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[Continued on next page]

(54) Title: USE OF AMINO ACIDS AS STABILIZING COMPOUNDS IN PHARMACEUTICAL COMPOSITIONS CONTAINING HIGH CONCENTRATIONS OF PROTEIN-BASED THERAPEUTIC AGENTS

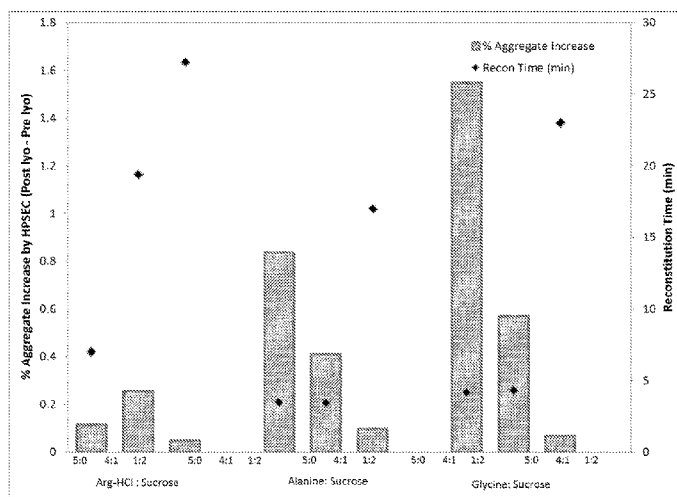


Figure 1

(57) Abstract: The present invention relates to improved pharmaceutical compositions that contain high concentrations of one or more protein biomolecule(s). In particular, the invention relates to such pharmaceutical compositions that include one or more amino acid molecules, particularly arginine, alanine, glycine, lysine or proline, or derivatives and salts thereof, or mixtures thereof, as stabilizing compounds. The inclusion of such stabilizing compounds decreases reconstitution time whilst improving and/or maintaining the long-term stability of the protein biomolecule, so as to facilitate the treatment, management, amelioration and/or prevention of a disease or condition by the pharmaceutical composition. The invention particularly pertains to such pharmaceutical compositions that lack, or substantially lack, a sugar stabilizing agent.



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Use of Amino Acids as Stabilizing Compounds in Pharmaceutical Compositions Containing High Concentrations of Protein-Based Therapeutic Agents

Field Of The Invention

[0001] The present invention relates to improved pharmaceutical compositions that contain high concentrations of one or more protein biomolecule(s). In particular, the invention relates to such pharmaceutical compositions that include one or more amino acid molecules, particularly arginine, alanine, glycine, lysine or proline, or derivatives and salts thereof, or mixtures thereof, as stabilizing compounds. The inclusion of such stabilizing compounds decreases reconstitution time whilst improving and/or maintaining the long-term stability of the protein biomolecule, so as to facilitate the treatment, management, amelioration and/or prevention of a disease or condition by the pharmaceutical composition. The invention particularly pertains to such pharmaceutical compositions that lack, or substantially lack, a sugar stabilizing agent.

Background Of The Invention

[0002] Protein-based therapeutic agents (*e.g.*, hormones, enzymes, cytokines, vaccines, immunotherapeutics, *etc.*) are becoming increasingly important to the management and treatment of human disease. As of 2014, more than 60 such therapeutics had been approved for marketing, with approximately 140 additional drugs in clinical trial and more than 500 therapeutic peptides in various stages of preclinical development (Fosgerau, K. *et al.* (2014) “*Peptide Therapeutics: Current Status And Future Directions*,” Drug Discov. Today 20(1):122-128; Kaspar, A.A. *et al.* (2013) “*Future Directions For Peptide Therapeutics Development*,” Drug Discov. Today 18:807–817).

[0003] One impediment to the use of such therapeutics is the physical instability that is often encountered upon their storage (US Patent No. 8,617,576; PCT Publications No. WO 2014/100143 and 2015/061584; Balcão, V.M. *et al.* (2014) “*Structural And Functional Stabilization Of Protein Entities: State-Of-The-Art*,” Adv. Drug Deliv. Rev. (Epub.): doi: 10.1016/j.addr.2014.10.005; pp. 1-17; Maddux, N.R. *et al.* (2011) “*Multidimensional Methods For The Formulation Of Biopharmaceuticals And Vaccines*,” J. Pharm. Sci. 100:4171-4197; Wang, W. (1999) “*Instability, Stabilization, And Formulation Of Liquid Protein Pharmaceuticals*,” Int. J. Pharm. 185:129-188; Kristensen, D. *et al.* (2011) “*Vaccine*

