein in reference to the pharmaceutical formulations, means that the antibodies within the pharmaceutical formulations retain an acceptable degree of chemical structure or biological function after storage under defined conditions. A formulation may be stable even though the antibody contained therein does not maintain 100% of its chemical structure or biological function after storage for a defined amount of time. Under certain circumstances, maintenance of about 90%, about 95%, about 96%, about 97%, about 98% or about 99% of an antibody's structure or function after storage for a defined amount of time may be regarded as "stable".

[0072] Stability can be measured, *inter alia*, by determining the percentage of native antibody that remains in the formulation after storage for a defined amount of time at a defined temperature. The percentage of native antibody can be determined by, *inter alia*, size exclusion chromatography (*e.g.*, size exclusion high performance liquid chromatography [SE-HPLC]), such that native means non-aggregated and non-degraded. An "acceptable degree of stability", as that phrase is used herein, means that at least 90% of the native form of the antibody can be detected in the formulation after storage for a defined amount of time at a given temperature. In certain embodiments, at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% of the native form of the antibody can be detected in the formulation after storage for a defined amount of time at a defined temperature. The defined amount of time after which stability is measured can be at least 14 days, at least 28 days, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least 18 months, at least 24 months, or more. The defined temperature at which the pharmaceutical formulation may be stored when assessing stability can be any temperature from about -80ºC to about 45ºC, *e.g.*, storage at about -80ºC, about -30ºC, about -20ºC, about 0ºC, about 4º-8°C, about 5ºC, about 25ºC, about 35ºC, about 37ºC, or about 45ºC. For example, a pharmaceutical formulation may be deemed stable if after nine months of storage at 5ºC, greater than about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may also be deemed stable if after six months of storage at 25ºC, greater than about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may also be

deemed stable if after 28 days of storage at 37ºC, greater than about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 45ºC, greater than about 93%, 94%, 95%, 96%, 97%, 98% or 99% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may also be deemed stable if after six months of storage at -20ºC, greater than about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may also be deemed stable if after six months of storage at -30ºC, greater than about greater than about 96%, 96.5%, 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC. A pharmaceutical formulation may also be deemed stable if after six months of storage at -80ºC, greater than about 97%, 97.5%, 98%, 98.5%, 99% or 99.5% of native antibody is detected by SE-HPLC.

[0073] Stability can be measured, *inter alia*, by determining the percentage of antibody that forms in an aggregate within the formulation after storage for a defined amount of time at a defined temperature, wherein stability is inversely proportional to the percent aggregate that is formed. The percentage of aggregated antibody can be determined by, *inter alia*, size exclusion chromatography (*e.g.*, size exclusion high performance liquid chromatography [SE-HPLC]). An "acceptable degree of stability", as that phrase is used herein, means that at most 6% of the antibody is in an aggregated form detected in the formulation after storage for a defined amount of time at a given temperature. In certain embodiments an acceptable degree of stability means that at most about 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% of the antibody can be detected in an aggregate in the formulation after storage for a defined amount of time at a given temperature. The defined amount of time after which stability is measured can be at least 2 weeks, at least 28 days, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least 18 months, at least 24 months, or more. The temperature at which the pharmaceutical formulation may be stored when assessing stability can be any temperature from about -80ºC to about 45ºC, *e.g.*, storage at about -80ºC, about -30ºC, about -20ºC, about 0ºC, about 4º-8°C, about 5ºC, about 25ºC, about 35ºC, about 37ºC or about 45ºC. For example, a pharmaceutical formulation may be deemed stable if after nine months of storage at 5ºC, less than about 2%, 1.75%, 1.5%, 1.25%, 1%, 0.75%, 0.5%, 0.25%, or 0.1% of the antibody is detected in an aggregated form. A pharmaceutical formulation may also be deemed stable if after six months of storage at 25ºC, less than about 2%, 1.75%, 1.5%, 1.25%, 1%, 0.75%, 0.5%, 0.25%, or 0.1% of the antibody is detected in an aggregated form. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 45ºC, less than about 4%, 3.5%, 3%, 2.5%, 2%, 1.5%, 1%, 0.5%, or 0.1% of the antibody is

detected in an aggregated form. A pharmaceutical formulation may also be deemed stable if after three months of storage at -20ºC, -30ºC, or -80ºC less than about 2%, 1.9%, 1.8%, 1.7%, 1.6%, 1.5%, 1%, 0.5%, or 0.1% of the antibody is detected in an aggregated form.

[0074] Stability can be measured, *inter alia*, by determining the percentage of antibody that migrates in a more acidic fraction during ion exchange ("acidic form") than in the main fraction of antibody ("main charge form"), wherein stability is inversely proportional to the fraction of antibody in the acidic form. While not wishing to be bound by theory, deamidation of the antibody may cause the antibody to become more negatively charged and thus more acidic relative to the non-deamidated antibody (see, *e.g.,* Robinson, N., Protein Deamidation, *PNAS*, April 16, 2002, 99(8):5283-5288). The percentage of "acidified" antibody can be determined by ion exchange chromatography (*e.g.*, cation exchange high performance liquid chromatography [CEX-HPLC]). An "acceptable degree of stability", as that phrase is used herein, means that at most 52% of the antibody is in a more acidic form detected in the formulation after storage for a defined amount of time at a defined temperature. In certain embodiments an acceptable degree of stability means that at most about 52%, 50%, 45%, 40%, 35%, 30%, 29%, 28%, 27%, 26%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.1% of the antibody can be detected in an acidic form in the formulation after storage for a defined amount of time at a given temperature. The defined amount of time after which stability is measured can be at least 2 weeks, at least 28 days, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, at least 18 months, at least 24 months, or more. The temperature at which the pharmaceutical formulation may be stored when assessing stability can be any temperature from about -80ºC to about 45ºC, *e.g.*, storage at about -80ºC, about -30ºC, about -20ºC, about 0ºC, about 4º-8°C, about 5ºC, about 25ºC, or about 45ºC. For example, a pharmaceutical formulation may be deemed stable if after three months of storage at -80ºC, -30ºC, or -20ºC less than about 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody is in a more acidic form. A pharmaceutical formulation may also be deemed stable if after nine months of storage at 5ºC, less than about 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody is in a more acidic form. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 25ºC, less than about 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody is in a more acidic form. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 37ºC, less than about

37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody is in a more acidic form. A pharmaceutical formulation may also be deemed stable if after 28 days of storage at 45ºC, less than about 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5% or 0.1% of the antibody can be detected in a more acidic form.

[0075] Measuring the binding affinity of the antibody to its target may also be used to assess stability. For example, a formulation of the present invention may be regarded as stable if, after storage at *e.g.*, -80ºC, -30ºC, -20ºC, 5ºC, 25ºC, 37ºC, 45ºC, etc. for a defined amount of time (*e.g.*, 14 days to 6 months), the anti-Ang-2 antibody contained within the formulation binds to Ang-2 with an affinity that is at least 84%, 90%, 95%, or more of the binding affinity of the antibody prior to said storage. Binding affinity may be determined by any method, such as *e.g.,* ELISA or plasmon resonance. Biological activity may be determined by an Ang-2 activity assay, such as by contacting a cell that expresses Ang-2 with the formulation comprising the anti Ang-2 antibody. The binding of the antibody to such a cell may be measured directly, such as via FACS analysis. Alternatively, the downstream activity of the Ang-2 system may be measured in the presence of the antibody, and compared to the activity of the Ang-2 system in the absence of antibody. In some embodiments, the Ang-2 may be endogenous to the cell. In other embodiments, the Ang-2 may be ectopically expressed (*i.e.,* heterologous expression) in the cell.

[0076] Additional methods for assessing the stability of an antibody in formulation are demonstrated in the Examples presented below.

CONTAINERS AND METHODS OF ADMINISTRATION

[0077] The pharmaceutical formulations of the present invention may be contained within any container suitable for storage or administration of medicines and other therapeutic compositions. For example, the pharmaceutical formulations may be contained within a sealed and sterilized plastic or glass container having a defined volume such as a vial, ampule, syringe, cartridge, bottle, or IV bag. Different types of vials can be used to contain the formulations of the present invention including, *e.g.,* clear and opaque (*e.g.*, amber) glass or plastic vials. Likewise, any type of syringe can be used to contain or administer the pharmaceutical formulations of the present invention.

