HIGH CONCENTRATION ANTIBODY FORMULATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority of U.S. Provisional Application No. 61/334,986 filed May 14, 2010, the disclosure of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Highly concentrated liquid antibody formulations are useful for delivering doses in smaller volume. However, highly concentrated protein formulations pose several problems. One problem is instability due to the formation of particulates. Another problem is increased viscosity as a result of numerous intermolecular interactions from the macromolecular nature of antibodies. Highly viscous formulations are difficult to manufacture, draw into a syringe, and inject. The use of force in manipulating the viscous formulations leads to excessive frothing, which can lead to denaturation and inactivation of active biologics.

[0003] U.S. Patent No. 6,875,432 and U.S. Patent Application Publication Nos. 2006/0182740, 2007/0172479, 2008/0160014 disclose antibody formulations and methods of making them. None of these publications disclose the antibodies referenced herein.

SUMMARY OF INVENTION

[0004] The present disclosure is based on the discovery that the addition of calcium acetate at low concentrations, e.g., 5-10 mM, reduced the effective viscosity in formulations comprising a high concentration of a selected anti-sclerostin antibody. In contrast, the same concentration of calcium acetate did not significantly reduce viscosity of other antibody formulations. In one aspect, the formulation is sterile and when in liquid or reconstituted liquid form comprises (a) an anti-sclerostin antibody at a concentration of at least 70 mg/mL, wherein the antibody comprises a set of six CDRs selected from the group consisting of SEQ ID NOs: 1-5 (Ab-A and Ab-1 CDRs), 15-20 (Ab-B CDRs), 25-30 (Ab-C CDRs), 35-40 (Ab-D CDRs), 45-50 (Ab-2 CDRs), 55-60 (Ab-3 and Ab-15 CDRs), 73-78 (Ab-4 and Ab-5 CDRs), 91-96 (Ab-6 CDRs), 101-106 (Ab-7 CDRs), 111-116 (Ab-8 CDRs), 121-126 (Ab-9 CDRs), 131-136 (Ab-10 CDRs), 141-146 (Ab-11 and Ab-16 CDRs), 159-164 (Ab-12 CDRs), 169-174 (Ab-13 and Ab-14 CDRs), 187-192 (Ab-17 and Ab-18 CDRs), 201-206 (Ab-19, Ab-20 and Ab-23 CDRs), 225-229 (Ab-21 and Ab-22 CDRs), or 239-244 (Ab-24 CDRs); and (b)

a calcium salt at a concentration ranging from about 1 mM to about 20 mM, or from about 5 mM to about 10 mM, wherein the formulation has an absolute viscosity of about 10 cP or less. Absolute viscosity as described herein is measured was measured using Brookfield LV-DVII cone and plate viscometer with a CPE-40 spindle with matching sample cup temperature regulated by a circulating water bath at constant 25°C.

[0005] In some embodiments, the calcium salt is selected from the group consisting of calcium acetate, calcium carbonate and calcium chloride. In one embodiment, the calcium salt is calcium acetate. Alternatively, in some embodiments, the calcium salt is present at a concentration that reduces viscosity of an antibody formulation by at least 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60% or more compared to the same formulation of antibody lacking the calcium salt.

[0006] In a related aspect, the formulation is sterile and when in liquid or reconstituted liquid form comprises (a) an anti-sclerostin antibody at a concentration of from about 70 mg/mL to about 200 mg/mL, wherein the antibody comprises a set of six CDRs selected from the group consisting of SEQ ID NOs: 1-5 (Ab-A and Ab-1 CDRs), 15-20 (Ab-B CDRs), 25-30 (Ab-C CDRs), 35-40 (Ab-D CDRs), 45-50 (Ab-2 CDRs), 55-60 (Ab-3 and Ab-15 CDRs), 73-78 (Ab-4 and Ab-5 CDRs), 91-96 (Ab-6 CDRs), 101-106 (Ab-7 CDRs), 111-116 (Ab-8 CDRs), 121-126 (Ab-9 CDRs), 131-136 (Ab-10 CDRs), 141-146 (Ab-11 and Ab-16 CDRs), 159-164 (Ab-12 CDRs), 169-174 (Ab-13 and Ab-14 CDRs), 187-192 (Ab-17 and Ab-18 CDRs), 201-206 (Ab-19, Ab-20 and Ab-23 CDRs), 225-229 (Ab-21 and Ab-22 CDRs), or 239-244 (Ab-24 CDRs); and (b) calcium acetate at a concentration ranging from about 5 mM to about 15 mM, or from about 5 mM to about 10 mM, wherein the formulation has an absolute viscosity of about 10 cP or less. Alternatively, in some embodiments, the calcium acetate is present at a concentration that reduces viscosity of an antibody formulation by at least 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%. 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60% or more compared to the same formulation of antibody lacking the calcium acetate.

[0007] Also provided is a method for reducing the viscosity of a protein formulation, the method comprising; adding calcium acetate at a concentration of between about 1 mM and about 20 mM, to an anti-sclerostin immunoglobulin formulation, wherein the formulation comprises an immunoglobulin at a concentration of from about 70 mg/mL to about 200 mg/mL, wherein the viscosity of the formulation with the calcium acetate is reduced compared to the viscosity of an antibody formulation without the calcium acetate.

[0008] In another aspect, the formulation is sterile and has an absolute viscosity of about 10 cP or less comprising: (a) Ab-5 at a concentration of at least 70 mg/mL to about 200 mg/mL; (b) calcium acetate at a concentration ranging from about 1 mM to about 20 mM; and (c) a polyol such as sucrose, for example, in an amount ranging from about 1% w/v to about 12% w/v. In certain embodiments, the polyol is in amount ranging from about 4% to 10%. In some embodiments, the immunoglobulin comprises the amino acid sequences of SEQ ID NO: 86 (Ab-5 heavy chain variable region) and/or SEQ ID NO: 84 (Ab-5 light chain variable region).

[0009] In another aspect, the formulation is sterile and has an absolute viscosity of about 10 cP or less and comprises (a) Ab-5 at a concentration of at least 70 mg/mL to about 200 mg/mL; (b) calcium acetate at a concentration ranging from about 1 mM to about 20 mM; and (c) a polyol such as sucrose, for example, in an amount ranging from about 4% w/v to about 6% w/v.

[0010] In any of the preceding aspects, in some embodiments, the formulation further comprises (c) an acetate buffer, for example, sodium acetate, at a concentration of from about 5 mM to about 15 mM, or from about 5 mM to about 10 mM. In some embodiments, the total concentration of acetate is about 10 mM to about 50 mM, or about 20 mM to about 40 mM.

[0011] In a different aspect, the formulation is sterile and when in liquid or reconstituted liquid form comprises (a) an anti-sclerostin antibody at a concentration of from about 70 mg/mL to about 200 mg/mL, wherein the antibody comprises a set of six CDRs selected from the group consisting of SEQ ID NOs: 1-5 (Ab-A and Ab-1 CDRs), 15-20 (Ab-B CDRs), 25-30 (Ab-C CDRs), 35-40 (Ab-D CDRs), 45-50 (Ab-2 CDRs), 55-60 (Ab-3 and Ab-15 CDRs), 73-78 (Ab-4 and Ab-5 CDRs), 91-96 (Ab-6 CDRs), 101-106 (Ab-7 CDRs), 111-116 (Ab-8 CDRs), 121-126 (Ab-9 CDRs), 131-136 (Ab-10 CDRs), 141-146 (Ab-11 and Ab-16 CDRs),

159-164 (Ab-12 CDRs), 169-174 (Ab-13 and Ab-14 CDRs), 187-192 (Ab-17 and Ab-18 CDRs), 201-206 (Ab-19, Ab-20 and Ab-23 CDRs), 225-229 (Ab-21 and Ab-22 CDRs), or 239-244 (Ab-24 CDRs); and (b) an acetate salt and/or acetate buffer at a concentration ranging from about 10 mM to about 50 mM acetate, or from about 20 mM to about 40 mM acetate, wherein the formulation has an absolute viscosity of about 10 cP or less. In some embodiments, the acetate salt and/or buffer comprises calcium acetate and/or sodium acetate. Alternatively, in some embodiments, the acetate salt and/or buffer is present at a concentration that reduces viscosity of an antibody formulation by at least 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%. 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60% or more compared to the same formulation of antibody lacking the acetate salt and/or buffer.

[0012] In any of the preceding aspects, in some embodiments, the total concentration of ions (cations and anions) in solution is about 20 mM to about 70 mM, or about 30 mM to about 60 mM. In any of these embodiments, the total osmolarity is less than about 400 mOsm/L or 350 mOsm/L, and is preferably close to isotonic, e.g. 250-350 mOsm/L. In some embodiments, the formulation is hypotonic. For example, in such embodiments, the osmolarity of the formulation is less than about 250 mOsm/L. In other embodiments, the formulation hypertonic. Thus, in such embodiments, the total osmolarity of the formulation is greater than about 350 mOsm/L.

[0013] In any of the formulations described herein, in some embodiments, an anti-sclerostin antibody in the formulation can comprise mature heavy and/or light chain variable regions of any of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-17, Ab-19, Ab-21, Ab-23 or Ab-24. Thus, in specific embodiments, the antibody comprises the amino acid sequences of: SEQ ID NO: 14 (Ab-1 heavy chain variable region), and/or SEQ ID NO: 12 (Ab-1 light chain variable region); or SEQ ID NO: 68 (Ab-15 heavy chain variable region), and/or SEQ ID NO: 66 (Ab-15 light chain variable region); or SEQ ID NO: 86 (Ab-5 heavy chain variable region), and/or SEQ ID NO: 84 (Ab-5 light chain variable region); or SEQ ID NO: 154 (Ab-16 heavy chain variable region), and/or SEQ ID NO: 152 (Ab-16 light chain variable region); or SEQ ID NO: 182 (Ab-14 heavy chain variable region) and/or SEQ ID NO: 180 (Ab-14 light chain variable region); or SEQ ID NO: 208 (Ab-19 heavy chain variable region) and/or SEQ ID NO: 207

(Ab-19 light chain variable region); or SEQ ID NO: 216 (Ab-20 heavy chain variable region) and/or SEQ ID NO: 214 (Ab-20 light chain variable region); or SEQ ID NO: 220 (Ab-23 heavy chain variable region) and/or SEQ ID NO: 218 (Ab-23 light chain variable region); or SEQ ID NO: 238 (Ab-22 heavy chain variable region) and/or SEQ ID NO: 236 (Ab-22 light chain variable region). In some embodiments, the antibody comprises the mature heavy and/or light chains of any of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-19, Ab-23 or Ab-24. In some embodiments, the antibody comprises amino acid sequences obtainable by expressing in mammalian host cells the cDNA encoding the heavy and/or light chain, or alternatively the heavy and/or light chain variable region, of any of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-19, Ab-23 or Ab-24, as described herein.

[0014] In any of the formulations described herein, in some embodiments, the anti-sclerostin antibody comprises the CDRs, or the mature heavy and light chain variable regions, or the mature heavy and light chains, of any of Ab-4 or Ab-5; Ab-13 or Ab-14; or Ab-19, Ab-20 or Ab-23. In any of the formulations described herein, in some embodiments, the antibody binds to sclerostin of SEQ ID NO: 1 with a K_D of 10^{-7} or less (lower numbers meaning higher binding affinity).

[0015] In any of the formulations described herein, in some embodiments, the antibody in the formulation is present at a concentration of at least 120 mg/mL, or at least 140 mg/mL. In any of the formulations described herein, in some embodiments, the absolute viscosity of the formulation is about 8 cP or less, or about 6 cP or less. In alternative embodiments, the antibody in the formulation is present at a concentration of about 70 mg/mL to about 130 mg/mL, wherein the formulation has an absolute viscosity of about 10 cP or less.