Stabilization: Research, Commercialization, And Potential Impact,” Vaccine 29:7122-7124; Kumru, O.S. *et al.* (2014) “*Vaccine Instability In The Cold Chain: Mechanisms, Analysis And Formulation Strategies*,” Biologicals 42:237-259). Such instability may comprise multiple aspects. A protein-based therapeutic agent may, for example experience operational instability, such as an impaired ability to survive processing operations (*e.g.*, sterilization, lyophilization, cryopreservation, *etc.*). Additionally or alternatively, proteins may experience thermodynamic instability such that a desired secondary or tertiary conformation is lost or altered upon storage. A further, and especially complex problem, lies in the stabilization of therapeutic agents that comprise multimeric protein subunits, with dissociation of the subunits resulting in the inactivation of the product. Kinetic instability is a measure of the capacity of a protein to resist irreversible changes of structure in *in vitro* non-native conditions. Protein aggregation and the formation of inclusion bodies is considered to be the most common manifestation of instability, and is potentially encountered in multiple phases of product development (Wang, W. (2005) “*Protein Aggregation And Its Inhibition In Biopharmaceutics*,” Int. J. Pharm. 289:1-30; Wang, W. (1999) “*Instability, Stabilization, And Formulation Of Liquid Protein Pharmaceuticals*,” Int. J. Pharm. 185:129-188; Arakawa, T. *et al.* (1993) “*Factors Affecting Short-Term And Long-Term Stabilities Of Proteins*,” Adv. Drug Deliv. Rev. 10:1-28; Arakawa, T. *et al.* (2001) “*Factors Affecting Short-Term And Long-Term Stabilities Of Proteins*,” Adv. Drug Deliv. Rev. 46:307-326). Such issues of instability can affect not only the efficacy of the therapeutic but its immunogenicity to the recipient patient. Protein instability is thus one of the major drawbacks that hinders the use of protein-based therapeutic agent (Balcão, V.M. *et al.* (2014) “*Structural And Functional Stabilization Of Protein Entities: State-Of-The-Art*,” Adv. Drug Deliv. Rev. (Epub.): doi: 10.1016/j.addr.2014.10.005; pp. 1-17).

[0004] Stabilization of protein-based therapeutic agents entails preserving the structure and functionality of such agents, and has been accomplished by establishing a thermodynamic equilibrium between such agents and their (micro)environment (Balcão, V.M. *et al.* (2014) “*Structural And Functional Stabilization Of Protein Entities: State-Of-The-Art*,” Adv. Drug Deliv. Rev. (Epub.): doi: 10.1016/j.addr.2014.10.005; pp. 1-17). One approach to stabilizing protein-based therapeutic agents involves altering the protein to contain additional covalent (*e.g.*, disulfide) bonds so as to increase the enthalpy associated with a desired conformation. Alternatively, the protein may be modified to contain additional polar groups so as to increase its hydrogen bonding with solvating water molecules (Mozhaev, V.V. *et al.* (1990) “*Structure-Stability Relationships In Proteins: A Guide To Approaches To Stabilizing Enzymes*,” Adv.

Drug Deliv. Rev. 4:387-419; Iyer, P.V. *et al.* (2008) “*Enzyme Stability And Stabilization — Aqueous And Non-Aqueous Environment*,” *Process Biochem.* 43:1019-1032).

[0005] A second approach to stabilizing protein-based therapeutic agents involves reducing the chemical activity of the water present in the protein’s microenvironment, for example by freezing the water, adding specific solutes, or lyophilizing the pharmaceutical composition (see, *e.g.*, Castronuovo, G. (1991) “*Proteins In Aqueous Solutions. Calorimetric Studies And Thermodynamic Characterization*,” *Thermochim. Acta* 193:363-390).

[0006] Employed solutes range from small molecular weight ions (*e.g.*, salts, buffering agents) to intermediate sized solutes (*e.g.*, amino acids, sugars) to larger molecular weight compounds (*e.g.*, polymers, proteins) (Kamerzell, T.J. *et al.* (2011) “*Protein–Excipient Interactions: Mechanisms And Biophysical Characterization Applied To Protein Formulation Development*,” *Adv. Drug Deliv. Rev.* 63:1118-1159).