[0078] The pharmaceutical formulations of the present invention may be contained within "normal tungsten" syringes or "low tungsten" syringes. As will be appreciated by persons of ordinary skill in the art, the process of making glass syringes generally involves the use of a

hot tungsten rod which functions to pierce the glass thereby creating a hole from which liquids can be drawn and expelled from the syringe. This process results in the deposition of trace amounts of tungsten on the interior surface of the syringe. Subsequent washing and other processing steps can be used to reduce the amount of tungsten in the syringe. As used herein, the term "normal tungsten" means that the syringe contains greater than or equal to 500 parts per billion (ppb) of tungsten. The term "low tungsten" means that the syringe contains less than 500 ppb of tungsten. For example, a low tungsten syringe, according to the present invention, can contain less than about 490, 480, 470, 460, 450, 440, 430, 420, 410, 390, 350, 300, 250, 200, 150, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10 or fewer ppb of tungsten.

[0079] The rubber plungers used in syringes, and the rubber stoppers used to close the openings of vials, may be coated to prevent contamination of the medicinal contents of the syringe or vial, or to preserve their stability. Thus, pharmaceutical formulations of the present invention, according to certain embodiments, may be contained within a syringe that comprises a coated plunger, or within a vial that is sealed with a coated rubber stopper. For example, the plunger or stopper may be coated with a fluorocarbon film. Examples of coated stoppers or plungers suitable for use with vials and syringes containing the pharmaceutical formulations of the present invention are mentioned in, *e.g.*, U.S. Patent Nos. 4,997,423; 5,908,686; 6,286,699; 6,645,635; and 7,226,554, the contents of which are incorporated by reference herein in their entireties. Particular exemplary coated rubber stoppers and plungers that can be used in the context of the present invention are commercially available under the tradename "FluroTec®", available from West Pharmaceutical Services, Inc. (Lionville, PA). FluroTec® is an example of a flurocarbon coating used to minimize or prevent drug product from adhering to the rubber surfaces.

[0080] According to certain embodiments of the present invention, the pharmaceutical formulations may be contained within a low tungsten syringe that comprises a fluorocarbon-coated plunger.

[0081] The pharmaceutical formulations can be administered to a patient by parenteral routes such as injection (*e.g.*, subcutaneous, intravenous, intramuscular, intraperitoneal, etc.) or percutaneous, mucosal, nasal, pulmonary or oral administration. Numerous reusable pen or autoinjector delivery devices can be used to subcutaneously deliver the pharmaceutical formulations of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN

PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany). Examples of disposable pen or autoinjector delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park, IL).

[0082] The use of a microinfusor to deliver the pharmaceutical formulations of the present invention is also contemplated herein. As used herein, the term "microinfusor" means a subcutaneous delivery device designed to slowly administer large volumes (*e.g.*, up to about 2.5 mL or more) of a therapeutic formulation over a prolonged period of time (*e.g.*, about 10, 15, 20, 25, 30 or more minutes). *See*, *e.g.*, U.S. 6,629,949; US 6,659,982; and Meehan *et al.*, *J. Controlled Release 46*:107-116 (1996). Microinfusors are particularly useful for the delivery of large doses of therapeutic proteins contained within high concentration (*e.g.*, about 100, 125, 150, 175, 200 or more mg/mL) or viscous solutions.

[0083] In one embodiment, the pharmaceutical formulation is administered via an IV drip, such that the formulation is diluted in an IV bag containing a physiologically acceptable solution. In one embodiment, pharmaceutical composition is a compounded sterile preparation in an intravenous infusion bag, such that a single dose of drug product is diluted into 100 mL, 250 mL (or other like amount suitable for intravenous drip delivery) of a physiological buffer (e.g., 0.9% saline). In some embodiments, the infusion bag is made of a polyvinyl chloride (e.g., VIAFLEX, Baxter, Deerfield, Illinois). In some embodiments, the infusion bag is made of a polyolefin (EXCEL IV Bags, Braun Medical Inc., Bethlehem, Pennsylvania).

THERAPEUTIC USES OF THE PHARMACEUTICAL FORMULATIONS

[0084] The pharmaceutical formulations of the present invention are useful, *inter alia*, for the treatment, prevention or amelioration of any disease or disorder associated with Ang-2 activity, including diseases or disorders mediated by Ang-2. Exemplary, non-limiting diseases and disorders that can be treated or prevented by the administration of the pharmaceutical formulations of the present invention include various diseases involving angiogenesis, which is the biological process whereby new blood vessels are formed. Aberrant angiogenesis is associated with several disease conditions including, *e.g.,* proliferative retinopathies, rheumatoid arthritis and psoriasis. In addition, it is well established that angiogenesis is critical for tumor growth and maintenance.

EXAMPLES

[0085] The following examples are presented so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by mole, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric pressure.

[0086] Initial formulation development activities involved empirical experiments and screening organic cosolvents, thermal stabilizers, and buffers in liquid and lyophilized formulations of anti-Ang-2 antibodies to identify excipients that are compatible with the protein and enhance its stability, while maintaining near physiologic osmolality and low viscosity for intravenous and subcutaneous injection. Buffer conditions were also examined to determine the optimal pH for maximum protein stability.

EXAMPLE 1: DEVELOPMENT OF ANTI-ANG-2 FORMULATION

[0087] Formulation development activities included the screening of buffers, organic cosolvents, and thermal stabilizers in liquid formulations of the anti-Ang-2 antibody to identify excipients that enhance the stability of the protein. Buffer conditions were also examined to determine the optimal pH for maximum protein stability. Results generated from these studies were used to develop a stable, liquid formulation suitable for clinical use. Anti-Ang-2 (*e.g.,* HIH685P) was formulated at 25 ± 3.75 mg/ml and 50 ± 7.5 mg/ml. In one embodiment, the anti-Ang-2 antibody is formulated in 10 ± 1.5 mM histidine (pH 6.0 ± 0.3), 0.2% ± 0.03% polysorbate 20, 10% ± 1.5% sucrose.

EXAMPLE 2: BUFFER AND PH

[0088] The effect of pH and buffer type on the stability of anti-Ang-2 was considered. HIH685P at 25 mg/mL was incubated at 45°C in either acetate (pH 5.0-5.5), citrate (pH 5.5-6.0), succinate (pH 6.0), histidine (pH 5.5-6.5), phosphate (pH 6.0-8.0), or Tris (pH 8.0) buffer to assess the effect of buffer and pH on the thermal stability of the protein (Table 1). Analysis of protein stability by size exclusion chromatography (SE-HPLC) revealed that acetate (pH 5.5), phosphate (pH 6.0), histidine (pH 5.5-6.5), and succinate performed better than the other buffer systems for the formulation of HIH685P. Maximum protein stability, as determined by cationic exchange chromatography (CEX HPLC), was observed when HIH685P was formulated in histidine buffer at pH 6.0. These analyses also revealed that degradation for the antibody resulted mostly in the formation of aggregates, cleavage products, and charge variants. The optimal pH for HIH685P stability in histidine buffer was

observed to be 6.0, although good stability was observed in the pH range of 5.5 to 6.5. Based on these results, 10 mM histidine buffer at pH 6.0 was chosen for the formulation of antibody drug product (DP).

[0089] Table 1: Effect of Buffer and pH on the Stability of HIH685P Incubated at 45°C for 28 Days

pH/Buffer	OD 405[1]	% Total (RP-HPLC)	% Native (SE-HPLC)	% Aggre-gate (SE-HPLC)	% Main Peak (CEX-HPLC)	% Acidic Peak (CEX-HPLC)	% Basic Peak (CEX-HPLC)
Starting Material[2] (no incubation)	0.00	100	96.4	2.5	59.6	28.9	11.5
pH 8.0, Tris	0.25	95	73.0	20.7	5.8	89.2	5.1
pH 8.0, Phosphate	0.54	95	52.7	40.2	2.4	94.0	3.6
pH 7.5, Phosphate	0.33	97	66.5	27.8	5.3	94.7	0.0
pH 7.0, Phosphate	0.05	101	85.5	10.8	16.8	77.5	5.7
pH 6.5, Phosphate	0.01	100	90.1	6.1	26.0	67.0	7.0
pH 6.0, Phosphate	0.00	100	92.3	4.0	31.0	57.5	11.5
pH 6.5, Histidine	0.01	100	92.6	4.3	31.5	58.7	9.8
pH 6.0, Histidine	0.01	100	92.3	4.3	34.9	52.2	13.0
pH 5.5, Histidine	0.00	100	92.4	4.0	33.1	50.4	16.5
pH 6.0, Succinate	0.00	99	92.3	4.5	32.3	56.4	11.4
pH 6.0, Citrate	0.03	100	89.3	7.4	31.1	57.6	11.3
pH 5.5, Citrate	0.05	100	88.6	8.0	27.3	58.7	14.1
pH 5.5, Acetate	0.00	101	92.8	3.5	32.5	54.3	13.2
pH 5.0, Acetate	0.00	100	91.6	3.7	29.5	52.1	18.4

[0090] For the results shown in Table 1, 0.35 mL of 25 mg/mL HIH685P in 10 mM test buffer in a 2 mL Type 1 borosilicate glass vial with a FluroTec® coated 4432/50 butyl rubber stopper were tested for 28 days at 45. [1]Turbidity was reported as the relative change in OD at 405 nm as compared to the starting material. [2]SE-HPLC and CEX-HPLC Starting Material Results were the average of the starting material for all 14 formulations. OD = Optical density; RP-HPLC = Reverse phase high performance liquid chromatography; SE-HPLC = Size exclusion high performance liquid chromatography; CEX-HPLC = cation exchange high performance liquid chromatography.

