

**METHODS OF BUFFER PREPARATION FOR A THERAPEUTIC PROTEIN
FORMULATION**

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0001] Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: XML file named "58404_SeqListing.xml," 13,970 bytes, created on July 16, 2024.

INCORPORATION BY REFERENCE

[0002] The following applications are hereby incorporated by reference in their entirety: International Patent Application No. PCT/US2012/049331, filed August 2, 2012, which claims priority to U.S. Provisional Patent Application No. 61/515,191, filed August 4, 2011; U.S. Patent Application No. 11/410,540, filed April 25, 2006, which claims priority to U.S. Provisional Patent Application No. 60/792,645, filed April 17, 2006, U.S. Provisional Patent Application No. 60/782,244, filed March 13, 2006, U.S. Provisional Patent Application No. 60/776,847, filed February 24, 2006, and U.S. Provisional Patent Application No. 60/677,583, filed May 3, 2005; and U.S. Patent Application No. 11/411,003 (issued as U.S. Patent No. 7,592,429), filed April 25, 2006, which claims priority to U.S. Provisional Patent Application No. 60/792,645, filed April 17, 2006, U.S. Provisional Patent Application No. 60/782,244, filed March 13, 2006, U.S. Provisional Patent Application No. 60/776,847, filed February 24, 2006, and U.S. Provisional Patent Application No. 60/677,583, filed May 3, 2005. The following applications also are hereby incorporated by reference: U.S. Patent Application No. 12/212,327, filed September 17, 2008, which claims priority to U.S. Provisional Patent Application No. 60/973,024, filed September 17, 2007; and U.S. Patent Application No. 12/811,171, filed June 29, 2010, which is a U.S. National Phase Application pursuant to 35 U.S.C. § 371 of International Patent Application No. PCT/US08/86864, filed on December 15, 2008, which claims priority to U.S. Provisional Patent Application No. 61/013,917, filed December 14, 2007.

BACKGROUND

[0003] Therapeutic protein formulations are known in the art. However, preparing a buffer for such therapeutic protein formulations in a streamlined and accelerated process that also optimizes and balances solubility of formulation components is needed.

SUMMARY OF THE INVENTION

[0004] In one aspect, described herein are materials and methods for preparing a buffer for a therapeutic protein formulation comprising (a) combining a calcium salt, an organic acid, and a disaccharide at a temperature of $\geq 22^{\circ}\text{C}$ (equal to or greater than 22°C) to

produce an admixture, and (b) adding sodium hydroxide to the admixture of (a) by bolus addition. In some embodiments, the calcium salt is calcium acetate. In some embodiments, the organic acid is acetic acid, such as glacial acetic acid. In some embodiments, the disaccharide is sucrose.

[0005] The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to”, and permit the presence of one or more additional features or components) unless otherwise noted. 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, unless context dictates otherwise. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein. Furthermore, “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term “and/or” as used in a phrase such as “A and/or B” herein is intended to include “A and B,” “A or B,” “A” (alone), and “B” (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0006] 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.

[0007] 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.

[0008] Unless defined otherwise herein, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary of Biochemistry and Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0009] All references cited herein are hereby incorporated by reference in their entireties.

DETAILED DESCRIPTION

[0010] The present disclosure is based on the discovery of an improved process for preparing a buffer for a therapeutic protein formulation that includes fixed mixing times at a constant mixing speed and at a constant temperature, and a sodium hydroxide bolus addition replacing an NaOH titration, to produce a buffer preparation for a therapeutic protein formulation. The process was designed to optimize and balance solubility of the different formulation components. Further benefits of the process provided herein are reduced addition steps of the components, the definition of fixed mixing speeds, the reduction of total mixing time, and the minimization of sample collection and analysis.

[0011] In one aspect, described herein is therefore a method of preparing a buffer for a therapeutic protein formulation comprising (a) combining a calcium salt, an organic acid, and a disaccharide at a temperature of $\geq 22^{\circ}\text{C}$ to produce an admixture, and (b) adding sodium hydroxide to the admixture of (a) by bolus addition. In one aspect, the calcium salt is calcium acetate. In one aspect, the organic acid is acetic acid, such as glacial acetic acid. In one aspect, the disaccharide is sucrose.

[0012] In some embodiments, step (a) comprises (i) mixing a calcium salt and an organic acid with water (e.g. water for injection or sterile water) for a period of ≤ 20 minutes (e.g., ranging from 16-20 minutes) to produce the admixture of (i), and (ii) adding the disaccharide to the admixture of (i) and further mixing for ≤ 20 minutes (e.g., ranging from 16-20 minutes) to produce the admixture of (ii). Step (a) occurs at a temperature of $\geq 22^{\circ}\text{C}$. In one aspect, steps (i) and (ii) of step (a) occur at the same temperature. In another aspect, steps (i) and (ii) of step (a) occur at different temperatures. In some embodiments, step (a) occurs at a

temperature between 22°C and 32°C. In some embodiments, step (a) occurs at 27°C. . In some embodiments, the calcium salt is calcium acetate. In some embodiments, the organic acid is acetic acid, such as glacial acetic acid. In some embodiments, the disaccharide is sucrose.

[0013] In step (b), sodium hydroxide (NaOH) is added to the admixture of (a) by bolus addition to produce the admixture of (b). In some embodiments, step (b) further comprises mixing for a period of ≤ 4 minutes (e.g. a period ranging from about 2 to about 4 minutes) following the bolus addition of sodium hydroxide. In some embodiments, step (b) comprises mixing the admixture for about 2 minutes, about 3 minutes or about 4 minutes following the bolus addition of sodium hydroxide.