[0007] For example, such solutes have included budesonide, dextran DMSO glycerol, glucose, inulin, lactose, maltose, mannitol, PEG, piroxicam, PLGA, PVA sorbitol, sucrose, trehalose and urea (Ohtake, S. *et al.* (2011) “*Trehalose: Current Use and Future Applications*,” *J. Pharm. Sci.* 100(6):2020-2053; Willart, J.F. *et al.* (2008) “*Solid State Amorphization of Pharmaceuticals*,” *Molec. Pharmaceut.* 5(6):905-920; Kumru, O.S. *et al.* (2014) “*Vaccine Instability In The Cold Chain: Mechanisms, Analysis And Formulation Strategies*,” *Biologicals* 42:237-259; Somero, G.N. (1995) “*Proteins And Temperature*,” *Annu. Rev. Physiol.* 57: 43-68; Sasahara, K. *et al.* (2003) “*Effect Of Dextran On Protein Stability And Conformation Attributed To Macromolecular Crowding*,” *J. Mol. Biol.* 326:1227-1237; Jain, N.K. *et al.* (2014) “*Formulation And Stabilization Of Recombinant Protein Based Virus-Like Particle Vaccines*,” *Adv. Drug Deliv. Rev.* (Epub.) doi: 10.1016/j.addr.2014.10.023; pp. 1-14; Kissmann, J. *et al.* (2011) “*H1N1 Influenza Virus-Like Particles: Physical Degradation Pathways And Identification Of Stabilizers*,” *J. Pharm. Sci.* 100:634-645; Kamerzell, T.J. *et al.* (2011) “*Protein–Excipient Interactions: Mechanisms And Biophysical Characterization Applied To Protein Formulation Development*,” *Adv. Drug Deliv. Rev.* 63:1118-1159).

[0008] Sugars such as sucrose and trehalose dihydrate are typically used as lyoprotectants and cryoprotectants in lyophilized therapeutic protein formulations to improve drug product stability, *e.g.*, for storage at 2-8 °C (US Patents No. 8,617,576 and 8,754,195). Trehalose, in particular, has been widely used as a stabilizing agent; it is used in a variety of research

applications and is contained in several commercially available therapeutic products, including HERCEPTIN®, AVASTIN®, LUCENTIS®, and ADVATE® (Ohtake, S. *et al.* (2011) “Trehalose: Current Use and Future Applications,” J. Pharm. Sci. 100(6):2020-2053).

[0009] The amino acids histidine, arginine, glutamate, glycine, proline, lysine and methionine have been mentioned as natural compounds that stabilize proteins. Human serum albumin (HSA) and gelatin have been mentioned as being protein stabilizers (US Patent No. 8,617,576; US Patent Publication No. 2015/0118249; Kamerzell, T.J. *et al.* (2011) “Protein–Excipient Interactions: Mechanisms And Biophysical Characterization Applied To Protein Formulation Development,” Adv. Drug Deliv. Rev. 63:1118-1159; Kumru, O.S. *et al.* (2014) “Vaccine Instability In The Cold Chain: Mechanisms, Analysis And Formulation Strategies,” Biologicals 42:237-259; Arakawa, T. *et al.* (2007) “Suppression Of Protein Interactions By Arginine: A Proposed Mechanism Of The Arginine Effects,” Biophys. Chem. 127:1-8; Arakawa, T. *et al.* (2007) “Biotechnology Applications Of Amino Acids In Protein Purification And Formulations,” Amino Acids 33:587-605; Chen, B. (2003) “Influence Of Histidine On The Stability And Physical Properties Of A Fully Human Antibody In Aqueous And Solid Forms,” Pharm. Res. 20:1952-1960; Tian, F. *et al.* (2007) “Spectroscopic Evaluation Of The Stabilization Of Humanized Monoclonal Antibodies In Amino Acid Formulations,” Int. J. Pharm. 335:20-31; Wade, A.M. *et al.* (1998) “Antioxidant Characteristics Of L-Histidine,” J. Nutr. Biochem. 9:308-315; Yates, Z. *et al.* (2010) “Histidine Residue Mediates Radical-Induced Hinge Cleavage Of Human IgG1,” J. Biol. Chem. 285:18662-18671; Lange, C. *et al.* (2009) “Suppression Of Protein Aggregation By L-Arginine,” Curr. Pharm. Biotechnol. 10:408-414; Nakakido, M. *et al.* (2009) “To Be Excluded Or To Bind, That Is The Question: Arginine Effects On Proteins,” Curr. Pharm. Biotechnol. 10:415-420; Shukla, D. *et al.* (2010) “Interaction Of Arginine With Proteins And The Mechanism By Which It Inhibits Aggregation,” J. Phys. Chem. B 114:13426-13438; Pyne, A. *et al.* (2001) “Phase Transitions Of Glycine In Frozen Aqueous Solutions And During Freeze-Drying,” Pharm. Res. 18:1448-1454; Lam, X.M. *et al.* (1997) “Antioxidants For Prevention Of Methionine Oxidation In Recombinant Monoclonal Antibody HER2,” J. Pharm. Sci. 86:1250-1255; Maeder, W. *et al.* (2011) “Local Tolerance And Stability Up To 24 Months Of A New 20% Proline-Stabilized Polyclonal Immunoglobulin For Subcutaneous Administration,” Biologicals 39:43-49; Kadoya, S. *et al.* (2010) “Freeze-Drying Of Proteins With Glass-Forming Oligosaccharide-Derived Sugar Alcohols,” Int. J. Pharm. 389:107-113; Golovanov, A.P. *et al.* (2004) “A Simple Method For Improving Protein Solubility And Long-Term Stability,” J. Am. Chem. Soc. 126:8933-8939).