EXAMPLE 3: SELECTION OF PROTECTANTS AGAINST AGITATION STRESS

[0091] The HIH685P anti-Ang-2 antibody (*i.e.,* Drug Substance or "DS") exhibited limited stability when subjected to agitation stress. Turbidity analysis of agitated DS demonstrated an increase in optical density (OD) at 405 nm when HIH685P was vortexed for 120 minutes (Table 2, see No Cosolvent data). This increase in turbidity indicates a significant formation of particulates as a result of the agitation stress. Agitation of the formulation in the absence of cosolvent also resulted in a significant increase in aggregate formation. Formulation with any of the evaluated cosolvents prevented the agitation dependent increase in turbidity and aggregate levels of HIH685P (Table 2). However, the addition of 0.2% Pluronic F68, 20% PEG 300, 10% PEG 300, and 20% propylene glycol to the formulation significantly decreased the thermal stability of HIH685P as determined by SE-HPLC and CEX-HPLC (Table 3). Formulations containing polysorbate 20, polysorbate 80, and PEG 3350 had no significant effect on the thermal stability of HIH685P as determined by SE-HPLC and CEX-HPLC, making these cosolvents suitable for the formulation of HIH685P (Table 3). Polysorbate 20 was chosen as the organic cosolvent for development of a HIH685P formulation because it stabilized the protein to agitation stress, had no effect on its thermal stability, and required lower co-solvent concentrations to stabilize the protein compared to polyethylene glycol.

[0092] For the antibody stability results shown in Table 2, 0.8 mL of 5 mg/mL HIH685P, in 10 mM histidine, pH 6.0 to 6.1 in a 2 mL Type 1 borosilicate glass vial with a FluroTec® coated 4432/50 butyl rubber stopper was combined with the organic cosolvents and subjected to 120 minutes of vortexing. [1]Turbidity was reported as the relative change in OD at 405 nm as compared to the starting material. [2]SE-HPLC and CEX-HPLC Starting Material Results were the average of the starting material for all nine formulations. OD = Optical density; RP-HPLC = Reverse phase high performance liquid chromatography; SE-HPLC = Size exclusion high performance liquid chromatography.

[0093] Table 2: Effect of Organic Cosolvents on Antibody Stability – Agitation

Organic Cosolvent	OD 405[1]	% Total (RP-HPLC)	% Native (SE-HPLC)	% Aggreg-ate (SE-HPLC)	% Main Peak (CEX-HPLC)	% Acidic Peak (CEX-HPLC)	% Basic Peak (CEX-HPLC)
Starting Material[2] (no vortexing)	0.00	100	98.3	0.6	59.3	32.8	7.9
No Cosolvent	0.25	103	76.6	22.3	60.4	30.9	8.7
0.2% Polysorbate 20	0.00	99	98.3	0.6	59.5	32.9	7.7
0.2% Polysorbate 80	0.00	99	98.2	0.6	59.4	32.8	7.8
0.2% Pluronic F68	0.00	98	98.3	0.7	59.4	32.7	7.9
3.0% PEG 3350	0.00	96	98.6	0.6	59.4	32.9	7.7
1.0% PEG 3350	0.00	99	98.3	0.7	59.7	32.8	7.6
20% PEG 300	0.01	101	98.1	0.8	57.6	33.1	9.3
10% PEG 300	0.01	100	97.9	0.9	59.0	32.7	8.2
20% Propylene Glycol	0.00	101	98.1	0.8	59.7	32.5	7.8

[0094] Table 3: Effect of Organic Cosolvents on Antibody Stability - Thermal

Organic Cosolvent	OD 405[1]	% Total (RP)	% Native (SE)	% Aggreg. (SE)	% Main Peak (CEX)	% Acidic Peak (CEX)	% Basic Peak (CEX)
Starting Material[2] (no incubation)	0.00	100	98.3	0.6	59.3	32.8	7.9
No Cosolvent	0.01	97	95.3	0.7	35.1	53.8	7.3
0.2% Polysorbate 20	0.01	97	95.3	0.7	34.9	53.7	7.4
0.2% Polysorbate 80	0.01	97	95.1	0.9	34.9	53.8	7.5
0.2% Pluronic F68	0.01	96	95.1	0.6	31.2	53.1	9.3
3.0% PEG 3350	0.01	96	95.7	0.8	35.0	52.4	7.9
1.0% PEG 3350	0.01	96	96.0	0.7	35.5	52.7	7.6
20% PEG 300	0.12	103	71.5	5.0	5.1	81.5	0.0
10% PEG 300	0.06	100	91.6	1.5	5.6	76.6	0.0
20% Propylene Glycol	0.00	98	92.2	0.8	34.3	52.5	8.2

[0095] For the antibody stability results shown in Table 3, 0.35 mL of 5 mg/mL HIH685P, in 10 mM histidine, pH 6.0 to 6.1 in a 2 mL Type 1 borosilicate glass vial with a FluroTec® coated 4432/50 butyl rubber stopper was combined with the organic cosolvents and subjected to 45°C for 28 days. [1]Turbidity was reported as the relative change in OD at 405 nm as compared to the starting material. [2]SE-HPLC and CEX-HPLC Starting Material Results were the average of the starting material for all nine formulations. OD = Optical density; RP = Reverse phase high performance liquid chromatography; SE = Size exclusion high performance liquid chromatography; CEX = cation exchange high performance liquid chromatography.

EXAMPLE 4: SELECTION OF PROTECTANTS AGAINST THERMAL STRESS

[0096] Stabilizers such as sugars, amino acids, and inorganic salts were examined for their ability to increase the thermal stability of HIH685P. A summary of the thermal stabilizers that were examined is presented in Table 4. Formulations containing 20% sucrose, 10% mannitol, and 20% trehalose had the least amount of HIH685P degradation as determined by SE-HPLC and CEX-HPLC analysis following thermal stress. However, formulation with mannitol destabilized the protein to multiple freezing and thawing cycles. HIH685P had similar stability to thermal stress when formulated with sucrose or trehalose. Sucrose was chosen as the thermal stabilizer for development of a liquid HIH685P formulation.

[0097] HIH685P exhibited maximal stability when formulated in the presence of histidine, polysorbate 20, and sucrose at pH 6.0. 10% sucrose was chosen for the HIH685P DP formulation, which is close to iso-osmolar. The main degradation pathways identified during the development of the HIH685P liquid formulation were the formation of aggregates, cleavage products, and charge variants, with the highest rate of degradation (increase in charge variants) being observed when the protein was incubated at 45°C.

[0098] For the antibody stability results shown in Table 4, 0.35 mL of 10 mM Histidine, pH 6.0 to 6.2, 0.2% Polysorbate 20, and 25 mg/mL HIH685P, plus the indicated thermal stabilizer, in a 2 mL Type 1 borosilicate glass vial with a FluroTec® coated 4432/50 butyl rubber stopper were subjected to 45°C for 28 days. [1]Turbidity was reported as the relative change in OD at 405 nm as compared to the starting material. [2]SE-HPLC and CEX-HPLC Starting Material Results represents the average values of the starting material for all ten formulations not incubated at 45°C. OD = Optical density; RP = Reverse phase high performance liquid chromatography; SE = Size exclusion high performance liquid chromatography; CEX = cation exchange high performance liquid chromatography.