[0014] In some embodiments, step (b) occurs at a temperature of $\geq 22^\circ\text{C}$. In some embodiments, step (b) occurs at a temperature between about 22°C and about 32°C. In some embodiments, step (b) occurs at a temperature of 22°C, 23°C, 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, 30°C, 31°C or 32°C. In some embodiments, step (b) occurs at a temperature of about 27°C.

[0015] In some embodiments, the mixing speed for step (a) (including steps (i) and (ii)) and step (b) ranges from about 135 rpm to about 170 rpm. In some embodiments, the mixing speed for step (a) and step (b) ranges from about 135 rpm to about 155 rpm or from about 140 rpm to about 150 rpm. In some embodiments, the mixing speed for step (a) and step (b) is about 145 rpm. In one aspect, step (a) and step (b) have the same mixing speed. In a further aspect, steps (i) and (ii) of step (a) have the same mixing speed. In yet another aspect, steps (i) and (ii) of step (a) and step (b) have the same mixing speed. In some embodiments, the mixing speed is about 135 rpm, or about 140 rpm, or about 145 rpm, or about 150 rpm, or about 155 rpm, or about 160 rpm, or about 165 rpm, or about 170 rpm. In some embodiments, the mixing speed is 135 rpm, 136 rpm, 137 rpm, 138 rpm, 139 rpm, 140 rpm, 141 rpm, 142 rpm, 143 rpm, 144 rpm, 145 rpm, 146 rpm, 147 rpm, 148 rpm, 149 rpm, 150 rpm, 151 rpm, 152 rpm, 153 rpm, 154 rpm, 155 rpm, 156 rpm, 157 rpm, 158 rpm, 159 rpm, 160 rpm, 161 rpm, 162 rpm, 163 rpm, 164 rpm, 165 rpm, 166 rpm, 167 rpm, 168 rpm, 169 rpm, or 170 rpm.

[0016] In some embodiments, the method comprises adding water to the admixture of step (b), preferably after mixing. This step may be required to obtain the desired concentrations of the different components in the (final) buffer. In some embodiments, the method comprises adding water to the admixture of step (b) and mixing. In some embodiments, the method comprises adding water to the admixture of step (b) and mixing before a cooling step. In some embodiments, the method comprises adding water to the

admixture of step (b) and mixing for ≤ 4 minutes (e.g. for about 2 to about 4 minutes). In some embodiments, the method comprises adding water to the admixture of step (b) and mixing for about 2 minutes, about 3 minutes or about 4 minutes. In one aspect, the water is water for injection or sterile water.

[0017] In one aspect, the mixing of the added water (referred to in the section above) occurs at a temperature of $\geq 22^{\circ}\text{C}$. In a further aspect, the mixing of the water occurs at a temperature between 22°C and 32°C . In some embodiments, the mixing of the water occurs at a temperature of 22°C , 23°C , 24°C , 25°C , 26°C , 27°C , 28°C , 29°C , 30°C , 31°C or 32°C . In some embodiments, the mixing of the water occurs at a temperature of about 27°C .

[0018] In one aspect, the mixing of the added water (referred to in the two sections above) occurs at a mixing speed ranging from about 135 rpm to about 155 rpm or from about 140 rpm to about 150 rpm. In some embodiments, the mixing speed is about 145 rpm. In some embodiments, the mixing speed is about 135 rpm, or about 140 rpm, or about 145 rpm, or about 150 rpm, or about 155 rpm. In some embodiments, the mixing speed is 135 rpm, 136 rpm, 137 rpm, 138 rpm, 139 rpm, 140 rpm, 141 rpm, 142 rpm, 143 rpm, 144 rpm, 145 rpm, 146 rpm, 147 rpm, 148 rpm, 149 rpm, 150 rpm, 151 rpm, 152 rpm, 153 rpm, 154 rpm, or 155 rpm.

[0019] In some embodiments, the method comprises a total mixing time (i.e. the mixing time for step (a), for step (b) and for mixing of the added water following step (b)) of less than about 60 minutes (e.g., about 10 minutes, about 15 minutes, about 20 minutes, about 25 minutes, about 30 minutes, about 35 minutes, about 40 minutes, about 45 minutes, about 50 minutes, about 55 minutes or about 60 minutes). In some embodiments, the method comprises a total mixing time ranging from about 36 minutes to about 48 minutes. In some embodiments, the method comprises a total mixing of about 36 minutes, or about 37 minutes, or about 38 minutes, or about 39 minutes, or about 40 minutes, or about 41 minutes, or about 42 minutes, or about 43 minutes, or about 44 minutes, or about 45 minutes, or about 46 minutes, or about 47 minutes, or about 48 minutes, or about 49 minutes, or about 50 minutes, or about 51 minutes, or about 52 minutes, or about 53 minutes, or about 54 minutes, or about 55 minutes, or about 56 minutes, or about 57 minutes, or about 58 minutes, or about 59 minutes or about 60 minutes. In some embodiments, the method comprises a total mixing time of about 36-48 minutes or about 38-46 minutes or about 40-44 minutes or about 42 minutes.

[0020] In some embodiments, the method comprises cooling the buffer prepared according to the method disclosed herein (i.e. either following step (b) or following the addition of water to the admixture of step (b)) to a temperature ranging from about 2°C to

about 8 °C. In some embodiments, the method comprises cooling the buffer to a temperature of 2 °C, 3 °C, 4 °C, 5 °C, 6 °C, 7 °C, or 8 °C. In some embodiments, the method comprises cooling the buffer to a temperature of about 5 °C.