[0016] In some embodiments, any of the formulations described herein further comprises a polyol such as sucrose, for example, in an amount ranging from about 4% w/v to about 6%. In some embodiments, the formulation comprises about 9% sucrose. In some embodiments, any of the formulations described herein optionally comprises other pharmaceutically acceptable excipients, e.g. salt, buffer, amino acid, stabilizer, polyol, other tonicity agent, surfactant, bulking agent, cryoprotectant, lyoprotectant, antioxidant, metal ion, chelating agent, and/or preservative. In some embodiments, the formulation has less than 0.05% by weight surfactant.

[0017] In any of the formulations described herein, in some embodiments, the formulation has a pH ranging from about 4.5 to about 6, or about 5 to about 6, or about 5 to about 5.5. In some embodiments, the formulation has a pH of 5.2.

[0018] Also described herein are methods of using the formulations described herein to treat any disorder associated with decreased bone density, including but not limited to, achondroplasia, cleidocranial dysostosis, enchondromatosis, fibrous dysplasia, Gaucher's Disease, hypophosphatemic rickets, Marfan's syndrome, multiple hereditary exotoses, neurofibromatosis, osteogenesis imperfecta, osteopetrosis, osteopoikilosis, sclerotic lesions, pseudoarthrosis, pyogenic osteomyelitis, periodontal disease, anti-epileptic drug induced bone loss, primary or secondary hyperparathyroidism, familial hyperparathyroidism syndromes, weightlessness induced bone loss, osteoporosis in men, postmenopausal bone loss, osteoarthritis, renal osteodystrophy, infiltrative disorders of bone, oral bone loss, osteonecrosis of the jaw, juvenile Paget's disease, melorheostosis, metabolic bone diseases, mastocytosis, sickle cell anemia/disease, organ transplant related bone loss, kidney transplant related bone loss, systemic lupus erythematosus, ankylosing spondylitis, epilepsy, juvenile arthritides, thalassemia, mucopolysaccharidoses, Fabry Disease, Turner Syndrome, Down Syndrome, Klinefelter Syndrome, leprosy, Perthe's Disease, adolescent idiopathic scoliosis, infantile onset multi-system inflammatory disease, Winchester Syndrome, Menkes Disease, Wilson's Disease, ischemic bone disease (such as Legg-Calve-Perthes disease or regional migratory osteoporosis), anemic states, conditions caused by steroids, glucocorticoid-induced bone loss, heparin-induced bone loss, bone marrow disorders, scurvy, malnutrition, calcium deficiency, osteoporosis, osteopenia, alcoholism, chronic liver disease, postmenopausal state, chronic inflammatory conditions, rheumatoid arthritis, inflammatory bowel disease, ulcerative colitis, inflammatory colitis, Crohn's disease, oligomenorrhea, amenorrhea, pregnancy, diabetes mellitus, hyperthyroidism, thyroid disorders, parathyroid disorders, Cushing's disease, acromegaly, hypogonadism, immobilization or disuse, reflex sympathetic dystrophy syndrome, regional osteoporosis, osteomalacia, bone loss associated with joint replacement, HIV associated bone loss, bone loss associated with loss of growth hormone, bone loss associated with cystic fibrosis, chemotherapy-associated bone loss, tumor-induced bone loss, cancer-related bone loss, hormone ablative bone loss, multiple myeloma, drug-induced bone loss, anorexia nervosa, disease-associated facial bone loss, disease-associated cranial bone loss, disease-associated bone loss of the jaw, disease-associated bone loss of the skull, bone loss associated with aging, facial bone loss associated with aging, cranial bone

loss associated with aging, jaw bone loss associated with aging, skull bone loss associated with aging, or bone loss associated with space travel.

[0019] The formulations described herein, in some embodiments, are useful for improving outcomes in orthopedic procedures, dental procedures, implant surgery, joint replacement, bone grafting, bone cosmetic surgery and bone repair such as fracture healing, nonunion healing, delayed union healing and facial reconstruction. One or more formulations may be administered before, during and/or after the procedure, replacement, graft, surgery or repair.

[0020] Such methods may comprise administering a formulation in a therapeutically effective amount, e.g. an amount effective to improve bone density, and may further comprise administering a second therapeutic agent.

[0021] Also disclosed herein is a vial, kit or container, e.g. a pre-filled syringe or injection device, comprising a formulation described herein and optionally a label comprising instructions to use the appropriate volume or amount of the formulation necessary to achieve a dose of from about 0.5-20 mg/kg, or 0.5-10 mg/kg of patient body weight.

[0022] It should be understood that while various embodiments in the specification are presented using "comprising" language, under various circumstances, a related embodiment may also be described using "consisting of" or "consisting essentially of" language. It is to be noted that the term "a" or "an", refers to one or more, for example, "an immunoglobulin molecule," is understood to represent one or more immunoglobulin molecules. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0023] It should also be understood that when describing a range of values, the characteristic being described could be an individual value found within the range. For example, "a pH from about pH 4 to about pH 6," could be, but is not limited to, pH 4, 4.2, 4.6, 5.1 5.5 etc. and any value in between such values. Additionally, "a pH from about pH 4 to about pH 6," should not be construed to mean that the pH of a formulation in question varies 2 pH units in the range from pH 4 to pH 6 during storage, but rather a value may be picked in that range for the pH of the solution, and the pH remains buffered at about that pH. In some embodiments, when the term "about" is used, it means the recited number plus or minus 5%, 10%, 15% or more of that recited number. The actual variation intended is determinable from the context.

[0024] In any of the ranges described herein, the endpoints of the range are included in the range. However, the description also contemplates the same ranges in which the lower and/or the higher endpoint is excluded. Additional features and variations of the invention will be apparent to those skilled in the art from the entirety of this application, including the drawing and detailed description, and all such features are intended as aspects of the invention. Likewise, features of the invention described herein can be re-combined into additional embodiments that also are intended as aspects of the invention, irrespective of whether the combination of features is specifically mentioned above as an aspect or embodiment of the invention. Also, only such limitations which are described herein as critical to the invention should be viewed as such; variations of the invention lacking limitations which have not been described herein as critical are intended as aspects of the invention.

DETAILED DESCRIPTION

[0025] Described herein are formulations comprising high concentrations of antibody that contain calcium salts and/or acetate salts or buffers to reduce viscosity, methods of using these formulations, and containers or kits comprising these formulations.

[0026] I. Antibodies in the formulation

[0027] In some embodiments, the anti-sclerostin antibody in the formulation is present at a concentration of at least about 70 mg/ml, about 71 mg/ml, about 72 mg/ml, about 73 mg/ml, about 74 mg/ml, about 75 mg/ml, about 76 mg/ml, about 77 mg/ml, about 78 mg/ml, about 79 mg/ml, about 80 mg/ml, about 81 mg/ml, about 82 mg/ml, about 83 mg/ml, about 84 mg/ml, about 85 mg/ml, about 86 mg/ml, about 87 mg/ml, about 88 mg/ml, about 89 mg/ml, about 90 mg/ml, about 91 mg/ml, about 92 mg/ml, about 93 mg/ml, about 94 mg/ml, about 95 mg/ml, about 96 mg/ml, about 97 mg/ml, about 98 mg/ml, about 99 mg/ml, about 100 mg/ml, about 101 mg/ml, about 102 mg/ml, about 103 mg/ml, about 104 mg/ml, about 105 mg/ml, about 106 mg/ml, about 107 mg/ml, about 108 mg/ml, about 109 mg/ml, about 110 mg/ml, about 111 mg/ml, about 112 mg/ml, about 113 mg/ml, about 114 mg/ml, about 115 mg/ml, about 116 mg/ml, about 117 mg/ml, about 118 mg/ml, about 119 mg/ml, about 120 mg/ml, about 121 mg/ml, about 122 mg/ml, about 123 mg/ml, about 124 mg/ml, about 125 mg/ml, about 126 mg/ml, about 127 mg/ml, about 128 mg/ml, about 129 mg/ml, about 130 mg/ml, about 131 mg/ml, about 132 mg/ml, about 132 mg/ml, about 133 mg/ml, about 134 mg/ml, about 135 mg/ml, about 136 mg/ml, about 137 mg/ml, about 138 mg/ml, about 139 mg/ml, about 140 mg/ml, about 141 mg/ml, about 142 mg/ml, about 143 mg/ml, about 144

mg/ml, about 145 mg/ml, about 146 mg/ml, about 147 mg/ml, about 148 mg/ml, about 149 mg/ml, about 150 mg/ml, about 151 mg/ml, about 152 mg/ml, about 153 mg/ml, about 154 mg/ml, about 155 mg/ml, about 156 mg/ml, about 157 mg/ml, about 158 mg/ml, about 159 mg/ml, or about 160 mg/ml, and may range up to , e.g., about 300 mg/ml, about 290 mg/ml, about 280 mg/ml, about 270 mg/ml, about 260 mg/ml, about 250 mg/ml, about 240 mg/ml, about 230 mg/ml, about 220 mg/ml, about 210 mg/ml, about 200 mg/ml, about 190 mg/ml, about 180 mg/ml, or about 170 mg/ml. Any range featuring a combination of the foregoing endpoints is contemplated, including but not limited to: about 70 mg/ml to about 250 mg/ml, about 70 mg/ml to about 200 mg/ml, about 70 mg/ml to about 160 mg/ml, about 100 mg/ml to about 250 mg/ml, about 100 mg/l to about 200 mg/ml, or about 100 mg/ml to about 180 mg/ml.

[0028] Antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23 and Ab-24 were previously described in U.S. Patent Application Publication No. 2007/0110747, the disclosure of which including sequence listing is incorporated herein by reference in its entirety.

[0029] The anti-sclerostin antibodies described herein bind to sclerostin of SEQ ID NO: 1 with a K_D of 10^{-6} or less, or 10^{-7} or less, or 10^{-8} or less, or 10^{-9} or less (lower numbers meaning higher binding affinity). Affinity can be determined by any means known in the art, including via Biacore technology.

[0030] In some exemplary embodiments, the antibody comprises the heavy and/or light chain of any of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-19, Ab-23 or Ab-24. The amino acid sequences of the mature full length light chain of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-17, Ab-19, Ab-23 and Ab-24, including the constant region, are set forth in SEQ ID NOs: 8, 22, 32, 42, 52, 62, 80, 88, 98, 108, 118, 128, 138, 148, 166, 176, 184, 70, 210 222, and 246, respectively. The amino acid sequences of the mature full length heavy chain of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-19, Ab-23 and Ab-24, including the constant region, are set forth in SEQ ID NOs: 10, 24, 34, 44, 54, 64, 82, 90, 100, 110, 120, 130, 140, 150, 168, 178, 186, 72, 224, and 248.

[0031] Corresponding cDNA sequences encoding the full length light chain of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-19, Ab-23 and Ab-24, including the constant region, are set forth in SEQ ID NOs: 7, 21, 31, 41, 51, 61, 79, 87, 97, 107, 117, 127, 137, 147, 165, 175, 183, 69, 209, 221 and 245, respectively. Corresponding cDNA sequences encoding the full length heavy chain, including the constant region of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-19, Ab-23 and Ab-24, are set forth in SEQ ID NOs: 9, 23, 33, 43, 53, 63, 81, 89, 99, 109, 119, 129, 139, 149, 167, 177, 185, 71, 211, 223, and 247, respectively.

[0032] In other exemplary embodiments, the antibody comprises the heavy and/or light chain variable region of any of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-17, Ab-19, Ab-21, Ab-23 or Ab-24. For example, the antibody comprises SEQ ID NO: 14 (Ab-1 heavy chain variable region), and/or SEQ ID NO: 12 (Ab-1 light chain variable region); SEQ ID NO: 68 (Ab-15 heavy chain variable region), and/or SEQ ID NO: 66 (Ab-15 light chain variable region); or SEQ ID NO: 86 (Ab-5 heavy chain variable region), and/or SEQ ID NO: 84 (Ab-5 light chain variable region); or SEQ ID NO: 154 (Ab-16 heavy chain variable region), and/or SEQ ID NO: 152 (Ab-16 light chain variable region); or SEQ ID NO: 182 (Ab-14 heavy chain variable region) and/or SEQ ID NO: 180 (Ab-14 light chain variable region); or SEQ ID NO: 208 (Ab-19 heavy chain variable region) and/or SEQ ID NO: 207 (Ab-19 light chain variable region); or SEQ ID NO: 216 (Ab-20 heavy chain variable region) and/or SEQ ID NO: 214 (Ab-20 light chain variable region); or SEQ ID NO: 220 (Ab-23 heavy chain variable region) and/or SEQ ID NO: 218 (Ab-23 light chain variable region); or SEQ ID NO: 238 (Ab-22 heavy chain variable region) and/or SEQ ID NO: 236 (Ab-22 light chain variable region).