[0099] Table 4: Effect of Thermal Stabilizers on the Stability

Thermal Stabilizer	OD 405 nm[1]	% Total (RP-HPLC)	% Native (SE-HPLC)	% Aggregate (SE-HPLC)	% Main Peak (CEX-HPLC)	% Acidic Peak (CEX-HPLC)	% Basic Peak (CEX-HPLC)
Starting Material[2]	0.00	100	97.7	1.5	58.7	31.5	9.8
No Stabilizer	0.02	100	92.9	3.7	32.0	55.2	12.8
150 mM NaCl	0.11	101	79.6	17.5	35.4	50.1	14.4
10% Sucrose	0.01	100	93.9	2.7	32.7	54.3	13.0
20% Sucrose	0.01	101	94.2	2.4	33.5	53.4	13.2
20% Sorbitol	0.10	101	94.4	1.9	24.5	62.7	12.9
10% Mannitol	0.01	99	94.4	2.3	33.1	53.1	13.8
20% Trehalose	0.01	100	94.7	2.0	33.6	52.9	13.6
5% Glycerol	0.09	103	88.0	8.1	4.7	92.1	3.1
3% Arginine	0.05	99	83.0	13.5	35.2	46.4	18.5
3% Glycine	0.01	102	93.7	3.0	29.1	58.2	12.7

EXAMPLE 5: STABLE LIQUID PHARMACEUTICAL FORMULATION

[00100] In conclusion, HIH685P DP was produced as a liquid in an optimized, aqueous buffered formulation containing 10 mM histidine, pH 6.0, 0.2% (w/v) polysorbate 20, 10% (w/v) sucrose, and 25 mg/mL HIH685P. HIH685P DP at 25 mg/mL was physically and chemically stable when subjected to various stress conditions (Table 5). There was no effect on pH, appearance, turbidity, or the amount of HIH685P recovered when the DP was vortexed, frozen and thawed multiple times, or incubated at 25°C for 14 days. After 28 days of incubation at 37°C, the DP was 0.6% more degraded relative to a control, unstressed sample, as determined by SE-HPLC analysis and 10.1% more degraded (decrease in % main peak) as determined by CEX-HPLC analysis. After 28 days of incubation at 45°C, the DP was 3.6% more degraded compared to unstressed control as determined by SE-HPLC analysis and 23.8% more degraded (decrease in % main peak) as determined by CEX-HPLC analysis. No significant loss of potency, as determined using the HIH685P binding assay described below, was observed for any of the stressed samples.

[00101] Table 5: Stress Stability of 25 mg/mL Anti-Ang-2 Antibody

Stress Test	No Stress	Agitation		45°C Incubation		37°C Incubation		25°C Incubation		Freeze/ Thaw
Length of Stress	0 min	60 min	120 min	14 days	28 days	14 days	28 days	14 days	28 days	8 cycles
OD 405 nm[1]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% Total (RP-HPLC)	100	100	99	101	103	101	103	101	102	97
% Native (SE-HPLC)	97.0	97.1	96.9	95.6	93.4	96.6	96.4	96.8	97.1	96.8
% Aggregate (SE-HPLC)	1.9	1.9	1.9	1.8	3.5	1.6	1.7	1.6	1.7	1.6
% Main Peak (CEX-HPLC)	56.0	56.1	55.9	42.8	32.2	50.4	45.9	55.1	53.5	55.7
% Acidic Peak (CEX-HPLC)	27.0	26.9	27.0	39.2	51.5	30.8	37.0	27.3	29.1	27.2
% Basic Peak (CEX-HPLC)	17.0	17.0	17.1	18.0	16.3	18.8	17.1	17.7	17.4	17.1
Binding Assay[2] (% Relative Potency)	105	NP	111	NP	99	NP	122	NP	107	110

[00102] Potency of the formulated HIH685P was determined by measuring the ability of HIH685P to block the binding of a human angiopoietin-2 – His-tagged fusion protein (hAng2-His) to a plate coated with a human Tie2-murine Fc-fusion protein (hTie2-mFc) via a competition sandwich ELISA (the binding assay). hAng2-His was titrated with varying amounts of HIH685P. hAng2-His concentration was calculated assuming a monomeric molecular weight of 50.1 kDa. The ligand-antibody complexes were incubated for 1 hour at 25°C before transfer to microtiter plates coated with the hTie2-mFc. After one-hour incubation, the wells were washed and bound hAng2-His was detected with an HRP-conjugated anti-His tag monoclonal antibody. The calculated IC_{50} value, defined as the concentration of antibody required to block 50% of His-tagged hAng2 to Tie2-mFc, was used as an indicator of blocking potency.

[00103] For the antibody stability results shown in Table 5, 0.35 mL of 10 mM Histidine, pH 6.0 to 6.1, 0.2% Polysorbate 20, 10% Sucrose, and 25 mg/mL HIH685P, in a 2 mL Type 1 borosilicate glass vial with a FluroTec® coated 4432/50 butyl rubber stopper was combined with the organic cosolvents and subjected to the various designated stresses.

[4051]Turbidity was reported as the relative change in OD at 405 nm as compared to the

starting material. [2]The acceptance criteria for the binding assay was 50-150% of reference standard. OD = Optical density; RP = Reverse phase high performance liquid chromatography; SE = Size exclusion high performance liquid chromatography; CEX = cation exchange high performance liquid chromatography.

EXAMPLE 6: STABILITY OF FORMULATED ANTI-ANG-2 ANTIBODY

[00104] Stability studies were performed to determine both the storage and stress stability of the anti-Ang-2 antibody formulation containing 25 mg/mL antibody, 10 mM histidine, pH 6.0 ± 0.3, 0.2% polysorbate 20, and 10% sucrose. Turbidity and RP-HPLC assays were used to assess the physical stability of the antibody. Physical stability is defined as the recovery of soluble forms of the anti-Ang-2 antibody in solution. Loss of protein could be due to either protein precipitation or surface adsorption. The presence of particulates in solution can be detected by visual inspection or by optical density (OD) measurements at 405 nm (turbidity measurements). In this latter assay, an increase in OD indicates an increase in turbidity due to the formation of particulates. The presence of particulates as determined by OD measurements indicates that the sample has failed to maintain stability. Recovery of antibody is measured by RP-HPLC. In the RP-HPLC assay, the anti-Ang-2 antibody is eluted from the reverse phase column as a single peak. The concentration of each test sample is determined from the area of the eluted antibody peak compared to a calibration curve generated using antibody standards of defined protein loads.

[00105] Chemical stability refers to the integrity of the chemical structure of the anti-Ang-2 antibody in a sample. Most chemical instability can be attributed to the formation of covalently modified forms of the antibody, (e.g. covalent aggregates, cleavage products, or charge variants) and non-covalently modified forms of the antibody (e.g. non-covalent aggregates). Thus far, the only degradation products of HIH685P that have been detected are species that differ in either molecular weight or charge. The higher and lower molecular weight degradation products can be separated from native antibody by SE-HPLC. The percentage of native in the size exclusion chromatographic method is determined by the ratio of the area of the native peak to the total area of all anti-Ang-2 antibody peaks.

[00106] Charge variant forms of the anti-Ang-2 antibody were resolved from native antibody using cation exchange chromatography. Peaks that eluted from the CEX-HPLC column with retention times earlier than that of the main peak were labeled “Acidic Peaks”, while those that eluted from the CEX-HPLC column with retention times later than that of the main peak were labeled “Basic Peaks”. The percentage of degraded anti-Ang-2 antibody in the cation exchange chromatographic method is determined by the change in the relative

percentage of the main, acidic, and basic peak areas compared to the total area of all anti-Ang-2 antibody peaks.

[00107] Evaluation of the antibody under accelerated conditions was performed by subjecting the antibody to a variety of stress tests. These tests represent the extreme handling conditions that the formulated drug substance may be subjected to during the manufacture, storage, or transportation of drug product. The formulated anti-Ang-2 antibody was filled in 5 mL polycarbonate vials for the agitation, cycles of freeze/thaw, and frozen storage conditions. The formulated antibody was filled in glass vials to examine stress stability at high temperatures.

EXAMPLE 7: STORAGE STABILITY

[00108] No change was observed in the physical and chemical stability of the 25 mg/mL HIH685P antibody formulation containing 10 mM histidine, pH 6.0 ± 0.3, 0.2% polysorbate 20, and 10% sucrose, when stored for six months at -80°C, -30°C, -20°C, and 5°C, and for nine months at 5°C. A slight diminution in binding activity (ELISA competition assay described above) was observed at 6 months at -80°C, -30°C, and -20°C, *i.e.,* ~86%, ~84%, and ~91% of baseline, respectively. See Table 6.