[0010] Typically, a protein-to-stabilizer compound ratio of 1:1 or 1:2 (w/w) has been used to achieve optimal stability for lower protein concentrations (< 50 mg/mL). However, for higher protein concentrations ($\geq$ 50 mg/mL), protein-to-stabilizer compound ratios in the 1:1 or 1:2 (w/w) range are less desirable. For example, such high sugar concentrations can result in high viscosity, which impose challenges during fill-finish operations and in drug-delivery and can require increased reconstitution times for lyophilized formulations. Moreover, the reconstituted formulations can exhibit high osmolality, far outside the desired isotonic range, especially if partial reconstitution is desired in order to achieve a higher protein concentration. Finally, high concentration protein formulations with protein-to-stabilizer compound ratios in the 1:1 or 1:2 (w/w) range can exhibit thermal characteristics that require unacceptably long lyophilization process times at much lower temperatures.

[0011] The need to reconstitute such protein-based therapeutic agents imposes a second impediment to their use. Factors that govern reconstitution time remain poorly understood (Beech, K.E. *et al.* (2015) “*Insights Into The Influence Of The Cooling Profile On The Reconstitution Times Of Amorphous Lyophilized Protein Formulations*,” Eur. J. Pharmaceut. Biopharmaceut. 96:247-254). The time needed to achieve full reconstitution of conventional compositions may be significant (*e.g.*, 20-40 minutes or more), and products that have not been fully reconstituted may be detrimental to recipient patients. Additionally, the reconstitution procedure can differ depending on the product, which can add further complexity to the administration process. For example, after the addition of a diluent, a product may require swirling at set intervals, or may require being left undisturbed, in order to achieve complete reconstitution (Beech, K.E. *et al.* (2015) “*Insights Into The Influence Of The Cooling Profile On The Reconstitution Times Of Amorphous Lyophilized Protein Formulations*,” Eur. J. Pharmaceut. Biopharmaceut. 96:247-254).

[0012] Thus, despite all of such advances, a need remains for formulations suitable for stabilizing protein-based pharmaceutical compositions, particularly without a sugar stabilizing agent, such that the pharmaceutical compositions would exhibit improved viscosity and reconstitution times and enhanced stability, both in lyophilized / cryopreserved form and following reconstitution. The present invention is directed to this and other goals.

Summary Of The Invention

[0013] The present invention relates to improved pharmaceutical compositions that contain high concentrations of one or more protein biomolecule(s). In particular, the invention relates

to such pharmaceutical compositions that include one or more amino acid molecules, particularly arginine, alanine, glycine, lysine or proline, or derivatives and salts thereof, or mixtures thereof, as stabilizing compounds. The inclusion of such stabilizing compounds decreases reconstitution time whilst improving and/or maintaining the long-term stability of the protein biomolecule, so as to facilitate the treatment, management, amelioration and/or prevention of a disease or condition by the pharmaceutical composition. The invention particularly pertains to such pharmaceutical compositions that lack, or substantially lack, a sugar stabilizing agent.