[0033] In some embodiments, the antibody comprises the CDRs set forth in SEQ ID NOs: 1-5 (Ab-A and Ab-1 CDRs), or 15-20 (Ab-B CDRs), or 25-30 (Ab-C CDRs), or 35-40 (Ab-D CDRs), or 45-50 (Ab-2 CDRs), or 55-60 (Ab-3 and Ab-15 CDRs), or 73-78 (Ab-4 and Ab-5 CDRs), or 91-96 (Ab-6 CDRs), or 101-106 (Ab-7 CDRs), or 111-116 (Ab-8 CDRs), or 121-126 (Ab-9 CDRs), or 131-136 (Ab-10 CDRs), or 141-146 (Ab-11 and Ab-16 CDRs), or 159-164 (Ab-12 CDRs), or 169-174 (Ab-13 and Ab-14 CDRs), or 187-192 (Ab-17 and Ab-18

CDRs), or 201-206 (Ab-19, Ab-20 and Ab-23 CDRs), or 225-229 (Ab-21 and Ab-22 CDRs), or 239-244 (Ab-24 CDRs).

[0034] In some embodiments, the antibody comprises amino acid sequences obtainable by expressing in mammalian host cells the cDNA encoding the heavy and/or light chain, or alternatively the heavy and/or light chain variable region, of any of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-19, Ab-23 or Ab-24, as described herein. In any of the formulations described herein, in some embodiments, the antibody is a tetrameric immunoglobulin consisting of two heavy chains and two light chains.

[0035] In some embodiments, the antibody comprises the CDRs of any of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-19, Ab-23 or Ab-24, and comprises a heavy and/or light chain comprising an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the heavy and/or light chain of antibody Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-19, Ab-23 or Ab-24, respectively. In some embodiments, the antibody comprises the CDRs of any of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-19, Ab-23 or Ab-24, and comprises a heavy and/or light chain comprising an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the heavy and/or light chain variable region of antibody Ab-A, Ab-B, Ab-C, Ab-D, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-15, Ab-16, Ab-19, Ab-23 or Ab-24, respectively.

[0036] In some embodiments, the antibody:

[0037] 1) retains any one, two, three, four, five, or six of CDRH1, CDRH2, CDRH3, CDRL1, CDRL2 and/or CDRL3 of any of antibodies Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23 or Ab-24, optionally including one or two mutations in such CDR(s),

[0038] 2) retains all of CDRH1, CDRH2, CDRH3, or the heavy chain variable region of, any of antibody Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-

8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23 or Ab-24, optionally including one or two mutations in such CDR(s),

[0039] 3) retains all of CDRL1, CDRL2, CDRL3, or the light chain variable region of, any of antibody Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23 or Ab-24, optionally including one or two mutations in such CDR(s),

[0040] 4) binds to the same epitope of sclerostin as antibody Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23 or Ab-24, e.g. as determined through X-ray crystallography, or an amino acid within a loop formed by amino acids 86-111 of SEQ ID NO: 249; and/or

[0041] 5) competes with antibody Ab-A, Ab-B, Ab-C, Ab-D, Ab-1, Ab-2, Ab-3, Ab-4, Ab-5, Ab-6, Ab-7, Ab-8, Ab-9, Ab-10, Ab-11, Ab-12, Ab-13, Ab-14, Ab-15, Ab-16, Ab-17, Ab-18, Ab-19, Ab-20, Ab-21, Ab-22, Ab-23 or Ab-24 for binding to sclerostin by more than about 75%, more than about 80%, or more than about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% or 95%.

[0042] In some embodiments, the antibody comprises all three light chain CDRs, the mature light chain variable region, all three heavy chain CDRs, the mature heavy chain variable region, all six CDRs, or both the mature light chain and the mature heavy chain variable region. In some exemplary embodiments, two light chain CDRs from an antibody may be combined with a third light chain CDR from a different antibody. Alternatively, a CDRL1 from one antibody can be combined with a CDRL2 from a different antibody and a CDRL3 from yet another antibody, particularly where the CDRs are highly homologous. Similarly, two heavy chain CDRs from an antibody may be combined with a third heavy chain CDR from a different antibody; or a CDRH1 from one antibody can be combined with a CDRH2 from a different antibody and a CDRH3 from yet another antibody, particularly where the CDRs are highly homologous.

[0043] The term "antibody" refers to an intact antibody or a binding fragment thereof. An antibody may comprise a complete antibody molecule (including polyclonal, monoclonal, chimeric, humanized, or human versions having full length heavy and/or light chains), or comprise an antigen binding fragment thereof. Antibody fragments include $F(ab')_2$, Fab,

Fab', Fv, Fc, and Fd fragments, and can be incorporated into single domain antibodies, single-chain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, e.g., Hollinger and Hudson, *Nature Biotechnology*, *23*(9):1126-1136 (2005)).

[0044] An "isolated" antibody refers to an antibody, as that term is defined herein, that has been identified and separated from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In certain embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated naturally occurring antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0045] An "immunoglobulin" or "native antibody" is a tetrameric glycoprotein. In a naturally-occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a "variable" ("V") region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Immunoglobulins can be assigned to different classes depending on the amino acid sequence of the constant domain of their heavy chains. Heavy chains are classified as mu (μ), delta (Δ), gamma (γ), alpha (α), and epsilon (ε), and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Several of these may be further divided into subclasses or isotypes, e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. Different isotypes have different effector functions; for example, IgG1 and IgG3 isotypes have antibody-dependent cellular cytotoxicity (ADCC) activity. Human light chains are classified as kappa (κ) and lambda (λ) light chains. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See

generally, Fundamental Immunology, Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)).

[0046] Allotypes are variations in antibody sequence, often in the constant region, that can be immunogenic and are encoded by specific alleles in humans. Allotypes have been identified for five of the human IGHC genes, the IGHG1, IGHG2, IGHG3, IGHA2 and IGHE genes, and are designated as G1m, G2m, G3m, A2m, and Em allotypes, respectively. At least 18 Gm allotypes are known: nG1m(1), nG1m(2), G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b5, b0, b3, b4, s, t, g1, c5, u, v, g5). There are two A2m allotypes A2m(1) and A2m(2).

[0047] The term "hypervariable" region refers to amino acid residues from a complementarity determining region or CDR (i.e., residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain as described by Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Even a single CDR may recognize and bind antigen, although with a lower affinity than the entire antigen binding site containing all of the CDRs.

[0048] An alternative definition of residues from a hypervariable "loop" is described by Chothia et al., *J. Mol.Biol. 196*: 901-917 (1987) as residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain .

[0049] "Framework" or FR residues are those variable region residues other than the hypervariable region residues.

[0050] "Antibody fragments" comprise a portion of an intact immunoglobulin, preferably an antigen binding or variable region of the intact antibody, and include multispecific (bispecific, trispecific, etc.) antibodies formed from antibody fragments. Fragments of immunoglobulins may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies.

[0051] Nonlimiting examples of antibody fragments include Fab, Fab', $F(ab')_2$, Fv (variable region), domain antibodies (dAb, containing a VH domain) (Ward et al., Nature 341:544-546, 1989), complementarity determining region (CDR) fragments, single-chain antibodies (scFv,

containing VH and VL domains on a single polypeptide chain) (Bird et al., Science 242:423-426, 1988, and Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883, 1988, optionally including a polypeptide linker; and optionally multispecific, Gruber et al., J. Immunol. 152: 5368 (1994)), single chain antibody fragments, diabodies (VH and VL domains on a single polypeptide chain that pair with complementary VL and VH domains of another chain) (EP 404,097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)), triabodies, tetrabodies, minibodies (scFv fused to CH3 via a peptide linker (hingeless) or via an IgG hinge) (Olafsen, et al., Protein Eng Des Sel. 2004 Apr;17(4):315-23), linear antibodies (tandem Fd segments (VH -CH1-VH -CH1) (Zapata et al., Protein Eng.,8(10):1057-1062 (1995)); chelating recombinant antibodies (crAb, which can bind to two adjacent epitopes on the sane antigen) (Neri et al., J Mol Biol. 246:367-73, 1995), bibodies (bispecific Fab-scFv) or tribodies (trispecific Fab-(scFv)(2)) (Schoonjans et al., J Immunol. 165:7050-57, 2000; Willems et al., J Chromatogr B Analyt Technol Biomed Life Sci. 786:161-76, 2003), intrabodies (Biocca, et al., EMBO J. 9:101–108, 1990; Colby et al., Proc Natl Acad Sci U S A. 101:17616-21, 2004) which may also comprise cell signal sequences which retain or direct the antibody intracellularly (Mhashilkar et al, EMBO J 14:1542-51, 1995; Wheeler et al., FASEB J. 17:1733-5, 2003), transbodies (cell-permeable antibodies containing a protein transduction domain (PTD) fused to scFv (Heng et al., Med Hypotheses. 64:1105-8, 2005), nanobodies (approximately 15kDa variable domain of the heavy chain) (Cortez-Retamozo et al., Cancer Research 64:2853-57, 2004), small modular immunopharmaceuticals (SMIPs) (WO03/041600, U.S. Patent publication 20030133939 and US Patent Publication 20030118592), an antigen-binding-domain immunoglobulin fusion protein, a camelized antibody (in which VH recombines with a constant region that contains hinge, CH1, CH2 and CH3 domains) (Desmyter et al., J. Biol. Chem. 276:26285-90, 2001; Ewert et al., Biochemistry 41:3628-36, 2002; U.S. Patent Publication Nos. 20050136049 and 20050037421), a VHH containing antibody, heavy chain antibodies (HCAbs, homodimers of two heavy chains having the structure H2L2), or variants or derivatives thereof, and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide, such as a CDR sequence, as long as the antibody retains the desired biological activity.

[0052] The term "variant" when used in connection with antibodies refers to a polypeptide sequence of an antibody that contains at least one amino acid substitution, deletion, or insertion in the variable region or the portion equivalent to the variable region, provided that

the variant retains the desired binding affinity or biological activity. In addition, the antibodies as described herein may have amino acid modifications in the constant region to modify effector function of the antibody, including half-life or clearance, ADCC and/or CDC activity. Such modifications can enhance pharmacokinetics or enhance the effectiveness of the antibody in treating cancer, for example. See Shields et al., J. Biol. Chem., 276(9):6591-6604 (2001), incorporated by reference herein in its entirety. In the case of IgG1, modifications to the constant region, particularly the hinge or CH2 region, may increase or decrease effector function, including ADCC and/or CDC activity. In other embodiments, an IgG2 constant region is modified to decrease antibody-antigen aggregate formation. In the case of IgG4, modifications to the constant region, particularly the hinge region, may reduce the formation of half-antibodies.

[0053] The term "modification" when used in connection with antibodies or polypeptides described herein, includes but is not limited to, one or more amino acid change (including substitutions, insertions or deletions); chemical modifications that do not interfere with hepcidin-binding activity; covalent modification by conjugation to therapeutic or diagnostic agents; labeling (e.g., with radionuclides or various enzymes); covalent polymer attachment such as pegylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of non-natural amino acids. In some embodiments, modified polypeptides (including antibodies) of the invention will retain the binding properties of unmodified molecules of the invention.