EXAMPLE 8: STRESS STABILITY

[00109] The stress stability of the 25 mg/mL anti-Ang-2 antibody (HIH685P) formulated in 10 mM histidine, pH 6.0 ± 0.3, 0.2% polysorbate 20, 10% sucrose was discerned by subjecting the formulation to agitation, thermal stress (45°C, 37°C, 25°C), and freeze thaw stress. The 0.35 mL of the formulation was filled in a 2 mL Type 1 borosilicate glass vial fitted with a FLUROTEC-coated 4432/50 butyl rubber stopper. The results are presented in Table 7.

EXAMPLE 9: MOLECULAR MASS DETERMINATION

[00110] A series of analytical, biochemical and biophysical techniques were used to characterize the formulated HIH685P antibody. The molecular weights of the heavy and light chains of glycosylated and deglycosylated HIH685P antibody samples were determined following Capillary Electrophoresis – Sodium Dodecyl Sulfate (CE-SDS) analysis under reducing conditions. Samples were denatured in 0.5% w/v sodium dodecyl sulfate (SDS) and 40 mM dithiothreitol (DTT) at 60°C for 10 min. Following denaturation and reduction, 1 x G7 buffer v/v (1% Nonidet 40 and 50 mM sodium phosphate pH 7.5) was added to the samples. Deglycosylation of HIH685P (50 µg) was accomplished by addition of 1250 units of Peptide N-glycosidase F (PNGase F) and incubation for three hours at 37°C. Untreated control samples were prepared similarly except that the PNGase F enzyme was omitted

from the three hour incubation. Samples were desalted following incubation to remove components of the reaction buffer that may interfere with CE-SDS analysis. Desalted samples were completely denatured in 1% w/v SDS and 4.5% w/v β-mercaptoethanol and incubated at 80°C for 10 min. A 10 kDa molecular weight standard (Beckman Coulter) was added to each sample and used as an internal standard to determine peak identity and calculate protein mobility.

[00111] Table 6: Stability of 25 mg/mL HIH685P antibody in 10 mM Histidine, pH 6.0, 0.2% Polysorbate 20, 10% Sucrose

Storage Condition	-80°C/6 mo.	-30°C/6 mo.	-20°C/6 mo.	5°C/6 mo.	5°C/9 mo.
Appearance	Pass	Pass	Pass	Pass	Pass
pH	6.1	6.1	6.1	6.0	6.1
Turbidity (OD 405 nm)[1]	0.00	0.00	0.00	0.00	0.00
% Total REGN910 Recovered (RP-HPLC)	105	105	107	102	105
Purity by Size-Exclusion-HPLC % main peak purity	97.3	96.8	96.8	97.1	96.6
% HMW species	1.8	1.9	1.9	1.8	1.7
Charged Variant Analysis by CE-HPLC % region 1 (acidic)	28.3	28.1	27.9	28.1	27.4
% region 2 (main)	55.5	55.9	55.8	55.6	56.3
% region 3 (basic)	16.2	16.0	16.3	16.4	16.2
Binding Assay (% Ref. Std.)	85.95	84.3	90.9	>100	NP

[00112] Three principal peaks were observed in the electrophoregrams of the HIH685P untreated control samples. Peak 1 represents reduced light chain with a calculated molecular weight of approximately 28 kDa (34.0-34.1% total peak area). Peaks 2 and 3 represent non-glycosylated heavy chain (~51 kDa; 3.1-3.8%) and glycosylated heavy chain (~56 kDa; 62.2-62.8% total peak area), respectively. The sum of heavy and light chains (peaks 1, 2, and 3) represent ≥99% of the total percentage of peaks detected by this method for both HIH685P lot samples.

[00113] Table 7: Stress Stability

Stress Test	No Stress	Agitation		45°C Incubation		37°C Incubation		25°C Incubation		Freeze/ Thaw
Length of Stress	-	60 min	120 min	14 days	28 days	14 days	28 days	14 days	28 days	8 cycles
Appearance	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass	Pass
pH	6.1	6.0	6.1	6.1	6.2	6.1	6.1	6.0	6.1	6.1
Turbidity (OD at 405 nm)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% Total REGN910 Recovered (RP-HPLC)	100	100	99	101	103	101	103	101	102	97
Purity by SE- HPLC % main peak purity	97.0	97.1	96.9	95.6	93.4	96.6	96.4	96.8	97.1	96.8
% HMW species	1.9	1.9	1.9	1.8	3.5	1.6	1.7	1.6	1.7	1.6
Charged Variant Analysis by CEX-HPLC % region 1 (acidic)	27.0	26.9	27.0	39.2	51.5	30.8	37.0	27.3	29.1	27.2
% region 2 (main)	56.0	56.1	55.9	42.8	32.2	50.4	45.9	55.1	53.5	55.7
% region 3 (basic)	17.0	17.0	17.1	18.0	16.3	18.8	17.1	17.7	17.4	17.1
Binding Assay (% Relative Potency)	100	NP	>100	NP	94.3	NP	>100	NP	>100	>100

[00114] In the samples treated with PNGase F, there was a substantial decrease in the intensity of peak 3 coupled with a concomitant increase in the intensity of peak 2 in each electropherogram, indicating removal of the glycan chain from the glycosylated heavy chain. The average corrected peak area percentage of peak 2 from the electropherograms generated following capillary electrophoresis of PNGase F treated HIH685P lot samples (66%) was equivalent to the sum of the mean peak area percentages of peaks 2 and 3 from the electropherograms generated following capillary electrophoresis of the control, non-PNGase F treated samples (66%), indicating complete deglycosylation of the heavy chain following PNGase F incubation. As expected, the molecular weight and relative migration time of the light chain was unchanged.

[00115] Multi-angle laser light scattering (MALLS) is an analytical method that provides an estimate of the molecular mass of a protein or glycoprotein. The molar mass of native HIH685P was analyzed by connecting the flow cell of a light scattering detector to a gel filtration column to allow molecular mass analysis of each separated drug substance

component (SEC-MALLS). The protein concentration was monitored by differential refractive index and absorbance detectors. Formulated HIH685P lot samples were injected directly onto a TSK Gel G3000SWxl (Tosoh Biosciences, cat 08541; column dimensions 0.78 cm x 30 cm, 5 µm particle size, and porosity of 250Å), pre-equilibrated in 10 mM sodium phosphate, 500 mM NaCl, pH 6.1 buffer (SEC buffer). The data were analyzed by Astra software (Wyatt Technology) using the specific refractive index increment, which is defined as the change in refractive index divided by the change in protein concentration (dn/dc), corrected for the high ionic strength of the mobile phase buffer. By SEC-MALLS analysis, the main peak (peak 4, elution volume of ~7.9 mL) for HIH685P corresponded to a molecular weight of approximately 151 kDa. The molar mass identified for the main peak (peak 4) corresponded to intact anti-Ang-2 antibody, which was the predominant species present in solution (96.1-96.9% of the total protein peak area). A very small percentage of two high molecular weight species, corresponding to peak 2 (elution volume of ~6.2 mL) and peak 3 (elution volume of 6.5 mL) and two low molecular weight species, corresponding to peak 5 (elution volume of ~8.4 mL) and peak 6 (elution volume of ~9.8 mL), were detected in all formulated HIH685P samples tested. The calculated molar mass from peak 3 (elution volume of ~6.5 mL) of approximately 300 kDa is consistent with a dimeric form of the HIH685P antibody, and represents a small fraction (1.5-1.9%) of the total antibody molecular weight forms present in solution. The calculated molecular weight for peak 5 (elution volume of ~8.4 mL) is approximately 66 kDa and represents ≤1.5% of the total molecular weight forms present in the sample. Peak 6 represents ~0.2-0.3% of the total peak area. The results from SEC-MALLS analysis of the HIH685P drug substance demonstrated that the majority of the protein exists as an intact antibody with an average molar mass of approximately 151 kDa.

[00116] Independent lots of formulated HIH685P were further analyzed to confirm the molecular weight of the intact proteins through mass measurement. 1.5 µg of each protein sample was injected onto a 1.7 µm BEH130 C18 column coupled to a Waters Synapt Mass Spectrometer for mass measurement. The ESI-TOF mass spectra were deconvoluted for the intact proteins using a maximum entropy algorithm with 11 iterations. Based on the heavy and light chain cDNA sequences of HIH685P, the intact antibody (with heavy chain C-terminal Lys removed) was predicted to have a molecular weight of 144604.4 Da. The deconvoluted mass spectra of the intact HIH685P lots showed a similar pattern, with each spectra containing multiple peaks differing by masses of either 146 dalton (fucose) or 162 dalton (galactose), suggesting the presence of glycosylation related micro-heterogeneity. These glycan masses were obtained by subtracting the predicted molecular weight of 144604.4 from the observed intact masses. For example, the 2nd ESI/MS peak has m/z

147493 Da, less of intact MAb MW at 144604.4 Da leaves the glycan mass at 2889 Da. Since the glycans are added on the both sides of Fc, the glycan mass should be 1445 Da.