[0021] In some embodiments, the final concentration of the disaccharide (e.g. of the sucrose) in the buffer is higher than the final concentration of the calcium salt (e.g. of the calcium acetate) in the buffer. In some embodiments, the disaccharide (e.g. the sucrose) has the highest final concentration of all components of the buffer (regardless of the concentration of the therapeutic protein which can later be added to the final buffer). In some embodiments, the disaccharide (e.g. the sucrose) is added to the admixture in an amount [e.g expressed in grams per 1 kg of buffer / final buffer] which is at least about 5 times, at least about 10 times, at least about 15 times, at least about 20 times, at least about 25 times or at least about 30 times higher than the amount of the calcium salt (e.g. of calcium acetate) added to the admixture.

[0022] The buffer preparation method disclosed herein comprises combining a calcium salt, an organic acid and a disaccharide, followed by the addition of NaOH. In some embodiments, the calcium salt (e.g. calcium acetate) is added in an amount of about 0.2 g to about 20 g, or about 0.4 g to about 15 g, or about 0.6 g to about 12 g, or about 0.8 g to about 10 g, or about 1 g to about 5 g, or about 1.5 g to about 4 g, or about 1.7 g to about 3 g, or about 2 g per 1 kg of final buffer. Any range featuring a combination of the foregoing endpoints is also contemplated.

[0023] In some embodiments, the disaccharide (e.g. sucrose) is added in an amount of about 6 g to about 180 g, about 10 g to about 150 g, about 10 g to about 120 g, about 20 g to about 100 g, about 30 g to about 90 g, about 40 g to about 80 g, about 50 g to about 70 g, about 55 g to about 65 g, about 59 g or about 60 g per 1 kg of final buffer. Any range featuring a combination of the foregoing endpoints is also contemplated.

[0024] In some embodiments, the organic acid (e.g. glacial acetic acid) is added in an amount of about 0.2 g to about 20 g, or about 0.4 g to about 15 g, or about 0.6 g to about 12 g, or about 0.5 g to about 5 g, or about 0.8 g to about 3 g, or about 1 g to about 2.5 g, or about 1.2 g to about 2.2 g, or about 1.4 g to about 2 g, or about 1.7 g per 1 kg of final buffer. Any range featuring a combination of the foregoing endpoints is also contemplated.

[0025] In some embodiments, the NaOH is added in an amount of about 0.2 mL to about 20 mL, or about 0.4 mL to about 15 mL, or about 0.6 mL to about 12 mL, or about 0.5 mL to about 5 mL, or about 0.8 mL to about 3 mL, or about 1 mL to about 2.5 mL, or about 1.2 mL to about 2.2 mL, or about 1.4 mL to about 2 mL, or about 1.65 mL per 1 kg of final buffer. Any range featuring a combination of the foregoing endpoints is also contemplated.

[0026] In some embodiments, the concentration of calcium in the buffer prepared according to the methods described herein ranges from 1 mM to 130 mM. In some embodiments, the concentration of calcium in the buffer ranges from 1 mM to 30 mM or ranges from 5 mM to 20 mM or ranges from 10 mM to 15 mM. In some embodiments, the calcium is present in the buffer at a concentration of at least 1 mM, at least 2 mM, at least 3 mM, at least 4 mM, at least 5 mM, at least 6 mM, at least 7 mM, at least 8 mM, at least 9 mM, at least 10 mM, at least 11 mM, or at least 12 mM. In some embodiments, the concentration of calcium in the buffer is about 13 mM. In certain embodiments, the concentration of calcium in the buffer is not greater than about 13 mM, not greater than about 14 mM, not greater than about 15 mM, not greater than about 16 mM, not greater than about 17 mM, not greater than about 18 mM, not greater than about 19 mM, not greater than about 20 mM, not greater than about 21 mM, not greater than about 22 mM, not greater than about 23 mM, not greater than about 24 mM, not greater than about 25 mM, not greater than about 26 mM, not greater than about 27 mM, not greater than about 28 mM, not greater than about 29 mM or not greater than about 30 mM. Any range featuring a combination of the foregoing endpoints is contemplated, including but not limited to, from about 5 mM to about 30 mM, or from about 10 mM to about 20 mM.

[0027] In some embodiments, the concentration of acetate in the buffer prepared according to the methods described herein ranges from about 5 mM to about 100 mM. In some embodiments, the concentration of acetate in the buffer ranges from about 10 mM to about 90 mM, or from about 20 mM to about 80 mM, or from about 30 mM to about 70 mM, or from about 35 mM to about 75 mM, or from about 40 mM to about 70 mM, or from about 45 mM to about 65 mM. In some embodiments, the concentration of acetate in the buffer ranges from about 50 mM to about 60 mM. In some embodiments, the acetate is present in the buffer at a concentration of at least about 5 mM, at least about 10 mM, at least about 15 mM, at least about 20 mM, at least about 25 mM, at least about 30 mM at least about 35 mM, at least about 40 mM, at least about 45 mM, or at least about 50 mM. In some embodiments, the concentration of acetate in the buffer is no greater than about 60 mM, no greater than about 65 mM, no greater than about 70 mM, no greater than about 75 mM, no greater than about 80 mM, no greater than about 85 mM, no greater than about 90 mM, no greater than about 100 mM, no greater than about 110 mM, no greater than about 120 mM, or no greater than about 130 mM. In some embodiments, the concentration of acetate in the buffer is about 55 mM. Any range featuring a combination of the foregoing endpoints is contemplated, including but not limited to from about 45 mM to about 65 mM.