[0054] The term "derivative" when used in connection with antibodies or polypeptides of the invention refers to antibodies or polypeptides that are covalently modified by conjugation to therapeutic or diagnostic agents, labeling (e.g., with radionuclides or various enzymes), covalent polymer attachment such as pegylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of non-natural amino acids. In some embodiments, derivatives of the invention will retain the binding properties of underivatized molecules of the invention.

[0055] Methods for making bispecific or other multispecific antibodies are known in the art and include chemical cross-linking, use of leucine zippers [Kostelny et al., J. Immunol. 148:1547-1553, 1992]; diabody technology [Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-48, 1993]; scFv dimers [Gruber et al., J. Immunol. 152: 5368, 1994], linear

antibodies [Zapata et al., Protein Eng. 8:1057-62, 1995]; and chelating recombinant antibodies [Neri et al., J Mol Biol. 246:367-73, 1995].

[0056] Proteins and non-protein agents may be conjugated to the antibodies by methods that are known in the art. Conjugation methods include direct linkage, linkage via covalently attached linkers, and specific binding pair members (e.g., avidin-biotin). Such methods include, for example, that described by Greenfield et al., Cancer Research 50, 6600-6607 (1990) for the conjugation of doxorubicin and those described by Arnon et al., Adv. Exp. Med. Biol. 303, 79-90 (1991) and by Kiseleva et al., Mol. Biol. (USSR) 25, 508-514 (1991) for the conjugation of platinum compounds.

[0057] In some embodiments, antibodies and antibody fragments described herein are obtained, for example, from naturally-occurring antibodies, or Fab or scFv phage display libraries. The phrase "humanized antibody" refers to an antibody derived from a sequence of a non-human antibody, typically a rodent monoclonal antibody, which comprises modifications that render the sequence more human-like. Alternatively, a humanized antibody may be derived from a chimeric antibody.

[0058] Antibody fragments include domain antibody (dAb) fragment (Ward et al., *Nature* 341:544-546, 1989) which consists of a V_H domain, "linear antibodies" comprise a pair of tandem Fd segments (V_H -C_H1-V_H -C_H1) which form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific (Zapata et al. Protein Eng. 8:1057-62 (1995)); "minibody" consisting of scFv fused to CH3 via a peptide linker (hingeless) or via an IgG hinge has been described in Olafsen, et al., Protein Eng Des Sel. 2004 Apr;17(4):315-23; "maxibody" refers to bivalent scFvs covalently attached to the Fc region of an immunoglobulin, see, for example, Fredericks et al, Protein Engineering, Design & Selection, 17:95-106 (2004) and Powers et al., Journal of Immunological Methods, 251:123-135 (2001); heavy-chain antibodies, e.g. the VH_H domain, or H_2L_2 (referred to as "heavy-chain antibodies" or "HCAbs"); or camelized V_{HH} (See, e.g., Reichman, etal., J Immunol Methods 1999, 231:25-38, Desmyter et al., *J. Biol. Chem.* 276:26285-90, 2001, Ewert et al., *Biochemistry* 41:3628-36, 2002; nanobody (Cortez-Retamozo et al., *Cancer Research* 64:2853-57, 2004);intrabodies are single chain antibodies which demonstrate intracellular expression and can manipulate intracellular protein function (Biocca, et al., *EMBO J.* 9:101–108, 1990; Colby et al., *Proc Natl Acad Sci U S A.* 101:17616-21, 2004, Mhashilkar et al, *EMBO J* 14:1542-51, 1995, Wheeler et al. (*FASEB J.* 17:1733-5. 2003); transbodies are cell-

permeable antibodies in which a protein transduction domains (PTD) is fused with single chain variable fragment (scFv) antibodies Heng et al., (*Med Hypotheses.* 64:1105-8, 2005); SMIPs or binding domain immunoglobulin fusion proteins specific for target protein are single-chain polypeptides comprising antigen binding domains fused to immunoglobulin domains necessary to carry out antibody effector functions. See e.g., WO03/041600, U.S. Patent publication 20030133939 and US Patent Publication 20030118592.

[0059] II. Calcium and acetate salts or buffers

[0060] It has been found that adding relatively low concentrations of calcium acetate to formulations of a selected antibody reduces the viscosity of the formulation. The term "viscosity" as used herein refers to "absolute viscosity." Absolute viscosity, sometimes called dynamic or simple viscosity, is the product of kinematic viscosity and fluid density: Absolute Viscosity=Kinematic Viscosity x Density. The dimension of kinematic viscosity is L^2/T where L is a length and T is a time. Commonly, kinematic viscosity is expressed in centistokes (cSt). The SI unit of kinematic viscosity is mm^2/s, which is 1 cSt. Absolute viscosity is expressed in units of centipoise (cP). The SI unit of absolute viscosity is the millipascal-second (mPa-s), where 1 cP=1 mPa-s.

[0061] Such viscosity measurements may be made hours (e.g., 1-23 hours), days (e.g., 1-10 days), weeks (e.g., 1-5 weeks), or months (e.g., 1-12 months), or years (e.g., 1-2 years, 1-3 years) after the addition of a viscosity reducing agent to an antibody formulation. Viscosity measurements may be made at a storage or administration temperature, e.g. 2-8°C or 25°C (room temperature). In some embodiments, absolute viscosity of the liquid or reconstituted liquid formulation at the storage and/or administration temperature is 15 cP or less, or 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, or 4 cP or less.

[0062] In some embodiments, the viscosity of the protein formulation is measured prior to and after the addition of the calcium salt, and/or acetate salt (and/or buffer). Methods of measuring viscosity are well known in the art and include, for example, using a capillary viscometer, or a cone-plate rheometer. Any methods may be used provided the same method is used to compare the test and reference formulations.

[0063] The viscosity of an antibody formulation can be reduced by the addition of a calcium salt, and/or an acetate salt (and/or buffer) to the formulation. Viscosity of an antibody formulation can be reduced by about 5%, about 10%, about 15%, about 20%, about 25%,

about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, and about 90% compared to the viscosity of a comparable antibody formulation lacking the calcium salt, and/or acetate salt (and/or buffer).

[0064] Exemplary calcium salts include, but are not limited to, calcium acetate, calcium carbonate and calcium chloride. In some embodiments, the calcium salt is at a concentration of at least 0.5 mM, 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM or 10 mM. In certain embodiments, the concentration of calcium salt is not greater than 11 mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23 mM, 24 mM, or 25 mM. Any range featuring a combination of the foregoing endpoints is contemplated, including but not limited to from about 0.5 mM to about 10 mM, about 5 mM to about 10 mM, or about 5 mM to about 15 mM. In some embodiments, the calcium salt is present at a concentration that reduces viscosity of an antibody formulation by at least 30%, 40%, 50%, 60% or more compared to the same formulation of antibody lacking the acetate salt and/or buffer, or that achieves a viscosity of 10 cP or less, or 9, 8, 7, 6, or 5 cP or less. In certain embodiments, the calcium salt is added at low concentrations so as not to negatively impact the protein formulation. For example, at calcium chloride or magnesium chloride concentrations of 20 mM or greater, proteins may form a gel at low storage temperatures (e.g., 2-8°C). Accordingly, a concentration of a calcium salt is generally selected for which the viscosity is reduced at the intended storage temperature of the reduced viscosity formulation.

[0065] In all of the ranges described herein, the concentration of cation, anion or salt described is the final concentration in the liquid or reconstituted liquid formulation that is to be administered. In any of the ranges described herein, the endpoints of the range are included in the range. However, the description also contemplates the same ranges in which the lower and/or the higher endpoint is excluded.

[0066] In some embodiments, a formulation described herein further comprises, in addition to the calcium salt, an acetate buffer at a concentration of at least 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, 10 mM, or 15 mM. In some embodiments, the concentration is no greater than 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM or 50 mM. Any range featuring a combination of the foregoing endpoints is contemplated, including but not limited to from about 5 mM to about 15 mM, or from about 5 mM to about 10 mM. The buffer is

preferably added to a concentration that maintains pH around 5-6 or 5-5.5 or 4.5-5.5. When the calcium salt in the formulation is calcium acetate, in some embodiments, the total concentration of acetate is about 10 mM to about 50 mM, or about 20 mM to about 40 mM.

[0067] In some aspects, the formulation comprises a total concentration of acetate that is at least about 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, or 50 mM. In some embodiments, the concentration of acetate is no greater than about 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, 85 mM, or 90 mM. Any range featuring a combination of the foregoing endpoints is contemplated, including but not limited to: about 10 mM to about 50 mM, about 20 mM to about 50 mM, about 20 mM to about 40 mM, about 30 mM to about 50 mM, or about 30 mM to about 75 mM. In some embodiments, the acetate salt or buffer comprises calcium acetate and/or sodium acetate. Alternatively, in some embodiments, the acetate salt and/or buffer is present at a concentration that reduces viscosity of an antibody formulation by at least 30%, 40%, 50%, 60% or more compared to the same formulation of antibody lacking the acetate salt and/or buffer, or that achieves a viscosity of 10 cP or less, or 9, 8, 7, 6, or 5 cP or less. By way of nonlimiting example, a solution containing 10 mM calcium acetate will have 20 mM acetate anion and 10 mM of calcium cation, because of the divalent nature of the calcium cation, while a solution containing 10 mM sodium acetate will have 10 mM sodium cation and 10 mM acetate anion.

[0068] In some embodiments, the total concentration of ions (cations and anions) in solution is at least 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, or 85 mM. In some embodiments, the total concentration of ions is no greater than about 30 mM, 35 mM, 40 mM, 45 mM, 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, 85 mM, 90 mM, 95 mM, 100 mM, 110 mM, 120 mM, 130 mM, 140 mM, 150 mM, 160 mM, 170 mM, 180 mM, 190 mM or 200 mM. Any range featuring a combination of the foregoing endpoints is contemplated, including but not limited to: about 30 mM to about 60 mM, or about 30 mM to about 70 mM, or about 30 mM to about 80 mM, or about 40 mM to about 150 mM, or about 50 mM to about 150 mM. By way of nonlimiting example, a solution of 10 mM calcium acetate will have a 30 mM total concentration of ions (10 mM cations and 20 mM anions).

[0069] In any of the formulations described herein, in some embodiments, the total osmolarity is no greater than 500 mOsm/L, 450 mOsm/L, 400 mOsm/L, or 350 mOsm/L, and is preferably close to isotonic, e.g. 250-350 mOsm/L.

[0070] Other excipients known in the art or described herein can be further included in the formulation.

[0071] III. Excipients in the formulation

[0072] Protein formulations are generally administered parenterally. When given parenterally, they must be sterile. Sterile diluents include liquids that are pharmaceutically acceptable (safe and non-toxic for administration to a human) and useful for the preparation of a liquid formulation, such as a formulation reconstituted after lyophilization. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution. Diluents can include aqueous solutions of salts and/or buffers.

[0073] Excipients are additives that are included in a formulation because they either impart or enhance the stability, delivery and manufacturability of a drug product. Regardless of the reason for their inclusion, excipients are an integral component of a drug product and therefore need to be safe and well tolerated by patients. For protein drugs, the choice of excipients is particularly important because they can affect both efficacy and immunogenicity of the drug. Hence, protein formulations need to be developed with appropriate selection of excipients that afford suitable stability, safety, and marketability.

[0074] The excipients described herein are organized either by their chemical type or their functional role in formulations. Brief descriptions of the modes of stabilization are provided when discussing each excipient type. Given the teachings and guidance provided herein, those skilled in the art will readily be able to vary the amount or range of excipient without increasing viscosity to an undesirable level. Excipients may be chosen to achieve a desired osmolality (i.e., isotonic, hypotonic or hypertonic) of the final solution, pH, desired stability, resistance to aggregation or degradation or precipitation, protection under conditions of freezing, lyophilization or high temperatures, or other properties. A variety of types of excipients are known in the art. Exemplary excipients include salts, amino acids, other tonicity agents, surfactants, stabilizers, bulking agents, cryoprotectants, lyoprotectants, anti-oxidants, metal ions, chelating agents and/or preservatives.