[00117] These glycan masses were used to assign the major HIH685P glycan structural forms. The analysis showed that these glycans are primarily composed of fucosylated complex bi-antennary structures, with 0, 1, and 2 galactoses at the glycan chain termini. The mass difference between the predicted and observed masses (4 Dalton) for the protein amino acid sequence was within the mass accuracy specification of the Synapt MS. These results confirmed the identity of HIH685P at the primary sequence level.

EXAMPLE 10: ISOELECTRIC POINT DETERMINATION

[00118] HIH685P was analyzed by one-dimensional isoelectric focusing (IEF) under native conditions to determine the isoelectric point (pI) of the intact antibody. A series of pI standards were included in the study, with protein bands visualized by Colloidal blue staining. All sample preparations of HIH685P exhibited a total of eight bands of varying intensity that migrated between a pI of 7.9 and 8.4, with the dominant form having a pI of 8.2. The band migrating at a pI of approximately 8.2, exhibited a slightly lower pI than the predicted pI of 8.6 for the intact antibody. This major band most likely represents fully glycosylated intact antibody lacking the C-terminal lysine (predicted pI of 8.54). This interpretation is consistent with mass spectrometry analysis of the heavy chain following proteolytic digestion and reverse phase separation. In the mass spectrometry analysis, the dominant C-terminal peptide from the HIH685P heavy chain was observed to be lacking the terminal lysine residue, predicted to be present from the cDNA sequence. Three minor basic variants (corresponding to a pI range of 8.3-8.4) were observed. While not wishing to be bound by theory, these minor species may represent partial forms of the antibody, such as an antibody form possessing only heavy chain dimer (predicted pI of 8.9), or heavy chain dimer lacking one or two C-terminal lysines. Alternatively, these species may be consistent with a small amount of a charged variant form of the intact antibody containing one or two C-terminal lysines. This interpretation is consistent with the mass spectral analysis of the heavy chain after tryptic digestion and separation of peptides by reverse phase chromatography. Approximately 2.5% of the HIH685P heavy chain was identified by mass spectrometry to contain the C-terminal lysine.

[00119] The pI of the native, monomeric form of the kappa light chain from HIH685P is predicted to be 6.4. No band corresponding to a pI of 6.4 was detected under the conditions employed, suggesting that minimal, if any, amounts of free light chain are present in the formulated HIH685P preparation. Although currently unidentified, four additional minor bands that migrated to a more acidic pH relative to the principal band (corresponding to a pI range of 7.9-8.1) were present in all tested samples, and may be speculated to represent

intact, glycosylated antibody with deamidated forms, incorrectly formed intermolecular disulfide bonds, or small amounts of truncated forms of the antibody.

[00120] The charge heterogeneity of formulated HIH685P was also quantitatively assessed by isoelectric focusing using a capillary electrophoresis (cIEF) based method. For this study, each HIH685P sample was diluted to 0.4 mg/mL (100 µg) in cIEF gel (Beckman Coulter, cat 477497) containing 39 mM arginine, 2.3 mM iminodiacetic acid, and 3-10 Pharmalytes. (GE Healthcare; 12 µL). A total of seven peaks of varying intensity were observed ranging from 7.77 to 8.48. The pI range of product related peaks observed by cIEF is slightly greater than the pI range observed by the gel-based isoelectric focusing method (pI range of 7.9 – 8.4) due to the increased resolution of the capillary. Highly similar qualitative peak patterns were observed from the electropherograms from each DS lot tested. There were a total of four major peaks (peaks 4-7) that represented approximately 92 – 93% of the total peak area. While not wishing to be bound by theory, these peaks may represent intact antibody lacking the C-terminal lysine, truncated or partial forms of the antibody, non-covalent intact forms of the antibody, or forms with incorrectly formed disulfides. It is possible that some of these peaks may arise during sample preparation or under the electrophoretic conditions employed. Peak 4 represents the dominant charge variant species with a pI of approximately 8.1 and average peak area percentages of 57.5% and 53.4% for each of two formulated HIH685P sample lots tested.

[00121] To further examine charge heterogeneity of the HIH685P formulated antibody, several samples were analyzed by two-dimensional gel electrophoresis. For the 2-D gel method, proteins were reduced and then separated on the basis of charge in the first dimension using isoelectric focusing. Subsequently, separated charged species were further resolved in the second dimension based on molecular weight using SDS-PAGE performed under reducing conditions. Each reduced HIH685P sample (2 µg), in the presence or absence of Bio-Rad 2-D pI standards, was loaded onto pH 3-10 immobilized pH gradient (IPG) strips for analysis in the first dimension. Following isoelectric focusing, the IPG strips were loaded onto 4-20% Novex Tris glycine gels for analysis in the second dimension. The second dimension was run in the presence of molecular weight standards. In addition, a series of internal standards of known pI were included in the isoelectric focusing analysis. All proteins were visualized by Coomassie Blue staining. Following 2-D gel electrophoresis and protein spot analysis, all HIH685P lot samples exhibited comparable spot patterns, with similar charge variant species present, likely due to equivalent heterogeneity of the heavy chain. A total of three HIH685P-related spots were observed in the gel that corresponded to an approximate mass of 55 kDa, with one highly abundant form exhibiting a pI corresponding to approximately 8.2. This spot (pI of ~8.2) is consistent with the reduced, glycosylated heavy chain polypeptide lacking the C-terminal lysine, detected by

mass spectrometry analysis. Two unidentified minor spots, detected in the 2-D gel, exhibited isoelectric points between approximately pH 7.5-8.0. In one theoretical scenario, it is possible that these spots consist of assay dependent deamidated protein forms or truncated forms of the heavy chain. Alternatively, it is possible that sample processing or gel artifacts may have led to spot spreading, resulting in the appearance of additional minor spots of varying isoelectric points.

[00122] As anticipated, the HIH685P light chain migrated within the 2 D gel as a single major spot, corresponding to a pI of approximately 6.0 with a molecular weight of approximately 25 kDa. An unidentified minor spot (pI of ~ 5.8) with a molecular weight of about 25 kDa was also observed. This spot may represent a small percentage of deamidated light chain, which was also detected in mass spectrometry analysis.

EXAMPLE 11: POST-TRANSLATIONAL MODIFICATIONS

[00123] Like most IgG1 antibodies, the HIH685P heavy chain terminates with the expected amino acid sequence, Pro-Gly-Lys, with the C-terminal Lys residue susceptible to removal by an unidentified basic carboxypeptidase during protein expression. Partial removal of the terminal Lys could lead to charge heterogeneity within the antibody drug substance. From the peptide mapping analysis of the reduced HIH685P, it was confirmed that the majority of the C-terminal Lys^{452} residue in the tryptic peptide was removed from the heavy chain, resulting in a C-terminal peptide with a sequence of ^{445}SLSLSPG^{451} (SEQ ID NO: 532). Only a very small percentage of tryptic peptide containing the C-terminal Lys residue (^{445}SLSLSPGK^{452}; SEQ ID NO: 533) was observed in the tryptic map. Based on the integrated peak areas of the two tryptic C-terminal heavy chain peptides from each lot of HIH685P, approximately 1.0% and 1.5% of the HIH685P antibody heavy chain contained C-terminal Lys^{452} in the toxicology and clinical lots, respectively.