[0028] In some aspects, the buffer prepared according to the methods described herein comprises a total concentration of acetate that is at least about 10 mM, at least about 15

mM, at least about 20 mM, at least about 25 mM, at least about 30 mM, at least about 35 mM, at least about 40 mM, at least about 45 mM, at least about 50 mM, at least 55 mM, at least 60 mM or at least 65 mM. In some embodiments, the concentration of acetate is no greater than about 30 mM, no greater than about 35 mM, no greater than about 40 mM, no greater than about 45 mM, no greater than about 50 mM, no greater than about 55 mM, no greater than about 60 mM, no greater than about 65 mM, no greater than about 70 mM, no greater than about 75 mM, no greater than about 80 mM, no greater than about 85 mM, or no greater than about 90 mM. Any range featuring a combination of the foregoing endpoints is contemplated, including but not limited to: about 10 mM to about 55 mM, about 20 mM to about 55 mM, about 20 mM to about 40 mM, about 30 mM to about 50 mM, or about 30 mM to about 75 mM. 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.

[0029] In some embodiments, the total concentration of ions (cations and anions) in the buffer prepared according to the methods described herein is at least about 10 mM, at least about 15 mM, at least about 20 mM, at least about 25 mM, at least about 30 mM, at least about 35 mM, at least about 40 mM, at least about 45 mM, at least about 50 mM, at least about 55 mM, at least about 60 mM, at least about 65 mM, at least about 70 mM, at least about 75 mM, at least about 80 mM, or at least about 85 mM. In some embodiments, the total concentration of ions in the buffer is no greater than about 30 mM, no greater than about 35 mM, no greater than about 40 mM, no greater than about 45 mM, no greater than about 50 mM, no greater than about 55 mM, no greater than about 60 mM, no greater than about 65 mM, no greater than about 70 mM, no greater than about 75 mM, no greater than about 80 mM, no greater than about 85 mM, no greater than about 90 mM, no greater than about 95 mM, no greater than about 100 mM, no greater than about 110 mM, no greater than about 120 mM, no greater than about 130 mM, no greater than about 140 mM, no greater than about 150 mM, no greater than about 160 mM, no greater than about 170 mM, no greater than about 180 mM, no greater than about 190 mM or no greater than about 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).

[0030] The buffer preparation method disclosed herein comprises combining a disaccharide with a calcium salt and an organic acid. Exemplary calcium salts include, but

are not limited to, calcium acetate, calcium carbonate and calcium chloride. In some embodiments, the calcium salt is calcium acetate.

[0031] The buffer preparation method disclosed herein comprises combining a disaccharide with a calcium salt and an organic acid. Exemplary disaccharides include, but are not limited to, sucrose, trehalose, lactose, and maltose. In some embodiments, the disaccharide is sucrose.

[0032] The buffer preparation method disclosed herein comprises combining a disaccharide with a calcium salt and an organic acid. Exemplary organic acids include, but are not limited to, acetic acid, citric acid, glutamic acid, histidine, lactic acid, succinic acid, and aspartic acid. In some embodiments, the organic acid is acetic acid, such as glacial acetic acid.

[0033] In some embodiments, the buffer prepared according to the methods described herein comprises a total concentration of a disaccharide (which may be sucrose according to one aspect of the invention) of about 0.1% to about 30% w/v, or about 0.5% to about 20% w/v, or about 1% to about 10% w/v. In some embodiments, the disaccharide is present in the buffer at a concentration of about 1% to about 12% w/v. In a yet further embodiment, the disaccharide is present in the buffer at a concentration of about 2% to about 10%. In a yet further embodiment, the disaccharide is present in the buffer at a concentration of about 3% to about 9%. In a yet further embodiment, the disaccharide is present in the buffer at a concentration of about 4% to about 8%. In a yet further embodiment, the disaccharide is present in the buffer at a concentration of about 5% to about 7%. In some embodiments, the disaccharide is present in the buffer at a concentration of about 6% w/v. In some embodiments, the disaccharide is present in the buffer at a concentration of at least 0.5, at least 1% w/v, at least 2% w/v, at least 3% w/v, at least 4% w/v, at least 5% w/v, or at least 6% w/v. In some embodiments, the disaccharide is present in the buffer at a concentration of no more than 30% w/v, no more than 25% w/v, no more than 20% w/v, no more than 15% w/v, no more than 14% w/v, no more than 13% w/v, no more than 12% w/v, no more than 11% w/v, no more than 10% w/v, no more than 9% w/v, no more than 8% w/v, no more than 7% w/v, or no more than 6% w/v. In some embodiments, the disaccharide is present in the buffer at a concentration of about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14% or about 15% w/v. Any range featuring a combination of the foregoing endpoints is contemplated. In some embodiments, the disaccharide is sucrose, and the above ranges and values equally apply to sucrose.

[0034] In some embodiments, the buffer prepared according to the methods described herein comprises 13 mM calcium, 55 mM acetate, and 6% (w/v) sucrose. In one aspect, this admixture has a pH of 5.2.

[0035] In some embodiments, the pH of the buffer prepared according to the methods described herein ranges from about 4 to about 6. In some embodiments, the pH of the buffer ranges from about 4.5 to about 6 or from about 4.8 to about 5.6. In some embodiments, the pH of the buffer ranges from about 5.0 to about 5.4 or from about 5.1 to about 5.3. In some embodiments, the pH of the buffer is 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9 or 6. In some embodiments, the pH of the buffer is 5.2.