[0075] Further, where a particular excipient is reported in a formulation by, e.g., percent (%) w/v, those skilled in the art will recognize that the equivalent molar concentration of that excipient is also contemplated.

[0076] A. Buffers

[0077] The pH range of optimal stability needs to be identified early during pre-formulation studies. Several approaches such as accelerated stability studies and calorimetric screening studies have been demonstrated to be useful in this endeavor (Remmele R.L. Jr., et al., Biochemistry, 38(16): 5241-7 (1999)). Once a formulation is finalized, the drug product must be manufactured and maintained within a predefined specification throughout its shelf-life. Hence, buffering agents are almost always employed to control pH in the formulation.

[0078] Organic acids, phosphates and Tris have been employed routinely as buffers in protein formulations (Table 1). The buffer capacity of the buffering species is maximal at a pH equal to the pKa and decreases as pH increases or decreases away from this value. Ninety percent of the buffering capacity exists within one pH unit of its pKa. Buffer capacity also increases proportionally with increasing buffer concentration.

[0079] Several factors need to be considered when choosing a buffer. First and foremost, the buffer species and its concentration need to be defined based on its pKa and the desired formulation pH. Equally important is to ensure that the buffer is compatible with the protein drug, other formulation excipients, and does not catalyze any degradation reactions. Recently, polyanionic carboxylate buffers such as citrate and succinate have been shown to form covalent adducts with the side chain residues of proteins. A third important aspect to be considered is the sensation of stinging and irritation the buffer may induce. For example, citrate is known to cause stinging upon injection (Laursen T, et al., Basic Clin Pharmacol Toxicol., 98(2): 218-21 (2006)). The potential for stinging and irritation is greater for drugs that are administered via the SC or IM routes, where the drug solution remains at the site for a relatively longer period of time than when administered by the IV route where the formulation gets diluted rapidly into the blood upon administration. For formulations that are administered by direct IV infusion, the total amount of buffer (and any other formulation component) needs to be monitored. For example, it has been reported that potassium ions administered in the form of the potassium phosphate buffer, can induce cardiovascular effects in a patient (Hollander-Rodriguez JC, et al., Am. Fam. Physician., 73(2): 283-90 (2006)).

Table 1: Commonly used buffering agents and their pK_a values

Buffer	pK_a	Example drug product
Acetate	4.8	Neupogen, Neulasta
Succinate	pK_{a1} = 4.8, pK_{a2} = 5.5	Actimmune
Citrate	pK_{a1} = 3.1, pK_{a2} = 4.8, pK_{a3} = 6.4	Humira
Histidine (imidazole)	6.0	Xolair
Phosphate	pK_{a1} = 2.15, pK_{a2} = 7.2, pK_{a3} = 12.3	Enbrel (liquid formulation)
Tris	8.1	Leukine

[0080] The buffer system present in the formulation is selected to be physiologically compatible and to maintain a desired pH.

[0081] The pH buffering compound may be present in any amount suitable to maintain the pH of the formulation at a predetermined level. The pH buffering agent, e.g. acetate, may be present at a concentration between 0.1 mM and 1000 mM (1 M). In one embodiment, the pH buffering agent is at least 0.1, 0.5, 0.7, 0.8 0.9, 1.0, 1.2, 1.5, 1.7, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 500, 700, or 900 mM. In another embodiment, the concentration of the pH buffering agent is between 1, 1.2, 1.5, 1.7, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, 40, 50, 60, 70, 80, or 90 mM and 100 mM. In still another embodiment, the concentration of the pH buffering agent is between 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, or 40 mM and 50 mM. In yet another embodiment, the concentration of the pH buffering agent is 10 mM.

[0082] Other exemplary pH buffering agents used to buffer the formulation as set out herein include, but are not limited to glycine, glutamate, succinate, phosphate, acetate, and aspartate. Amino acids such as histidine and glutamic acid can also be used as buffering agents.

[0083] B. Stabilizers and bulking agents

[0084] Stabilizers include a class of compounds that can serve as cryoprotectants, lyoprotectants, and glass forming agents. Cryoprotectants act to stabilize proteins during freezing or in the frozen state at low temperatures. Lyoprotectants stabilize proteins in the freeze-dried solid dosage form by preserving the native-like conformational properties of the protein during dehydration stages of freeze-drying. Glassy state properties have been

classified as "strong" or "fragile" depending on their relaxation properties as a function of temperature. It is important that cryoprotectants, lyoprotectants, and glass forming agents remain in the same phase with the protein in order to impart stability. Sugars, polymers, and polyols fall into this category and can sometimes serve all three roles.

[0085] Polyols encompass a class of excipients that includes sugars, (e.g. mannitol, sucrose, sorbitol), and other polyhydric alcohols (e.g., glycerol and propylene glycol). The polymer polyethylene glycol (PEG) is included in this category. Polyols are commonly used as stabilizing excipients and/or isotonicity agents in both liquid and lyophilized parenteral protein formulations. Polyols can protect proteins from both physical and chemical degradation pathways.

[0086] Exemplary C3-C6 polyols include propylene glycol, glycerin (glycerol), threose, threitol, erythrose, erythritol, ribose, arabinose, arabitol, lyxose, maltitol, sorbitol, sorbose, glucose, mannose, mannitol, levulose, dextrose, maltose, trehalose, fructose, xylitol, inositol, galactose, xylose, fructose, sucrose, 1,2,6-hexanetriol and the like. Higher order sugars include dextran, propylene glycol, or polyethylene glycol. Reducing sugars such as fructose, maltose or galactose oxidize more readily than do non-reducing sugars. Additional examples of sugar alcohols are glucitol, maltitol, lactitol or iso-maltulose. Additional exemplary lyoprotectants include glycerin and gelatin, and the sugars mellibiose, melezitose, raffinose, mannotriose and stachyose. Examples of reducing sugars include glucose, maltose, lactose, maltulose, iso-maltulose and lactulose. Examples of non-reducing sugars include non-reducing glycosides of polyhydroxy compounds selected from sugar alcohols and other straight chain polyalcohols. Monoglycosides include compounds obtained by reduction of disaccharides such as lactose, maltose, lactulose and maltulose.

[0087] In some embodiments, the formulations described herein also comprise a stabilizer (or a combination of stabilizers) is added to the formulation. The term "stabilizer" means an excipient capable of preventing aggregation or other physical degradation, as well as chemical degradation (for example, autolysis, deamidation, oxidation, etc.) in an aqueous and solid state. Stabilizers that are conventionally employed in pharmaceutical compositions include, but are not limited to, sucrose, trehalose, mannose, maltose, lactose, glucose, raffinose, cellobiose, gentiobiose, isomaltose, arabinose, glucosamine, fructose, mannitol, sorbitol, glycine, arginine HCL, poly-hydroxy compounds, including polysaccharides such as dextran, starch, hydroxyethyl starch, cyclodextrins, N-methyl pyrollidene, cellulose and

hyaluronic acid, sodium chloride, [Carpenter et al., Develop. Biol. Standard 74:225, (1991)]. In one embodiment, the stabilizer is incorporated in a concentration of about 0% to about 40% w/v. In another embodiment, the stabilizer is incorporated in a concentration of at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, or 40% w/v. In another embodiment, the stabilizer is incorporated in a concentration of about 1, 2, 3, 4, 5, 6, 7, 8, 9% to about 10% w/v. In still another embodiment, the stabilizer is incorporated in a concentration of about 2% to about 6% w/v. In yet another embodiment, the stabilizer is incorporated in a concentration of about 4% w/v. In yet another embodiment, the stabilizer is incorporated in a concentration of about 6% w/v.

[0088] If desired, the formulations also include appropriate amounts of bulking and osmolarity regulating agents suitable for forming a lyophilized "cake". Bulking agents may be either crystalline (for example, mannitol, glycine) or amorphous (for example, sucrose, polymers such as dextran, polyvinylpyrolidone, carboxymethylcellulose). Other exemplary bulking agents include lactose, sorbitol, trehalose, or xylitol. In a further embodiment, the bulking agent is incorporated in a concentration of about 0% to about 10% w/v. In another embodiment, the bulking agent is incorporated in a concentration of at least 0.2, 0.5, 0.7, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, or 9.5% w/v. In a yet further embodiment the bulking agent is in a concentration of about 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5% to 5.0% w/v, to produce a mechanically and pharmaceutically stable cake.

[0089] C. Surfactants

[0090] Protein molecules have a high propensity to interact with surfaces making them susceptible to adsorption and denaturation at air-liquid, vial-liquid, and liquid-liquid (silicone oil) interfaces. This degradation pathway has been observed to be inversely dependent on protein concentration and result in either the formation of soluble and insoluble protein aggregates or the loss of protein from solution via adsorption to surfaces. In addition to container surface adsorption, surface-induced degradation is exacerbated with physical agitation, as would be experienced during shipping and handling of the product.

[0091] Surfactants are commonly used in protein formulations to prevent surface-induced degradation. Surfactants are amphipathic molecules with the capability of out-competing proteins for interfacial positions. Hydrophobic portions of the surfactant molecules occupy interfacial positions (e.g., air/liquid), while hydrophilic portions of the molecules remain

oriented towards the bulk solvent. At sufficient concentrations (typically around the detergent's critical micellar concentration), a surface layer of surfactant molecules serve to prevent protein molecules from adsorbing at the interface. Thereby, surface-induced degradation is minimized. The most commonly used surfactants are fatty acid esters of sorbitan polyethoxylates, i.e. polysorbate 20 and polysorbate 80 (e.g., Avonex®, Neupogen®, Neulasta®). The two differ only in the length of the aliphatic chain that imparts hydrophobic character to the molecules, C-12 and C-18, respectively. Accordingly, polysorbate-80 is more surface-active and has a lower critical micellar concentration than polysorbate-20. The surfactant poloxamer 188 has also been used in several marketed liquid products such Gonal-F ®, Norditropin ®, and Ovidrel ®.

[0092] Detergents can also affect the thermodynamic conformational stability of proteins. Here again, the effects of a given excipient will be protein specific. For example, polysorbates have been shown to reduce the stability of some proteins and increase the stability of others. Detergent destabilization of proteins can be rationalized in terms of the hydrophobic tails of the detergent molecules that can engage in specific binding with partially or wholly unfolded protein states. These types of interactions could cause a shift in the conformational equilibrium towards the more expanded protein states (i.e. increasing the exposure of hydrophobic portions of the protein molecule in complement to binding polysorbate). Alternatively, if the protein native state exhibits some hydrophobic surfaces, detergent binding to the native state may stabilize that conformation.

[0093] Another aspect of polysorbates is that they are inherently susceptible to oxidative degradation. Often, as raw materials, they contain sufficient quantities of peroxides to cause oxidation of protein residue side-chains, especially methionine. The potential for oxidative damage arising from the addition of stabilizer emphasizes the point that the lowest effective concentrations of excipients should be used in formulations. For surfactants, the effective concentration for a given protein will depend on the mechanism of stabilization. It has been postulated that if the mechanism of surfactant stabilization is related to preventing surface-denaturation the effective concentration will be around the detergent's critical micellar concentration. Conversely, if the mechanism of stabilization is associated with specific protein-detergent interactions, the effective surfactant concentration will be related to the protein concentration and the stoichiometry of the interaction (Randolph T.W., et al., *Pharm Biotechnol.*, 13:159-75 (2002)).