[0014] In detail, the invention concerns a pharmaceutical composition comprising a protein biomolecule as an active agent or component thereof, wherein the composition comprises:

- (A) (1) an aqueous carrier;
- (2) a protein biomolecule;
- (3) a buffer;
- (4) a stabilizing compound selected from the group consisting of arginine, alanine, glycine, lysine or proline, or a derivative or salt thereof, or mixtures thereof, in a total concentration of from about 1% (w/v) to about 6% (w/v);

or

- (B) a lyophilisate of (A).

[0015] The invention further concerns the embodiment of the above-indicated pharmaceutical composition wherein the composition substantially lacks a sugar stabilizing compound.

[0016] The invention further concerns the embodiment of either of the above-indicated pharmaceutical compositions wherein the composition comprises from about 10 mg/mL to about 200 mg/mL of a protein biomolecule, and wherein the composition comprises 50 mg/mL, 75 mg/mL, 100 mg/mL, 150 mg/mL or 200 mg/mL of a protein biomolecule.

[0017] The invention further concerns the embodiment of all of the above-indicated pharmaceutical compositions wherein the protein biomolecule is an antibody or an antibody-based immunotherapeutic, enzyme, or a hormone/factor.

[0018] The invention further concerns the embodiment of the above-indicated pharmaceutical compositions wherein the protein biomolecule is an antibody or an antibody-based immunotherapeutic, and the antibody is selected from the antibodies of Table 1.

[0019] The invention further concerns the embodiment of the above-indicated pharmaceutical compositions wherein the protein biomolecule is a hormone/factor, and the hormone/factor is selected from the hormone/factors of Table 2.

[0020] The invention further concerns the embodiment of any of the above-indicated pharmaceutical compositions wherein the composition comprises at least two protein biomolecules.

[0021] The invention further concerns the embodiments of any of the above-indicated pharmaceutical compositions wherein the stabilizing compound is arginine or a derivative or salt thereof, and wherein the arginine is present at a concentration from about 2.0% (w/v) to about 5.0% (w/v), preferably at a concentration of 2.0% (w/v), a concentration of 3.5% (w/v) or a concentration of 5.5% (w/v).

[0022] The invention further concerns the embodiments of any of the above-indicated pharmaceutical compositions wherein the stabilizing compound is alanine or a derivative or salt thereof, and wherein the alanine is present at a concentration from about 2.5% (w/v) to about 5.5% (w/v), preferably at a concentration of about 2.5% (w/v), about 3.5% (w/v), about 4.0% (w/v), or about 5.5% (w/v). The invention further concerns the embodiment of such pharmaceutical compositions wherein arginine is additionally present at a concentration of about 1.25% (w/v), about 1.75% (w/v), about 2.0% (w/v) or about 2.75% (w/v).

[0023] The invention further concerns the embodiments of any of the above-indicated pharmaceutical compositions wherein the stabilizing compound is glycine or a derivative or salt thereof, and wherein the glycine is present at a concentration from about 2.5% (w/v) to about 5.5% (w/v), preferably at a concentration of about 2.5% (w/v), about 3.5% (w/v), about 4.0% (w/v) or about 5.5% (w/v). The invention further concerns the embodiment of such pharmaceutical compositions wherein arginine is additionally present at a concentration of about 1.25% (w/v), about 1.75% (w/v), about 2.0% (w/v) or about 2.75% (w/v).

[0024] The invention further concerns the embodiment of any of the above-indicated pharmaceutical compositions wherein the composition comprises at least two stabilizing compounds.

[0025] The invention further concerns the embodiment of any of the above-indicated pharmaceutical compositions wherein the pH of the pharmaceutical composition is from about

3 to about 11, from about 4 to about 9, from about 5 to about 8, from about 5 to about 7.5, preferably 6.0 or 7.4.

[0026] The invention further concerns the embodiment of any of the above-indicated pharmaceutical compositions wherein the buffer is present in a range from about 5 mM to about 50 mM, about 20 mM to about 30 mM, or about 23 mM to about 27 mM, preferably wherein the buffer is present at 25mM.

[0027] The invention further concerns the embodiments of any of the above-indicated pharmaceutical compositions wherein the buffer comprises histidine, phosphate, acetate, citrate, succinate, Tris, or a combination thereof, and wherein the buffer is histidine / histidine-HCl.