[0036] Therapeutic protein

[0037] A “therapeutic protein” is a protein-based drug. The term can include medicines that are genetically engineered versions of naturally occurring proteins, e.g. human proteins. Therapeutic proteins can be used to replace a protein that is abnormal or deficient in a particular disease. They can also augment the supply of a beneficial protein to the body to help reduce the impact of disease or chemotherapy. Genetically engineered proteins can closely resemble the natural proteins they replace, or they can be enhanced by adding sugars or other molecules that extend the duration of the protein activity.

[0038] In some embodiments, the therapeutic protein is a signaling protein, a cytokine, an enzyme or enzyme replacement, a CD protein or its extracellular domain, a growth factor, a growth factor receptor or its extracellular domain, a cell adhesion molecule or its extracellular domain, a hormone or hormone analog, a coagulation factor, a coagulation-related protein, a colony stimulating factor, a receptor thereof, or biologically active fragments or analogs or variants or receptors or receptor fragments of any of the foregoing.

[0039] In some embodiments, a therapeutic protein is added to the prepared buffer to obtain a concentration of the therapeutic protein of at least about 10 mg/ml, at least about 15 mg/ml, at least about 20 mg/ml, at least about 25 mg/ml, at least about 30 mg/ml, at least about 35 mg/ml, at least about 40 mg/ml, at least about 45 mg/ml, at least about 50 mg/ml, at least about 55 mg/ml, at least about 60 mg/ml, at least about 65 mg/ml, at least about 70 mg/ml, at least about 75 mg/ml, at least about 80 mg/ml, at least about 81 mg/ml, at least about 82 mg/ml, at least about 83 mg/ml, at least about 84 mg/ml, at least about 85 mg/ml, at least about 86 mg/ml, at least about 87 mg/ml, at least about 88 mg/ml, at least about 89 mg/ml, or at least about 90 mg/ml. In some embodiments, a therapeutic protein is added to the prepared buffer to obtain a concentration of the therapeutic protein of no more than about 90 mg/ml, no more than about 91 mg/ml, no more than about 92 mg/ml, no more than

about 93 mg/ml, no more than about 94 mg/ml, no more than about 95 mg/ml, no more than about 96 mg/ml, no more than about 97 mg/ml, no more than about 98 mg/ml, no more than about 99 mg/ml, no more than about 100 mg/ml, no more than about 105 mg/ml, no more than about 110 mg/ml, no more than about 115 mg/ml, no more than about 120 mg/ml, no more than about 125 mg/ml, no more than about 130 mg/ml, no more than about 135 mg/ml, no more than about 140 mg/ml, no more than about 145 mg/ml, no more than about 150 mg/ml, no more than about 155 mg/ml, no more than about 160 mg/ml, no more than about 165 mg/ml, no more than about 170 mg/ml, no more than about 175 mg/ml, or no more than about 180 mg/ml. In some embodiments, the therapeutic protein is added to the prepared buffer to obtain a concentration of the therapeutic protein of 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, or about 190 mg/ml. Any range featuring a combination of the foregoing endpoints is contemplated, including but not limited to: about 50 mg/ml to about 150 mg/ml, about 60 mg/ml to about 120 mg/ml, about 65 mg/mL to about 115 mg/mL, about 70 mg/ml to about 110 mg/ml, about 75 mg/ml to about 105 mg/ml, about 80 mg/l to about 100 mg/ml, or about 85 mg/ml to about 95 mg/ml. In some embodiments, a therapeutic protein is added to the prepared buffer to obtain a concentration of the therapeutic protein of about 90 mg/ml.

[0040] In some embodiments, the therapeutic protein is an antigen binding protein. An “antigen binding protein” refers to a protein that specifically binds a specified antigen. Examples of antigen binding proteins include but are not limited to antibodies, peptibodies, antibody fragments, antibody constructs, multispecific (such as bispecific or trispecific) antibodies or antibody constructs, BiTE® molecules and fusion proteins. The term encompasses intact antibodies that comprise at least two full-length heavy chains and two full-length light chains (including intact bispecific antibodies), as well as derivatives, variants, fragments, and mutations thereof. An antigen binding protein also includes domain antibodies such as nanobodies and scFvs.

[0041] In some embodiments, the antigen binding protein is an antibody. As used herein, the term “antibody” refers to a protein having a conventional immunoglobulin format, comprising heavy and light chains, and comprising variable and constant regions. An antibody has a variable region and a constant region. In IgG formats, the variable region is generally about 100-110 or more amino acids, comprises three complementarity determining regions (CDRs), is primarily responsible for antigen recognition, and substantially varies among other antibodies that bind to different antigens. The constant region allows the antibody to recruit cells and molecules of the immune system. The variable region is made of the N-terminal regions of each light chain and heavy chain, while the constant region is

made of the C-terminal portions of each of the heavy and light chains. (Janeway et al., "Structure of the Antibody Molecule and the Immunoglobulin Genes", Immunobiology: The Immune System in Health and Disease, 4th ed. Elsevier Science Ltd./Garland Publishing, (1999)).