[0094] Surfactants may also be added in appropriate amounts to prevent surface related aggregation phenomenon during freezing and drying [Chang, B, J. Pharm. Sci. 85:1325, (1996)]. Exemplary surfactants include anionic, cationic, nonionic, zwitterionic, and amphoteric surfactants including surfactants derived from naturally-occurring amino acids. Anionic surfactants include, but are not limited to, sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate, chenodeoxycholic acid, N-lauroylsarcosine sodium salt, lithium dodecyl sulfate, 1-octanesulfonic acid sodium salt, sodium cholate hydrate, sodium deoxycholate, and glycodeoxycholic acid sodium salt. Cationic surfactants include, but are not limited to, benzalkonium chloride or benzethonium chloride, cetylpyridinium chloride monohydrate, and hexadecyltrimethylammonium bromide. Zwitterionic surfactants include, but are not limited to, CHAPS, CHAPSO, SB3-10, and SB3-12. Non-ionic surfactants include, but are not limited to, digitonin, Triton X-100, Triton X-114, TWEEN-20, and TWEEN-80. In another embodiment, surfactants include lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 40, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, soy lecithin and other phospholipids such as DOPC, DMPG, DMPC, and DOPG; sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose.

[0095] Formulations described herein may further comprise these surfactants, either individually or as a mixture in different ratios. In one embodiment, the surfactant is incorporated in a concentration of about 0% to about 5% w/v. In another embodiment, the surfactant is incorporated in a concentration of at least 0.001, 0.002, 0.005, 0.007, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, or 4.5% w/v. In another embodiment, the surfactant is incorporated in a concentration of about 0.001% to about 0.5% w/v. In still another embodiment, the surfactant is incorporated in a concentration of about 0.004, 0.005, 0.007, 0.01, 0.05, or 0.1% w/v to about 0.2% w/v. In yet another embodiment, the surfactant is incorporated in a concentration of about 0.01% to about 0.1% w/v.

[0096] In some embodiments, viscosity reduction is achieved with relatively little or no surfactant, e.g. 0.1% or less total surfactant, or 0.05% or less, or 0.01% or less.

[0097] D. Amino acids

[0098] Amino acids have found versatile use in protein formulations as buffers, bulking agents, stabilizers and antioxidants. Histidine and glutamic acid are employed to buffer protein formulations in the pH range of 5.5 – 6.5 and 4.0 – 5.5 respectively. The imidazole group of histidine has a pKa = 6.0 and the carboxyl group of glutamic acid side chain has a pKa of 4.3 which makes them suitable for buffering in their respective pH ranges. Glutamic acid is found in some formulations (e.g., Stemgen®). Histidine is commonly found in marketed protein formulations (e.g., Xolair®, Herceptin®, Recombinate®). It provides a good alternative to citrate, a buffer known to sting upon injection. Interestingly, histidine has also been reported to have a stabilizing effect when used at high concentrations in both liquid and lyophilized presentations (Chen B, et al., *Pharm Res.*, 20(12): 1952-60 (2003)). Histidine (up to 60 mM) was also observed to reduce the viscosity of a high concentration formulation of this antibody. However, in the same study, the authors observed increased aggregation and discoloration in histidine containing formulations during freeze-thaw studies of the antibody in stainless steel containers. The authors attributed this to an effect of iron ions leached from corrosion of steel containers. Another note of caution with histidine is that it undergoes photo-oxidation in the presence of metal ions (Tomita M, et al., *Biochemistry*, 8(12): 5149-60 (1969)). The use of methionine as an antioxidant in formulations appears promising; it has been observed to be effective against a number of oxidative stresses (Lam XM, et al., *J Pharm Sci.*, 86(11): 1250-5 (1997)).

[0099] The amino acids glycine, proline, serine and alanine stabilize proteins. Glycine is also a commonly used bulking agent in lyophilized formulations (e.g., Neumega ®, Genotropin®, Humatrope®). Arginine has been shown to be an effective agent in inhibiting aggregation and has been used in both liquid and lyophilized formulations (e.g., Activase®, Avonex®, Enbrel® liquid).

[00100] E. Antioxidants

[00101] Oxidation of protein residues arises from a number of different sources. Beyond the addition of specific antioxidants, the prevention of oxidative protein damage involves the careful control of a number of factors throughout the manufacturing process and storage of the product such as atmospheric oxygen, temperature, light exposure, and chemical contamination. The most commonly used pharmaceutical antioxidants are reducing agents, oxygen/free-radical scavengers, or chelating agents. Antioxidants in therapeutic protein formulations must be water-soluble and remain active throughout the product shelf-life.

Reducing agents and oxygen/free-radical scavengers work by ablating active oxygen species in solution. Chelating agents such as EDTA can be effective by binding trace metal contaminants that promote free-radical formation. For example, EDTA was utilized in the liquid formulation of acidic fibroblast growth factor to inhibit the metal ion catalyzed oxidation of cysteine residues. EDTA has been used in marketed products like Kineret® and Ontak®.

[00102] However, antioxidants themselves can induce other covalent or physical changes to the protein. A number of such cases have been reported in the literature. Reducing agents (like glutathione) can cause disruption of intramolecular disulfide linkages, which can lead to disulfide shuffling. In the presence of transition metal ions, ascorbic acid and EDTA have been shown to promote methionine oxidation in a number of proteins and peptides (Akers MJ, and Defelippis MR. Peptides and Proteins as Parenteral Solutions. In: Pharmaceutical Formulation Development of Peptides and Proteins. Sven Frokjaer, Lars Hovgaard, editors. Pharmaceutical Science. Taylor and Francis, UK (1999)); Fransson J.R., *J. Pharm. Sci.* 86(9): 4046-1050 (1997); Yin J, et al., *Pharm Res.*, 21(12): 2377-83 (2004)). Sodium thiosulfate has been reported to reduce the levels of light and temperature induced methionine-oxidation in rhuMab HER2; however, the formation of a thiosulfate-protein adduct was also reported in this study (Lam XM, Yang JY, et al., *J Pharm Sci.* 86(11): 1250-5 (1997)). Selection of an appropriate antioxidant is made according to the specific stresses and sensitivities of the protein.

[00103] F. Metal Ions

[00104] In general, transition metal ions are undesired in protein formulations because they can catalyze physical and chemical degradation reactions in proteins. However, specific metal ions are included in formulations when they are co-factors to proteins and in suspension formulations of proteins where they form coordination complexes (e.g., zinc suspension of insulin). Recently, the use of magnesium ions (10 –120 mM) has been proposed to inhibit the isomerization of aspartic acid to isoaspartic acid (WO 2004/039337).

[00105] Two examples where metal ions confer stability or increased activity in proteins are human deoxyribonuclease (rhDNase, Pulmozyme®), and Factor VIII. In the case of rhDNase, Ca^{+2} ions (up to 100 mM) increased the stability of the enzyme through a specific binding site (Chen B, et al., *J Pharm Sci.*, 88(4): 477-82 (1999)). In fact, removal of calcium

ions from the solution with EGTA caused an increase in deamidation and aggregation. However, this effect was observed only with Ca^{+2} ions; other divalent cations – Mg^{+2}, Mn^{+2} and Zn^{+2} were observed to destabilize rhDNase. Similar effects were observed in Factor VIII. Ca^{+2} and Sr^{+2} ions stabilized the protein while others like Mg^{+2}, Mn^{+2} and Zn^{+2}, Cu^{+2} and Fe^{+2} destabilized the enzyme (Fatouros, A., et al., *Int. J. Pharm.*, 155, 121–131 (1997). In a separate study with Factor VIII, a significant increase in aggregation rate was observed in the presence of Al^{+3} ions (Derrick TS, et al., *J. Pharm. Sci.*, 93(10): 2549-57 (2004)). The authors note that other excipients like buffer salts are often contaminated with Al^{+3} ions and illustrate the need to use excipients of appropriate quality in formulated products.

[00106] G. Preservatives

[00107] Preservatives are necessary when developing multi-use parenteral formulations that involve more than one extraction from the same container. Their primary function is to inhibit microbial growth and ensure product sterility throughout the shelf-life or term of use of the drug product. Commonly used preservatives include phenol, benzyl alcohol, meta-cresol, alkyl parabens such as methyl paraben or propyl paraben, benzalkonium chloride, and benzethonium chloride. Other examples of compounds with amtimicrobial preservative activity include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride. Other types of preservatives include aromatic alcohols such as butyl alcohol, phenol, benzyl alcohol; atechol, resorcinol, cyclohexanol, 3-pentanol. Although preservatives have a long history of use, the development of protein formulations that includes preservatives can be challenging. Preservatives almost always have a destabilizing effect (aggregation) on proteins, and this has become a major factor in limiting their use in multi-dose protein formulations (Roy S, et al., *J Pharm Sci.*, 94(2): 382-96 (2005)).

[00108] Multi-use injection pen presentations include preserved formulations. For example, preserved formulations of hGH are currently available on the market. Norditropin® (liquid, Novo Nordisk), Nutropin AQ® (liquid, Genentech) & Genotropin (lyophilized – dual chamber cartridge, Pharmacia & Upjohn) contain phenol while Somatrope® (Eli Lilly) is formulated with m-cresol.

[00109] Several aspects need to be considered during the formulation development of preserved dosage forms. The effective preservative concentration in the drug product must be optimized. This requires testing a given preservative in the dosage form with

concentration ranges that confer anti-microbial effectiveness without compromising protein stability. For example, three preservatives were successfully screened in the development of a liquid formulation for interleukin-1 receptor (Type I), using differential scanning calorimetry (DSC). The preservatives were rank ordered based on their impact on stability at concentrations commonly used in marketed products (Remmele RL Jr., et al., *Pharm Res.*, 15(2): 200-8 (1998)).

[00110] Some preservatives can cause injection site reactions, which is another factor that needs consideration when choosing a preservative. In clinical trials that focused on the evaluation of preservatives and buffers in Norditropin, pain perception was observed to be lower in formulations containing phenol and benzyl alcohol as compared to a formulation containing m-cresol (Kappelgaard A.M., *Horm Res.* 62 Suppl 3:98-103 (2004)). Interestingly, among the commonly used preservative, benzyl alcohol possesses anesthetic properties (Minogue SC, and Sun DA., *Anesth Analg.*, 100(3): 683-6 (2005)).

[00111] IV. Kits

[00112] As an additional aspect, the described herein are kits which comprise one or more formulations described herein packaged in a manner which facilitates their use for administration to subjects. In one embodiment, such a kit includes a formulation described herein (e.g., a composition comprising any of the antibodies described therein), packaged in a container such as a sealed bottle, vessel, single-use or multi-use vial, prefilled syringe, or prefilled injection device, optionally with a label affixed to the container or included in the package that describes use of the compound or composition in practicing the method. In one aspect, the compound or composition is packaged in a unit dosage form. The kit may further include a device suitable for administering the composition according to a specific route of administration. Preferably, the kit contains a label that describes use of an antibody described herein or formulation described herein.

[00113] V. Dosages

[00114] The dosage regimen involved in a method for treating a condition described herein will be determined by the attending physician, considering various factors which modify the action of drugs, e.g. the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. In various aspects, the daily regimen is in the range of 0.1-50 mg of a preparation of antibody per kilogram of body

weight (calculating the mass of the protein alone, without chemical modification). In some embodiments, the dosage is about 0.5 mg/kg to 20 mg/kg, or about 0.5-10 mg/kg.

[00115] The formulations are generally administered parenterally, e.g. intravenously, subcutaneously, intramuscularly, via aerosol (intrapulmonary or inhalational administration), or via depot for long-term release. In some embodiments, the formulation is administered intravenously by an initial bolus followed by a continuous infusion to maintain therapeutic circulating levels of drug product. In other embodiments, the formulation is administered as a one-time dose. Those of ordinary skill in the art will readily optimize effective dosages and administration regimens as determined by good medical practice and the clinical condition of the individual patient. The frequency of dosing will depend on the pharmacokinetic parameters of the agents and the route of administration. The optimal pharmaceutical formulation will be determined by one skilled in the art depending upon the route of administration and desired dosage. See for example, Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712, the disclosure of which is hereby incorporated by reference. Such formulations may influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of the administered agents. Depending on the route of administration, a suitable dose may be calculated according to body weight, body surface area or organ size. Further refinement of the calculations necessary to determine the appropriate dosage for treatment involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art without undue experimentation, especially in light of the dosage information and assays disclosed herein, as well as the pharmacokinetic data observed in the human clinical trials discussed above. Appropriate dosages may be ascertained through use of established assays for determining blood level dosages in conjunction with appropriate dose-response data. The final dosage regimen will be determined by the attending physician, considering various factors which modify the action of drugs, e.g. the drug's specific activity, the severity of the damage and the responsiveness of the patient, the age, condition, body weight, sex and diet of the patient, the severity of any infection, time of administration and other clinical factors. As studies are conducted, further information will emerge regarding the appropriate dosage levels and duration of treatment for various diseases and conditions.