[00124] Non-enzymatic deamidation of asparagine is another common modification that is frequently observed in antibodies. Deamidation, which occurs via the formation of a succinimide intermediate, followed by hydrolysis, results in the formation of isoaspartate and aspartate. Each deamidation event introduces one additional negative charge to an antibody and generates charge heterogeneity. An asparagine residue followed by glycine or serine within the primary amino acid sequence is by far the most susceptible site for deamidation within a protein. In HIH685P, two asparagine residues in the heavy chain (Asn^{320} and Asn^{389}) are directly followed by glycine residues and are therefore candidate sites for deamidation. Mass spectrometry analysis indicated that little deamidation occurred at Asn^{320} in any of the HIH685P sample lots. Focused mass analysis revealed that Asn^{389} was identified within a peptide eluted as peak 53. A peptide containing a deamidated form of Asn^{389} eluted within peak 52. Due to other co-eluting peptides within peak 52, it was not

possible to obtain a quantitative analysis of the relative amount of deamidation based on the UV signal. However, using ion intensities from the mass spectra, the relative percentage of deamidated Asn^{389} in either of the lots of HIH685P was determined to be less than 2%. In the reduced peptide map of HIH685P, mass spectrometry analysis also identified peak 49 as a light chain tryptic peptide that contained a native form of asparagine 94 followed by serine. An equivalent peptide, containing a deamidated form of Asn^{94} from the light chain, was also identified (peak 48). Integration of these two peaks revealed that approximately 4.2% of the total amount of this asparagine was present in the deamidated form in both the toxicology and clinical lots. Light chain asparagine 94 was identified as the only Asn-Ser containing peptide that exhibited deamidation end products in HIH685P. The light chain Asn^{94} is located within the third complementarity-determining region (CDR). Due to the structurally disruptive conditions used to prepare the peptide mapping samples, it cannot be ruled out that the deamidation observed at Asn^{94} may have been generated during the tryptic digestion procedure. No deamidation was observed at any other asparagine residue to any detectable level. The result suggests that deamidation of asparagine is not expected to occur to any appreciable level during the manufacture of HIH685P.

[00125] The potential for oxidation of surface methionine residues, which can potentially affect HIH685P stability and activity, was also examined. HIH685P contains three methionine residues located within the heavy chain. Some of the methionine residues are predicted to be located on the surface of the antibody making them susceptible for oxidation. Oxidation of the methionine side chain to the sulfide form increases the side chain mass by 16 daltons, and makes the side chain more polar. Extensive mass data analysis, focused on the methionine-containing peptides, revealed no oxidation at any of the three methionine residues in any of the tested lots of HIH685P, suggesting that expression and purification processes as well as storage conditions of the drug substances does not result in chemical changes to the protein.

EXAMPLE 12: GLYCOSYLATION PATTERNS

[00126] Glycosylation is a major post-translational modification that can lead to molecular mass heterogeneity of antibodies. Human IgG1 isotype antibodies contain a single canonical asparagine-linked (N-linked) glycosylation site located within the heavy chain constant region (Fc domain). Analysis of the reduced HIH685P tryptic map identified two glycopeptides corresponding to two tryptic peptides from the Fc domain (amino acid sequences of ^{298}EEQFNSTYR^{306} (SEQ ID NO: 534) and ^{294}TKPREEQFNSTYR^{306} (SEQ ID NO: 535)). The major glycan forms on these glycopeptides were elucidated based on MS analysis, and are summarized in Table 8. Glycopeptide mass analysis revealed that the HIH685P N-linked sugar is predominantly composed of a complex, bi-antennary structure,

with a core fucose and zero, one, or two galactose residues at the glycan chain termini. These structures are consistent with the typical glycan forms found on recombinant antibodies expressed from mammalian cells. The LC/MS analysis also revealed a peptide containing the Fc glycosylation site (Asn^{302}) but lacking glycan occupancy (peak 18 and peak 23). Integration of the peak areas in the UV chromatograms showed the 4.8% and 5.2% of N-linked sites at the Fc exhibited no glycan occupancy in the toxicology and clinical lots, respectively. These results are in agreement with the capillary electrophoresis analysis. The MS analysis of each of the peptides generated from the tryptic map reveals that HIH685P has no other N- linked or O linked glycosylation sites within the HIH685P antibody molecule.

[00127] Table 8: Peptide Assignments From Tryptic Map

Peak No.[a]	Retention Time (min)[b]	Fragment Identity[c]	Observed Peptide Mass[d] (Da)	Expected Peptide Mass[e] (Da)	Comments
1	8.8	H220-223	471.294	471.269	
2	9.0	H340-343	447.287	447.269	
3	9.9	H326-331	734.390	734.374	
4	11.3	H219-223	599.390	599.364	
5	11.9	L184-188	624.303	624.275	
6	12.9	L184-190	889.461	889.429	
7	15.1	L208-211	522.279	522.255	
8	15.3	H361-365	604.334	604.306	
9	17.2	H216-223	941.611	941.554	
10	18.0	L19-24	706.372	706.343	
11	18.5	L208-214	868.376	868.349	
12	19.2	L104-107	487.331	487.300	
13	20.3	H415-421	817.490	817.465	
14	20.4	H415-419	574.348	574.332	
15	20.6	L104-108	643.428	643.401	
16	21.0	H298-306	2957.177	2957.144	$Fuc(GlcNAc)_2(Man)_3(GlcNAc)_2(Gal)_2$
		H298-306	2795.150	2795.091	$Fuc(GlcNAc)_2(Man)_3(GlcNAc)_2(Gal)$
		H298-306	2592.198	2592.011	$Fuc(GlcNAc)_2(Man)_3(GlcNAc)_1(Gal)_1$
		H298-306	2429.972	2429.959	$Fuc(GlcNAc)_2(Man)_3(GlcNAc)$
		H298-306	2405.031	2404.927	$(GlcNAc)_2(Man)_5$
17	21.1	H298-306	2633.086	2633.038	$Fuc(GlcNAc)_2(Man)_3(GlcNAc)_2$
		H298-306	2487.066	2486.980	$(GlcNAc)_2(Man)_3(GlcNAc)_2$
		H298-306	2283.996	2283.901	$(GlcNAc)_2(Man)_3(GlcNAc)$
		H298-306	2267.995	2267.917	$Fuc(GlcNAc)_2(Man)_2(GlcNAc)$
		H298-306	2226.959	2226.879	$Fuc(GlcNAc)_2(Man)_3$
		H298-306	2121.904	2121.848	$(GlcNAc)_2(Man)_2(GlcNAc)$
		H298-306	2080.957	2080.821	$(GlcNAc)_2(Man)_3$
18	22.0	H298-306	1188.547	1188.505	non-glycosylated
19	22.6	L56-62	728.413	728.381	

Peak No.[a]	Retention Time (min)[b]	Fragment Identity[c]	Observed Peptide Mass[d] (Da)	Expected Peptide Mass[e] (Da)	Comments
20	23.0	H294-306	3277.467	3277.387	$Fuc(GlcNAc)_2(Man)_3(GlcNAc)_2(Gal)_1$
		H294-306	3439.492	3439.440	$Fuc(GlcNAc)_2(Man)_3(GlcNAc)_2(Gal)_2$
21	23.1	H294-306	3115.411	3115.335	$Fuc(GlcNAc)_2(Man)_3(GlcNAc)_2$
		H294-306	2969.325	2969.277	$(GlcNAc)_2(Man)_3(GlcNAc)_2$
		H294-306	2766.276	2766.198	$(GlcNAc)_2(Man)_3(GlcNAc)_1$
22	23.8	L146-149	559.340	559.311	
23	24.2	H294-306	1670.876	1670.801	non-glycosylated
24	27.9	L150-169	2135.024	2134.961	
25	28.8	H67-75	1064.579	1064.561	
26	29.5	H87-97	1245.589	1245.544	
27	29.9	H65-75	1277.733	1277.683	
28	30.6	H65-71	835.491	835.466	
29	30.9	H445-452	787.493	787.443	Heavy Chain with C-terminal K (1.5%)
30	32.8	H445-451	659.364	659.349	Heavy Chain C-terminal K Removal (=98.5%)
31	33.2	H254-260	834.459	834.426	
		H332-339	837.515	837.495	
32	35.9	L189-207	2140.139	2140.073	
33	37.0	L191-207	1874.980	1874.919	
34	37.4	L184-207	2746.385	2746.338	
35	38.0	L47-55	978.584	978.549	
36	40.6	H344-365	2509.374	2509.328	
		L146-169	2676.313	2676.262	
37	41.1	H350-365	1872.004	1871.962	
		H139-152	1320.696	1320.670	
38	44.6	H366-375	1160.680	1160.622	
		H127-138	1185.692	1185.639	
39	45.8	L170-183	1501.801	1501.750	
40	46.3	L63-78	1631.823	1631.778	
		L25-46	2452.250	2452.213	
		H1-19	1881.052	1880.995	
41	47.2	H76-86	1337.715	1337.675	
42	47.9	L150-183	3618.780	3618.701	
		H280-293	1676.822	1676.794	
43	48.5	L1-18	1883.018	1882.999	
44	48.9	H261-279	2138.056	2138.019	
45	49.8	H420-444	3043.452	3043.392	
46	50.1	H422-444	2800.328	2800.259	
47	51.0	L56-78	2342.233	2342.150	
48	51.9	L79-103	2997.316	2997.300	$N_{94}S$ partial deamidation (4.2%)
49	52.3	L79-103	2996.373	2996.292	
50	52.7	L146-183	4160.162	4161.003	
51	53.0	L79-108	3621.812	3621.683	
52	53.4	H376-397	2544.165	2544.131	$N_{389}G$ partial deamidation
53	53.7	H376-397	2543.220	2543.123	
54	54.6	H39-64	2651.450	2651.322	