[0042] The general structure and properties of CDRs of antibodies have been described in the art. Briefly, in an antibody scaffold, the CDRs are embedded within a framework in the heavy and light chain variable region where they constitute the regions largely responsible for antigen binding and recognition. A variable region comprises at least three heavy or light chain CDRs (Kabat et al., 1991, Sequences of Proteins of Immunological Interest, Public Health Service N.I.H., Bethesda, Md.; see also Chothia and Lesk, 1987, J. Mol. Biol. 196:901-917; Chothia et al., 1989, Nature 342: 877-883), within a framework region (designated framework regions 1-4, FR1, FR2, FR3, and FR4, by Kabat et al., 1991; see also Chothia and Lesk, 1987, supra).

[0043] Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. IgM has subclasses, including, but not limited to, IgM1 and IgM2. Embodiments of the invention include all such classes or isotypes of antibodies. The light chain constant region can be, for example, a kappa- or lambda-type light chain constant region, e.g., a human kappa- or lambda-type light chain constant region. The heavy chain constant region can be, for example, an alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant regions, e.g., a human alpha-, delta-, epsilon-, gamma-, or mu-type heavy chain constant region. Accordingly, in exemplary embodiments, the antibody is an antibody of isotype IgA, IgD, IgE, IgG, or IgM, including any one of IgG1, IgG2, IgG3 or IgG4. IgG antibodies and in particular IgG2 antibodies represent one preferred embodiment of the disclosure.

[0044] The antibody may be a monoclonal antibody or a polyclonal antibody. In some embodiments, the antibody comprises a sequence that is substantially similar to a naturally-occurring antibody produced by a mammal, e.g., mouse, rabbit, goat, horse, chicken, hamster, human, and the like. In this regard, the antibody may be considered as a mammalian antibody, e.g., a mouse antibody, rabbit antibody, goat antibody, horse antibody, chicken antibody, hamster antibody, human antibody, and the like. In certain aspects, the monoclonal antibody is a human antibody. In certain aspects, the monoclonal antibody is a chimeric antibody or a humanized antibody. The term "chimeric antibody" is used herein to refer to an antibody containing constant domains from one species and the variable domains from a second, or more generally, containing stretches of amino acid sequence from at least

two species. The term "humanized" when used in relation to antibodies refers to antibodies having at least CDR regions from a non-human source which are engineered to have a structure and immunological function more similar to true human antibodies than the original source antibodies. For example, humanizing can involve grafting CDR from a non-human antibody, such as a mouse antibody, into a human antibody. Humanizing also can involve select amino acid substitutions to make a non-human sequence look more like a human sequence.

[0045] Advantageously, the methods disclosed herein for preparing a buffer for a therapeutic protein formulation (such as an antibody formulation) are not limited to the antigen-specificity of the antibody. Accordingly, the antibody (or antibody fragment or antibody protein product) can have any binding specificity for virtually any antigen. In exemplary aspects, the antibody binds to a hormone, growth factor, cytokine, a cell-surface receptor, or any ligand thereof.

[0046] In some embodiments, the antibody is an anti-sclerostin antibody. An "anti-sclerostin antibody" or an "antibody that binds to sclerostin" is an antibody that binds to sclerostin of SEQ ID NO: 1 or portions thereof. Recombinant human sclerostin/SOST is commercially available from, e.g., R&D Systems (Minneapolis, Minn., USA; 2006 Catalog #1406-ST-025). U.S. Patent Nos. 6,395,511 and 6,803,453, and U.S. Patent Publication Nos. 2004/0009535 and 2005/0106683 (hereby incorporated by reference) refer to anti-sclerostin antibodies generally. Examples of anti-sclerostin antibodies suitable for use in the context of the disclosure also are described in U.S. Patent Publication Nos. 2007/0110747 and 2007/0072797, which are hereby incorporated by reference. Suitable anti-sclerostin antibodies, including an antibody designated as Ab-5, are further described in US 8,715,663 and US 7,592,429 (hereby incorporated by reference). Additional information regarding materials and methods for generating sclerostin antibodies can be found in U.S. Patent Publication No. 20040158045 (hereby incorporated by reference). In some embodiments, the anti-sclerostin antibody is romosozumab, blosozumab, or setrusumab. In some embodiments, the anti-sclerostin antibody is romosozumab.

[0047] "Specifically binds" as used herein means that the antibody preferentially binds the antigen over other proteins. In some embodiments, "specifically binds" means the antibody has a higher affinity for the antigen than for other proteins.

[0048] In some or any embodiments, the antibody binds to sclerostin of SEQ ID NO: 1, or a naturally occurring variant thereof, with an affinity (Kd) of less than or equal to 1×10^{-7} M, less than or equal to 1×10^{-8} M, less than or equal to 1×10^{-9} M, less than or equal to 1×10^{-10} M, less than or equal to 1×10^{-11} M, or less than or equal to 1×10^{-12} M. Affinity is

determined using a variety of techniques, an example of which is an affinity ELISA assay. In various embodiments, affinity is determined by a BIAcore assay. In various embodiments, affinity is determined by a kinetic method. In various embodiments, affinity is determined by an equilibrium/solution method. U.S. Patent Publication No. 2007/0110747 (the disclosure of which is incorporated herein by reference) contains additional description of affinity assays suitable for determining the affinity (K_d) of an antibody for sclerostin.

[0049] In various aspects, the anti-sclerostin antibody comprises at least one CDR sequence having at least 75% identity (e.g., at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity) to a CDR selected from CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 wherein CDR-H1 has the sequence given in SEQ ID NO: 2, CDR-H2 has the sequence given in SEQ ID NO: 3, CDR-H3 has the sequence given in SEQ ID NO: 4, CDR-L1 has the sequence given in SEQ ID NO: 5, CDR-L2 has the sequence given in SEQ ID NO: 6 and CDR-L3 has the sequence given in SEQ ID NO: 7. The anti-sclerostin antibody, in various aspects, comprises three of the CDRs, four of the CDRs, five of the CDRs or six of the CDRs.