[00116] VI. Therapeutic Uses of the Formulation

[00117] The formulations described herein are useful for treating or preventing bone-related disorders, such as bone-related disorders associated with abnormal osteoblast or osteoclast activity. In some embodiments, the formulation is administered to a subject suffering from a bone related disorder selected from the group consisting of achondroplasia, cleidocranial dysostosis, enchondromatosis, fibrous dysplasia, Gaucher's Disease, hypophosphatemic rickets, Marfan's syndrome, multiple hereditary exotoses, neurofibromatosis, osteogenesis imperfecta, osteopetrosis, osteopoikilosis, sclerotic lesions, pseudoarthrosis, pyogenic osteomyelitis, periodontal disease, anti-epileptic drug induced bone loss, primary and secondary hyperparathyroidism, familial hyperparathyroidism syndromes, weightlessness induced bone loss, osteoporosis in men, postmenopausal bone loss, osteoarthritis, renal osteodystrophy, infiltrative disorders of bone, oral bone loss, osteonecrosis of the jaw, juvenile Paget's disease, melorheostosis, metabolic bone diseases, mastocytosis, sickle cell anemia/disease, organ transplant related bone loss, kidney transplant related bone loss, systemic lupus erythematosus, ankylosing spondylitis, epilepsy, juvenile arthritides, thalassemia, mucopolysaccharidoses, Fabry Disease, Turner Syndrome, Down Syndrome, Klinefelter Syndrome, leprosy, Perthe's Disease, adolescent idiopathic scoliosis, infantile onset multi-system inflammatory disease, Winchester Syndrome, Menkes Disease, Wilson's Disease, ischemic bone disease (such as Legg-Calve-Perthes disease and regional migratory osteoporosis), anemic states, conditions caused by steroids, glucocorticoid-induced bone loss, heparin-induced bone loss, bone marrow disorders, scurvy, malnutrition, calcium deficiency, osteoporosis, osteopenia, alcoholism, chronic liver disease, postmenopausal state, chronic inflammatory conditions, rheumatoid arthritis, inflammatory bowel disease, ulcerative colitis, inflammatory colitis, Crohn's disease, oligomenorrhea, amenorrhea, pregnancy, diabetes mellitus, hyperthyroidism, thyroid disorders, parathyroid disorders, Cushing's disease, acromegaly, hypogonadism, immobilization or disuse, reflex sympathetic dystrophy syndrome, regional osteoporosis, osteomalacia, bone loss associated with joint replacement, HIV associated bone loss, bone loss associated with loss of growth hormone, bone loss associated with cystic fibrosis, chemotherapy-associated bone loss, tumor-induced bone loss, cancer-related bone loss, hormone ablative bone loss, multiple myeloma, drug-induced bone loss, anorexia nervosa, disease-associated facial bone loss, disease-associated cranial bone loss, disease-associated bone loss of the jaw, disease-associated bone loss of the skull, bone loss associated with aging, facial bone loss associated with aging, cranial bone loss associated with aging, jaw bone loss associated with aging, skull bone loss associated with aging, and bone loss associated with space travel.

[00118] In some embodiments, the formulations described herein are useful for improving outcomes in orthopedic procedures, dental procedures, implant surgery, joint replacement, bone grafting, bone cosmetic surgery and bone repair such as fracture healing, nonunion healing, delayed union healing and facial reconstruction. One or more compositions may be administered before, during and/or after the procedure, replacement, graft, surgery or repair.

[00119] The formulation need not cure the subject of the disorder or completely protect against the onset of a bone-related disorder to achieve a beneficial biological response. The formulation may be used prophylactically, meaning to protect, in whole or in part, against a bone-related disorder or symptom thereof. The formulation also may be used therapeutically to ameliorate, in whole or in part, a bone-related disorder or symptom thereof, or to protect, in whole or in part, against further progression of a bone-related disorder or symptom thereof. Indeed, the materials and methods of the invention are particularly useful for increasing bone mineral density and maintaining the increased bone mineral density over a period of time.

[00120] One or more administrations of a formulation described herein may be carried out over a therapeutic period of, for example, about 1 month to about 12 months (e.g., about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 7 months, about 8 months, about 9 months, about 10 months, or about 11 months). In some embodiments, a subject is administered one or more doses of the formulation to maintain bone mineral density. The term "maintain bone mineral density" as used herein means that the increased bone mineral density resulting the initial dose of the formulation does not fall more than about 1% to about 5% over the course of about 6 months, about 9 months about 1 year, about 18 months, about 2 years, or over the course of the patient's life). It will be appreciated that a patient can require alternate treatment phases for increasing bone density and maintaining bone density.

[00121] In addition, it may be advantageous to administer multiple doses of the formulation or space out the administration of doses, depending on the therapeutic regimen selected for a particular subject. The formulation can be administered periodically over a time period of one year or less (e.g., 9 months or less, 6 months or less, or 3 months or less). In this regard, the formulation can be administered to the human once every about 7 days, or 2 weeks, or 3 weeks, or 1 month, or 5 weeks, or 6 weeks, or 7 weeks, or 2 months, or 9 weeks, or 10 weeks, or 11 weeks, or 3 months, or 13 weeks, or 14 weeks, or 15 weeks, or 4 months, or 17 weeks,

or 18 weeks, or 19 weeks, or 5 months, or 21 weeks, or 22 weeks, or 23 weeks, or 6 months, or 12 months.

[00122] VII. Combination therapy

[00123] Treatment of a pathology by combining two or more agents that target the same pathogen or biochemical pathway sometimes results in greater efficacy and diminished side effects relative to the use of the therapeutically relevant dose of each agent alone. In some cases, the efficacy of the drug combination is additive (the efficacy of the combination is approximately equal to the sum of the effects of each drug alone), but in other cases the effect can be synergistic (the efficacy of the combination is greater than the sum of the effects of each drug given alone). As used herein, the term "combination therapy" means the two compounds can be delivered in a simultaneous manner, e.g. concurrently, or wherein one of the compounds is administered first, followed by the second agent, e.g., sequentially. The desired result can be either a subjective relief of one or more symptoms or an objectively identifiable improvement in the recipient of the dosage.

[00124] In some embodiments, the formulation is administered along with a standard of care therapeutic for the treatment of decreased bone mineral density. As used herein, the term "standard of care" refers to a treatment that is generally accepted by clinicians for a certain type of patient diagnosed with a type of illness. In some embodiments, the standard of care therapeutic is selected from the group consisting of an anti-resorptive drug, a bone-forming agent, an estrogen receptor antagonist (including, but not limited to, raloxifene, bazedoxifene and lasofoxifene)and a drug that has a stimulatory effect on osteoclasts. In some embodiments, the anti-resorptive drug includes, but is not limited to, a bisphosphonate (including, but not limited to, alendronate, risedronate, ibandronate and zoledronate), an estrogen or estrogen analogue, a selective estrogen receptor modulator (SERM) and a calcium source, Tibolone, calcitonin, a calcitriol and hormone replacement therapy. In some embodiments, the bone-forming agent includes, but is not limited to parathyroid hormone (PTH) or a peptide fragment thereof, PTH-related protein (PTHrp), bone morphogenetic protein, osteogenin, NaF, a PGE_2 agonist, a statin, and a RANK ligand (RANKL). In some embodiments, the drug having a stimulatory effect on osteoclasts includes, but it not limited to, vitamin D, or a vitamin D derivative or mimic thereof.

[00125] In some embodiments, the formulation is administered to a subject when treatment of a standard of care therapeutic described herein is contraindicated.

EXAMPLES

Example 1 – Calcium acetate reduced the effective viscosity of sclerostin antibody formulations

[00126] 10 ml of a selected anti-sclerostin antibody (75.7 mg/ml) was dialyzed against 2 liters of 10 mM Na(OAc) and 9% sucrose at 4°C for 2 hours. A selected anti-sclerostin antibody (75.7 mg/ml) was concentrated to approximately 160 mg/ml and diluted with water to approximately 140 mg/ml and 120 mg/ml. Absorbance of the diluted samples were determined to be 120, 142 and 157 mg/ml, respectively

[00127] 10 µl 1.0M $Ca(OAc)_2$ was added to 1 ml of the 120 mg/ml, 140 mg/ml and 160 mg/ml samples. Absolute viscosity, pH and osmolarity of the samples were determined (See Table 2). Absolute viscosity of the samples (500µl) was measured using Brookfield LV-DVII cone and plate viscometer with a CPE-40 spindle with matching sample cup temperature regulated by a circulating water bath at constant 25°C. .

[00128] Table 2

Sample	Viscosity (cP)	pH	Osmolarity
120 mg/ml (Control)	18	5.3	375
120 mg/ml + 10 mM $Ca(OAc)_2$	8.4	5.4	398
142 mg/ml + 10 mM $Ca(OAc)_2$	17	5.4	450
157 mg/ml + 10 mM $Ca(OAc)_2$	36	5.4	610

[00129] Results indicated that 10 mM $Ca(OAc)_2$ spiked into a liquid composition of the selected antibody reduced viscosity by about half. This experiment is performed for each of antibodies Ab-4, Ab-5, Ab-13, Ab-14, Ab-19, Ab-20 and Ab-23.

Example 2 – Formulations adjustment

[00130] 10 ml of a selected anti-sclerostin antibody (75.7 mg/ml) was dialyzed against 2 liters of 10 mM Na(OAc), 6% sucrose or 4% sucrose at 4°C for 2 hours. Each sucrose formulation was then concentrated using Amicons to approximately 140 mg/ml then diluted with water back down to the targeted concentrations (i.e., 120 mg/ml, 140 mg/ml and 160 mg/ml). Absorbance values of the diluted samples were determined to be 124 mg/ml (4% sucrose), 119.5 mg/ml (6% sucrose), 137.5 mg/ml (4% sucrose) and 142 mg/ml (6% sucrose), respectively.

[00131] 10 µl 1.0M $Ca(OAc)_2$ was added to 1 ml of the samples. Viscosity, osmolarity and pH of the samples were determined (See Table 3)

Table 3

Sample	mM	Mg/mL	pH	Osmolarity	Viscosity (cP)
120 mg/mL + 10 mM CaOAC + 4% sucrose	10	124	5.285	214	6.2
120 mg/mL + 10 mM CaOAC + 6% sucrose	10	119.5	5.25	282	5.7
140 mg/mL + 10 mM CaOAC + 4% sucrose	10	137.5	5.303	231	9.5
140 mg/mL + 10 mM CaOAC + 6% sucrose	10	142	5.307	294	11

[00132] The assay was repeated as follows: 10 ml of a selected anti-sclerostin antibody (75.7 mg/ml) was dialyzed against 2 liters of 10 mM Na(OAc), 6% sucrose or 4% sucrose at 4°C for 2 hours. Each sucrose formulation was then concentrated using Amicon filter to approximately 140 mg/ml then diluted with water back down to the targeted concentrations (i.e., 70 mg/ml, 100 mg/ml and 120 mg/ml). Absorbance values of the diluted samples were determined to be 71 mg/ml (4% sucrose), 68.2 mg/ml (6% sucrose), 99.4 mg/ml (4% sucrose), 100.5 (6% sucrose), 122 mg/ml (4% sucrose) and 113 mg/ml (6% sucrose), respectively.

[00133] pH, osmolarity and viscosity of the samples were determined. See Table 4.