Peak No.[a]	Retention Time (min)[b]	Fragment Identity[c]	Observed Peptide Mass[d] (Da)	Expected Peptide Mass[e] (Da)	Comments
55	55.0	H350-375	3014.715	3014.574	
56	56.3	H398-414	1872.973	1872.914	
		H44-66	2166.157	2166.063	
57	57.7	H20-38	2203.060	2203.015	
58	58.5	L109-126	1945.065	1945.019	
59	61.5	H224-253	3333.696	3333.634	
60	62.8	H228-253	2843.510	2843.450	
61	63.6	H307-325	2227.254	2227.199	
62	64.2	L127-142	1796.938	1796.887	
63	65.3	H307-322	1807.042	1806.999	
64	65.7	H228-260	3659.800	3659.866	
65	66.4	H376-414	4398.082	4398.027	
66	69.5	L150-183	3618.780	3618.701	
67	73.1	H153-223	7635.962	7635.851	
68	73.5	H153-219	7182.596	7182.592	
69	74.0	H153-218	7054.603	7054.497	
70	74.4	H153-215	6712.410	6712.307	
71	76.4	H139-223	8938.581	8938.511	
72	76.8	H139-219	8485.280	8485.252	
73	77.3	H139-218	8357.136	8357.157	
74	77.6	H139-215	8014.698	8014.967	
75	78.9	H98-126	1985.620	2985.548	

[00128] [a]Column 1: Peak numbers corresponding to the chromatographic peak numbers. [b]Column 2: Retention time of each peptide peak in minutes. [c]Column 3: Location of peptide within the HIH685P sequences. H and L denote sequences located within HIH685P heavy chain and light chain, respectively. [d]Column 4: Experimentally determined peptide mass from LC/MS and LC/MS/MS analysis. [e]Column 5: Theoretical peptide mass calculated from predicted trypsin digestion cleavage sites within the amino acid sequence of HIH685P sequence.

What is claimed is:

1. A stable pharmaceutical formulation comprising:

(i) an antibody at a concentration of from 20 ± 3 mg/ml to 60 ± 9 mg/ml that binds specifically to human angiopoietin 2 (human Ang-2), wherein the antibody comprises a heavy chain variable domain (HCVD) having the amino acid sequence of SEQ ID NO:18 and a light chain variable domain (LCVD) having the amino acid sequence of SEQ ID NO:20;

(ii) histidine at a concentration of 10 ± 1.5 mM;

(iii) polysorbate 20 in an amount of 0.2% w/v ± 0.03% w/v; and

(iv) sucrose in an amount of 10% w/v ± 1.5% w/v,

wherein the formulation has a pH of 6 ± 0.3, and at least 92% of the antibody has native conformation when the formulation is kept at about 45°C for up to about 28 days.

2. The pharmaceutical formulation of claim 1, wherein

(a) over 96% of the antibodies have a molecular weight of 151 kDa ± 1 kDa;

(b) at least 53% of the antibodies have an isoelectric point of about 8.13 ± 0.01; and

(c) from about 90% to about 92% of the antibodies are fucosylated.

3. The pharmaceutical formulation of claim 1, wherein the antibody concentration is about 25 mg/mL ± 0.375 mg/mL.

4. The pharmaceutical formulation of claim 1, wherein the antibody concentration is about 50 mg/mL ± 7.5 mg/mL.

5. The pharmaceutical formulation of claim 1, wherein at least 93% of the antibody has native conformation after 28 days at 45°C.

6. The pharmaceutical formulation of claim 1, wherein at least 32% of the antibody is the main charge variant of the antibody after 28 days at 45°C.

7. The pharmaceutical formulation of claim 1, wherein at least 97% of the antibody has native conformation after 28 days at 25°C.

8. The pharmaceutical formulation of claim 1, wherein at least 53% of the antibody is the main charge variant of the antibody after 28 days at 25°C.

9. The pharmaceutical formulation of claim 1, wherein at least 96% of the antibody has native conformation after 28 days at 37°C.

10. The pharmaceutical formulation of claim 1, wherein at least 45% of the antibody is the main charge variant of the antibody after 28 days at 37°C.

11. The pharmaceutical formulation of claim 1, wherein at least 97% of the antibody has native conformation after six months at 5°C.

12. The pharmaceutical formulation of claim 1, wherein at least 55% of the antibody is the main charge variant of the antibody after six months at 5°C.

13. The pharmaceutical formulation of claim 1, wherein the percent relative potency of the antibody after six months at 5°C is at least 100% of the potency of the antibody prior to storage.

14. The pharmaceutical formulation of claim 1, wherein at least 97% of the antibody has native conformation after six months at -80°C.

15. The pharmaceutical formulation of claim 1, wherein at least 55% of the antibody is the main charge variant of the antibody after six months at -80°C.

16. The pharmaceutical formulation of claim 1, wherein the percent relative potency of the antibody after six months at -80°C is at least 85% of the potency of the antibody prior to storage.

17. The pharmaceutical formulation of claim 1, wherein at least 96% of the antibody has native conformation after six months at -30°C.

18. The pharmaceutical formulation of claim 1, wherein at least 55% of the antibody is the main charge variant of the antibody after six months at -30°C.

19. The pharmaceutical formulation of claim 1, wherein the percent relative potency of the antibody after six months at -30°C is at least 84% of the potency of the antibody prior to storage.

20. The pharmaceutical formulation of claim 1, wherein at least 96% of the antibody has native conformation after six months at -20°C.

21. The pharmaceutical formulation of claim 1, wherein at least 55% of the antibody is the main charge variant of the antibody after six months at -20°C.

22. The pharmaceutical formulation of claim 1, wherein the percent relative potency of the antibody after six months at -20°C is at least 90% of the potency of the antibody prior to storage.

23. A pharmaceutical formulation comprising (a) 25 mg/mL ± 3.75 mg/mL of an anti-Ang-2 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose, wherein:

(a) the antibody comprises an HCVD of SEQ ID NO: 18 and an LCVD of SEQ ID NO: 20;

(b) over 96% of the antibodies have a molecular weight of 151 kDa ± 1 kDa;

(c) at least 53% of the antibodies have an isoelectric point of about 8.13 ± 0.01; and

(d) from about 90% to about 92% of the antibodies are fucosylated.

24. The pharmaceutical formulation of claim 23 consisting of (a) 25 mg/mL ± 3.75 mg/mL of the antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose, in water.

25. A pharmaceutical formulation comprising (a) 50 mg/mL ± 7.5 mg/mL of an anti-Ang-2 antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose, wherein:

(a) the antibody comprises an HCVD of SEQ ID NO: 18 and an LCVD of SEQ ID NO: 20;

(b) over 96% of the antibodies have a molecular weight of 151 kDa ± 1 kDa;

(c) at least 53% of the antibodies have an isoelectric point of about 8.13 ± 0.01; and

(d) from about 90% to about 92% of the antibodies are fucosylated.

26. The pharmaceutical formulation of claim 25 consisting of (a) 50 mg/mL ± 7.5 mg/mL of the antibody, (b) 10 mM ± 1.5 mM histidine, pH 6 ± 0.3, (c) 0.2% w/v ± 0.03% polysorbate 20, and (d) 10% w/v ± 1.5% sucrose, in water.

27. A pharmaceutical composition comprising the formulation of claim 1, wherein said composition is contained in a container.

28. The pharmaceutical composition of claim 27, wherein the container is a vial.

29. The pharmaceutical composition of claim 28, wherein the vial is glass.

30. The pharmaceutical composition of claim 27, wherein the container is an intravenous drip bag.

31. The pharmaceutical composition of claim 30, wherein the bag is made of polyvinyl chloride.

32. The pharmaceutical composition of claim 30, wherein the bag is made of polyolefin.

33. A kit comprising a pharmaceutical composition of claim 27, and instructions.

34. The kit of claim 33, wherein the container is a glass vial fitted with a fluorocarbon coated rubber stopper.