[0050] In a preferred embodiment, the anti-sclerostin antibody comprise a set of six CDRs as follows: CDR-H1 of SEQ ID NO: 2, CDR-H2 of SEQ ID NO: 3, CDR-H3 of SEQ ID NO: 4, CDR-L1 of SEQ ID NO: 5, CDR-L2 of SEQ ID NO: 6 and CDR-L3 of SEQ ID NO: 7.

[0051] In some or any embodiments, the anti-sclerostin antibody comprises a light chain variable region comprising an amino acid sequence having at least 75% identity (e.g., at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity) to the amino acid sequence set forth in SEQ ID NO: 8 and a heavy chain variable region comprising an amino acid sequence having at least 75% identity (e.g., at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity) to the amino acid sequence set forth in SEQ ID NO: 9. In various aspects, the difference in the sequence compared to SEQ ID NO: 8 or 9 lies outside the CDR region in the corresponding sequences. In some or any embodiments, the anti-sclerostin antibody comprises a light chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 8 and a heavy chain variable region comprising an amino acid sequence set forth in SEQ ID NO: 9.

[0052] In some or any embodiments, the anti-sclerostin antibody comprises all or part of a heavy chain (e.g., two heavy chains) comprising an amino acid sequence having at least 75% identity (e.g., at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity) to the amino acid sequence set forth in SEQ ID NO: 10 and all or part of a light chain (e.g., two light chains) comprising an amino

acid sequence having at least 75% identity (e.g., at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity) to the amino acid sequence set forth in SEQ ID NO: 11.

[0053] In some or any embodiments, the anti-sclerostin antibody comprises all or part of a heavy chain (e.g., two heavy chains) comprising an amino acid sequence having at least 75% identity (e.g., at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity) to the amino acid sequence set forth in SEQ ID NO: 12 and all or part of a light chain (e.g., two light chains) comprising an amino acid sequence having at least 75% identity (e.g., at least 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity) to the amino acid sequence set forth in SEQ ID NO 13.

[0054] Examples of other anti-sclerostin antibodies include, but are not limited to, the anti-sclerostin antibodies disclosed in International Patent Publication NOs. WO 2008/092894, WO 2008/115732, WO 2009/056634, WO 2009/047356, WO 2010/100200, WO 2010/100179, WO 2010/115932, and WO 2010/130830 (each of which is incorporated by reference herein in its entirety).

[0055] It will be understood by one skilled in the art that some proteins, such as antibodies, may undergo a variety of posttranslational modifications. The type and extent of these modifications often depends on the host cell line used to express the protein as well as the culture conditions. Such modifications may include variations in glycosylation, methionine oxidation, diketopiperazine formation, aspartate isomerization and asparagine deamidation. A frequent modification is the loss of a carboxy-terminal basic residue (such as lysine or arginine) due to the action of carboxypeptidases (as described in Harris, R.J. *Journal of Chromatography* 705:129-134, 1995).

[0056] Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, *Proteins: Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco, pp. 79-86 [1983], entirely incorporated by reference), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[0057] A therapeutic protein as described above can be added to the buffer prepared according to the method described herein, thereby producing a pharmaceutical composition. Such pharmaceutical composition may be placed within containers (e.g., vials or syringes), along with packaging material and/or material that provides instructions regarding the use of such pharmaceutical compositions. Generally, such instructions will include a tangible

expression describing the therapeutic protein concentration, as well as within certain embodiments, relative amounts of excipient ingredients or diluents (e.g., water, saline or PBS) that may be necessary to reconstitute the pharmaceutical composition.

EXAMPLE

[0058] This Example describes a representative buffer preparation method for a therapeutic protein formulation. Calcium acetate and glacial acetic acid were combined with water for injection (WFI) to produce a first admixture which was mixed at 145 ± 10 rpm for 16-20 minutes at $27^\circ\text{C} \pm 5^\circ\text{C}$. Then, sucrose was added to produce a second admixture which was then mixed at 145 ± 10 rpm for another 16-20 minutes at $27^\circ\text{C} \pm 5^\circ\text{C}$. Next, a bolus amount of sodium hydroxide (NaOH) was added resulting in a third admixture which was then mixed at 145 ± 10 rpm for 2-4 minutes at $27^\circ\text{C} \pm 5^\circ\text{C}$. Water for injection was finally added and mixed at 145 ± 10 rpm for 2-4 minutes at $27^\circ\text{C} \pm 5^\circ\text{C}$, resulting in the final buffer. The buffer was then cooled down to $5^\circ\text{C} \pm 3^\circ\text{C}$.

[0059] The buffer preparation method in the present example blends 2.012 g/kg of calcium acetate, 1.704 g/kg of glacial acetic acid, 58.708 g/kg of sucrose and 1.65 mL/kg of sodium hydroxide (all indicated amounts referring to 1 kg of buffer). The final buffer of the present Example comprises 13 mM calcium, 55 mM acetate and 6% (w/v) sucrose at pH 5.2. The temperature during addition and mixing of the ingredients was kept constant at $27^\circ\text{C} \pm 5^\circ\text{C}$. Likewise, the mixing speed was kept constant throughout the process at 145 ± 10 rpm.