Table 4

Sample	mM	Mg/mL	pH	Osmolarity	Viscosity (cP)
70 mg/mL + 4% sucrose	10	71	5.205	154	3.5
70 mg/mL + 10 mM CaOAC + 4% sucrose	10	71	5.233	183	2.2
70 mg/mL 6% sucrose	10	68.2	5.201	231	3.4
70 mg/mL + 10 mM CaOAC + 6% sucrose	10	68.2	5.279	256	2.4
100 mg/mL + 4% sucrose	10	99.4	5.265	165	8.1
100 mg/mL + 10 mM CaOAC + 4% sucrose	10	99.4	5.288	191	4.1
100 mg/mL + 6% sucrose	10	100.5	5.273	241	8.4
100 mg/mL + 10 mM CaOAC + 6% sucrose	10	100.5	5.303	270	4.3
120 mg/mL + 4% sucrose	10	122	5.295	177	15.6
120 mg/mL + 10 mM CaOAC + 4% sucrose	10	122	5.306	202	6.9

120 mg/mL + 6% sucrose	10	113	5.3	249	15.4
120 mg/mL + 10 mM CaOAC + 6% sucrose	10	113	5.311	274	6.6

[00134] Lowering pH of $Ca(OAc)_2$ buffer to 5.2 kept all final formulation pHs between 5.25 and 5.307. The 4% sucrose formulations were below the isotonic range (250-350 mOsm/kg), but the 6% sucrose formulations were near the middle of the isotonic range.

[00135] To further assess the effect of 6% sucrose with 10 mM $Ca(OAc)_2$ in reducing viscosity, the assay above was repeated with further concentrations of anti-sclerostin antibody up to 160 mg/ml.

[00136] Samples were prepared as described above with the following concentrations: 120 mg/ml, 140 mg/ml and 160 mg/ml. 10 µl of 1.0M $Ca(OAc)_2$, pH 5.2, was added to each of the samples. pH, osmolarity and viscosity of the samples were determined. See Table 5.

[00137] Table 5

Sample	mM	Mg/mL	pH	Osmolarity	Viscosity (cP)
100 mg/mL + 10 mM CaOAC + 6% sucrose	10	107	5.285	271	4.3
100 mg/mL + 10 mM CaOAC + 6% sucrose	10	107	5.285	277	4.3
120 mg/mL + 10 mM CaOAC + 6% sucrose	10	120	5.311	279	6.1
120 mg/mL + 10 mM CaOAC + 6% sucrose	10	120	5.311	278	6
140 mg/mL + 10 mM CaOAC + 6% sucrose	10	145	5.329	X	12
140 mg/mL + 10 mM CaOAC + 6% sucrose	10	145	5.329	309	11.7
160 mg/mL + 10 mM CaOAC + 6% sucrose	10	168.7	5.343	X	18.8
160 mg/mL + 10 mM CaOAC + 6% sucrose	10	168.7	5.343	X	18.8

[00138] The above-described experiments are performed for each of antibodies Ab-4, Ab-5, Ab-13, Ab-14, Ab-19, Ab-20 and Ab-23.

Example 3 – Effect of calcium acetate in other high protein concentration formulations.

[00139] The following Example determined whether calcium acetate reduces the viscosity of formulations containing high concentration of protein other than a sclerostin antibody.

[00140] Non-sclerostin antibodies #1-#5 were determined to have a concentration of 131.6 mg/ml, 94 mg/ml, 113.2 mg/ml, 50 mg/ml and 106.3, respectively. The term "non-sclerostin antibody" as used herein means an antibody other than a sclerostin antibody described herein.

[00141] 10 µl 1.0M $Ca(OAc)_2$ was added to 1 ml of the 5 samples discussed above. Viscosity, pH and osmolarity of the samples were determined (See Table 6).

[00142] Table 6

Sample	Mg/mL	Viscosity (cP)
Non-sclerostin antibody #1	94	6.8
Non-sclerostin antibody #1+ 10 mM $Ca(OAc)_2$	94	5.10
Non-sclerostin antibody #2	135	9.8
Non-sclerostin antibody #2 + 10 mM $Ca(OAc)_2$	135	8.3
Protein #1	50	3.3
Protein #1 + 10 mM $Ca(OAc)_2$	50	3.2
Protein #1	106.3	16.6
Protein #1 + 10 mM $Ca(OAc)_2$	106.3	15.6

[00143] Calcium acetate did not significantly reduce the viscosity of any of the samples.

Example 4 – Effect of non-calcium salts on the viscosity of high concentration anti-sclerostin antibody formulation.

[00144] The following experiment was performed to determine whether non-calcium salts would be capable of reducing the viscosity of an anti-sclerostin antibody formulation.

[00145] A selected anti-sclerostin antibody (the same as in Examples 1-2 above) was concentrated to ~130 mg/mL. 10 µl of either 1.0M $(NH_4)_2SO_4$ or 1.0M $MgSO_4$ was added to 1 ml of antibody sample. Viscosity of the control was determined to be 30 cP. $MgSO_4$ was determined to significantly reduce viscosity of the sample ($MgSO_4$ + sample = 16 cP). $(NH_4)_2SO_4$ did not significantly reduce viscosity of the sample.

Example 5 – Effect of other calcium salts on the viscosity of high concentration anti-sclerostin antibody formulation.

[00146] The following experiment was performed to determine whether calcium salts other than calcium acetate would be capable of reducing the viscosity of an anti-sclerostin antibody formulation.

[00147] A selected anti-sclerostin antibody (the same as in Examples 1-2 above) was concentrated to ~125 mg/mL. 10 µl of either 25 mM $CaCl_2$ or 25 mM $MgCl_2$ was added to 1 ml of antibody sample. Viscosity of the control was determined to be 18.5 cP. $CaCl_2$ and $MgCl_2$ were determined to significantly reduce viscosity of the sample ($CaCl_2$ + sample = 9 cP and $MgCl_2$ + sample = 8).

Example 6 – Effect of calcium acetate on another anti-sclerostin antibody.

[00148] The following experiment was performed to determine whether calcium acetate would be capable of reducing the viscosity of an anti-sclerostin antibody formulation comprising a different anti-sclerostin antibody than in Examples 1-2 above.

[00149] A selected anti-sclerostin antibody was concentrated to ~131 mg/mL. 10 µl 1.0M $Ca(OAc)_2$ was added to 1 ml of antibody sample. Viscosity of the control was determined to be 17.3 cP. $Ca(OAc)_2$ was determined to slightly reduce viscosity of the sample (15.3 cP)

[00150] Numerous modifications and variations in the practice of the invention are expected to occur to those of skill in the art upon consideration of the presently preferred embodiments thereof. Consequently, the only limitations which should be placed upon the scope of the invention are those which appear in the appended claims.

[00151] All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification and/or listed in the Application Data Sheet, are incorporated herein by reference, in their entirety.

[00152] From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

Statements of invention

1. A sterile liquid formulation that has an absolute viscosity of about 10 cP or less comprising: (a) an anti-sclerostin immunoglobulin at a concentration of at least 70 mg/mL; and (b) calcium acetate at a concentration ranging from about 1 mM to about 20 mM.

2. The formulation of claim 1, wherein the immunoglobulin comprises amino acid sequences of: SEQ ID NO: 14 and/or SEQ ID NO: 12; or SEQ ID NO: 86 and/or SEQ ID NO: 84; or SEQ ID NO: 68 and/or SEQ ID NO: 66; or SEQ ID NO: 154 and/or SEQ ID NO: 152; or SEQ ID NO: 182 and/or SEQ ID NO: 180; or SEQ ID NO: 208 and/or SEQ ID NO: 207; or SEQ ID NO: 216 and/or SEQ ID NO: 214; or SEQ ID NO: 238 and/or SEQ ID NO: 236.

3. The formulation of claim 1, further comprising (c) an acetate buffer, for example, sodium acetate, at a concentration of from about 5 mM to about 15 mM.

4. A sterile liquid formulation that has an absolute viscosity of about 10 cP or less comprising: (a) an anti-sclerostin immunoglobulin at a concentration of from about 70 mg/mL to about 200 mg/mL; and (b) an acetate salt and/or acetate buffer at a concentration ranging from about 10 mM to about 50 mM acetate, wherein the immunoglobulin comprises amino acid sequences of: SEQ ID NO: 14 and/or SEQ ID NO: 12; or SEQ ID NO: 86 and/or SEQ ID NO: 84; or SEQ ID NO: 68 and/or SEQ ID NO: 66; or SEQ ID NO: 154 and/or SEQ ID NO: 152; or SEQ ID NO: 182 and/or SEQ ID NO: 180; or SEQ ID NO: 208 and/or SEQ ID NO: 207; or SEQ ID NO: 216 and/or SEQ ID NO: 214; or SEQ ID NO: 238 and/or SEQ ID NO: 236.

5. The formulation of any one of claims 1-4, that has a total osmolarity of less than about 350 mOsm/L.

6. The formulation of any one of claims 1-5, wherein the immunoglobulin is present at a concentration of at least 120 mg/mL, or optionally 140 mg/mL.

7. The formulation of any one of claims 1-6, wherein the absolute viscosity of the formulation is about 8 cP or less, or optionally about 6 cP or less.

8. The formulation of any one of claims 1-7, wherein the formulation further comprises a polyol such as sucrose, for example, in an amount ranging from about 4% w/v to about 6% w/v.

9. The formulation of any one of claims 1-8, wherein the formulation has a pH ranging from about 4.5 to about 6, or optionally about 5 to about 5.5.

10. A method for reducing the viscosity of a protein formulation, the method comprising; adding calcium acetate at a concentration of between about 1 mM and about 20 mM, to an anti-sclerostin immunoglobulin formulation, wherein the formulation comprises an immunoglobulin at a concentration of from about 70 mg/mL to about 200 mg/mL, wherein the viscosity of the formulation with the calcium acetate is reduced compared to the viscosity of an antibody formulation without the calcium acetate.

11. The method of claim 10, wherein the immunoglobulin comprises the amino acid sequences of SEQ ID NO: 86 and/or SEQ ID NO: 84.

12. A sterile liquid formulation that has an absolute viscosity of about 10 cP or less comprising: (a) Ab-5 at a concentration of at least 70 mg/mL to about 200 mg/mL; (b) calcium acetate at a concentration ranging from about 1 mM to about 20 mM; and (c) a polyol such as sucrose, for example, in an amount ranging from about 4% w/v to about 6% w/v.

13. A sterile liquid formulation comprising:

(a) an anti-sclerostin immunoglobulin at a concentration of at least 70 mg/mL to about 200 mg/mL, wherein the immunoglobulin comprises amino acid sequences of: SEQ ID NO: 14 and/or SEQ ID NO: 12; or SEQ ID NO: 86 and/or SEQ ID NO: 84; or SEQ ID NO: 68 and/or SEQ ID NO: 66; or SEQ ID NO: 154 and/or SEQ ID NO: 152; or SEQ ID NO: 182 and/or SEQ ID NO: 180; or SEQ ID NO: 208 and/or SEQ ID NO: 207; or SEQ ID NO: 216 and/or SEQ ID NO: 214; or SEQ ID NO: 220 and/or SEQ ID NO: 218; or SEQ ID NO: 238 and/or SEQ ID NO: 236; and

(b) calcium acetate at a concentration ranging from about 1 mM to about 20 mM, wherein the calcium acetate reduces the absolute viscosity of the formulation by at least 10% compared to the viscosity of an antibody formulation without the calcium acetate.

14. A method of using a therapeutically effective amount of the formulation of any one of claim 1-9, 12 and 13 to treat a patient with suffering from orthopedic procedures, dental procedures, implant surgery, joint replacement, bone grafting, bone cosmetic surgery and bone repair such as fracture healing, nonunion healing, delayed union healing and facial reconstruction.

CLAIMS

1. A sterile liquid formulation that has an absolute viscosity of about 10 cP or less comprising:

(a) an anti-sclerotin immunoglobulin at a concentration of at least 70 mg/m; and

(b) calcium chloride at a concentration ranging from about 1 mM to about 20 mM.