[0060] The final buffer prepared in the present Example is suitable e.g. for an antibody, in particular for an antibody of the IgG isotype, such as an IgG2 antibody or specifically romosozumab.

[0061] The method described herein has several benefits as compared to the standard practice of preparing a buffer for a therapeutic protein formulation, which will be discussed in the following.

[0062] The state of the art is to produce the buffer at cold or room temperature. It is not standard practice to produce a buffer at higher temperatures and then cool it down to an acceptable temperature prior to adding the therapeutic protein. The buffer preparation temperature disclosed herein ensures the dissolution of the solid excipients during mixing process. Preparing the buffer at higher temperatures requires a special understanding of how the different components dissolve in liquid. Calcium acetate and sucrose are the main components impacted by buffer preparation at a higher temperature. Sucrose solubility increases with higher temperatures, whereas calcium acetate solubility decreases. However, the ingredient with the highest per quantity in the buffer is sucrose, and sucrose

constitutes the highest percentage of its solubility limit, making it the most important excipient in the buffer to ensure dissolution, while also aiming at reducing the mixing time. An optimal temperature was presently obtained for sucrose solubility while still warranting calcium acetate solubility. In other words, buffer preparation at the indicated temperature instead of commonly used lower temperatures favors solubility of sucrose (i.e. the excipient with the highest final concentration) in the aqueous buffer solution, without affecting calcium acetate solubility (which decreases with temperature increments), to successfully formulate the buffer solution at a reduced mixing time compared to previous buffer preparation processes. The average total mixing time could be reduced by 30% from 60 min down to 42 min.

[0063] Due to the increased temperature, it was also possible to define a fixed mixing speed for the addition and mixing of all components. It was not necessary to adjust (e.g. increase) the mixing speed after the addition of sucrose or to adjust (e.g. decrease) the mixing speed again after the addition of NaOH.

[0064] It is also standard practice to mix one ingredient at a time, which corresponds to a less efficient process as compared to the present disclosure. Buffer preparation process as described herein reduces excipient addition steps because calcium acetate and glacial acetic acid were combined in one single step.

[0065] In the (previous) standard process, the mixing time after addition of the individual buffer components varied and depended on the actual dissolution of the components. The newly disclosed process is balanced and optimized in a way that no visual confirmation (e.g. to control excipient dissolution completion) is required, which also allows to set up a fixed mixing time.

[0066] Furthermore, NaOH bolus amount addition speeds up buffer preparation process because NaOH does not need to be titrated in a multi-step process, hence reducing the required mixing time after NaOH addition (from about 20 min to about 2-4 min) while adjusting buffer pH to the desired value (here: 5.2) in a single step.

[0067] The method disclosed herein efficiently utilizes resources as it minimizes sampling collection and analysis.

What is claimed is:

1. A method of preparing a buffer for a therapeutic protein formulation comprising
 - (a) combining a calcium salt, an organic acid and a disaccharide at a temperature equal to or greater than 22°C to produce an admixture, and
 - (b) adding sodium hydroxide to the admixture of (a) by bolus addition.
2. The method of claim 1, wherein the method occurs at a temperature ranging from about 22°C to about 32°C, preferably at a temperature of about 27°C.
3. The method of claim 1 or 2, wherein step (a) comprises
 - (i) mixing the calcium salt and the organic acid with water for about 16-20 minutes to produce the admixture of (i), and
 - (ii) adding the disaccharide to the admixture of (i) and further mixing for about 16-20 minutes.
4. The method of any one of claims 1-3, wherein step (b) further comprises mixing for about 2-4 minutes following the bolus addition of sodium hydroxide.
5. The method of claim 3 or 4, wherein the mixing speed for step (a) and/or step (b) ranges between about 135 rpm and about 155 rpm, preferably wherein the mixing speed for step (a) and/or step (b) is about 145 rpm.
6. The method of any one of claims 1-5, wherein step (b) is followed by the addition of water.
7. The method of claim 6, further comprising mixing for about 2-4 minutes following the addition of the water.
8. The method of claim 7, wherein the total mixing time for step (a), step (b) and the mixing of the added water is less than about 60 minutes, preferably in the range of about 36 minutes to about 48 minutes.
9. The method of any one of claims 1-8, further comprising cooling the buffer to a temperature ranging from about 2°C to about 8°C, preferably to a temperature of about 5°C.
10. The method of any one of claims 1-9, wherein the disaccharide is added to the admixture in an amount which is at least about 5 times, at least about 10 times, at least about 15 times, at least about 20 times, at least about 25 times or at least about 30 times higher than the amount of the calcium salt.

11. The method of any one of claims 1-10, wherein the disaccharide is selected from the group consisting of sucrose, trehalose, lactose and maltose, preferably wherein the disaccharide is sucrose.
12. The method of any one of claims 1-11, wherein the calcium salt is selected from the group consisting of calcium acetate, calcium carbonate and calcium chloride, preferably wherein the calcium salt is calcium acetate.
13. The method of any one of claims 1-12, wherein the organic acid is selected from the group consisting of acetic acid, citric acid, glutamic acid, histidine, lactic acid, succinic acid, and aspartic acid, preferably wherein the organic acid is acetic acid.
14. The method of any one of claims 1-13, wherein the pH of the buffer ranges from about 5.0 to about 5.4.
15. The method of any one of claims 1-14, wherein the buffer comprises 13 mM calcium, 55 mM acetate, and 6% (w/v) sucrose at pH 5.2.