[0028] The invention further concerns the embodiment of any of the above-indicated pharmaceutical compositions wherein the pharmaceutical composition additionally comprises a non-ionic detergent, and especially wherein the non-ionic detergent is polysorbate-80 (PS-80). The invention further concerns the embodiment of such pharmaceutical compositions wherein such polysorbate-80 (PS-80) is present at a concentration of between 0.005 and 0.1% (w/v), preferably at a concentration of 0.02% (w/v).

[0029] The invention further concerns the embodiment of any of the above-indicated pharmaceutical compositions wherein the pharmaceutical composition is the lyophilisate.

[0030] The invention further concerns the embodiment of any of the above-indicated pharmaceutical compositions wherein the presence of the stabilizing compound(s) causes the reconstitution time of a lyophilisate of the pharmaceutical composition to be less than 20 minutes, less than 15 minutes, less than 10 minutes, less than 8 minutes, less than 5 minutes, or less than 2 minutes.

[0031] The invention further concerns the embodiment of any of the above-indicated pharmaceutical compositions wherein the presence of the stabilizing compound(s) enhances a stability characteristic of the pharmaceutical composition by more than 400%, by more than 200%, by more than 100%, by more than 50%, or by more than 10%, relative to such stability characteristic as observed in the complete absence of the amino acid stabilizing compound(s).

[0032] The invention further concerns the embodiment of any of the above-indicated pharmaceutical compositions wherein the presence of the stabilizing compound(s) enhances a stability characteristic of the pharmaceutical composition by more than 50%, by more than 20%, by more than 10%, by more than 5%, or by more than 1%, relative to such stability characteristic as observed in the complete absence of a sugar stabilizing compound.

[0033] The invention further concerns an ampoule, vial, cartridge, syringe or sachette that contains any of the above-indicated pharmaceutical compositions.

[0034] The invention further concerns a method of treating a disease or disorder by administering any of the above-indicated pharmaceutical compositions.

[0035] The invention further concerns of the above-indicated pharmaceutical compositions for use in medicine.

[0036] The invention further concerns a use of one or more amino acids, such as arginine, alanine, glycine, lysine or proline, as a replacement of one or more sugars in a pharmaceutical formulation to decrease reconstitution time.

Brief Description Of The Drawings

[0037] **Figure 1** shows the observed reconstitution times and degree of aggregation (assessed by high performance size-exclusion chromatography (HPSEC)) of a lyophilized formulation of a pharmaceutical composition containing a high concentration (100 mg/mL) of an exemplary protein biomolecule (a human IgG1 monoclonal antibody) with various amino acid-sugar combinations. Percent aggregate increase post-lyophilization (left axis) is shown as bars, reconstitution times (right axis) are shown as diamonds.

[0038] **Figure 2** shows the effects of protein concentration and amino acid excipients on reconstitution times for the indicated lyophilized formulations of pharmaceutical compositions containing a high concentration (50 mg/mL, 75 mg/mL or 100 mg/mL) of a human IgG1 monoclonal antibody. The human IgG1 monoclonal antibody was employed as an exemplary protein biomolecule.

[0039] **Figures 3A-3B** show the percent monomer purity over time, as determined by HPSEC for formulations of pharmaceutical compositions containing 75 mg/mL or 100 mg/mL of a human IgG1 monoclonal antibody with various added excipients as shown, at 40 °C, 60%

relative humidity (**Figure 3A**) and at 25 °C, 75% relative humidity (**Figure 3B**). The human IgG1 monoclonal antibody was employed as an exemplary protein biomolecule.

[0040] **Figure 4** shows the reconstitution times for lyophilized formulations, as shown, of pharmaceutical compositions containing a high concentration (75 mg/mL or 100 mg/mL) of a human IgG1 monoclonal antibody, which was employed as an exemplary protein biomolecule. The results are the averages of 10 replicate experiments.

[0041] **Figures 5A-5C** show reconstitution times of lyophilized formulations prepared with 75 mg/mL or 100 mg/mL of one of three different exemplary proteins with various amino acid excipients. **Figure 5A** shows reconstitution times for a human IgG1 monoclonal antibody. **Figure 5B** shows reconstitution times for a Tn3-HSA fusion protein. **Figure 5C** shows reconstitution times for a humanized IgG4 monoclonal antibody.

Detailed Description Of The Invention

[0042] The present invention relates to improved pharmaceutical compositions that contain high concentrations of one or more protein biomolecule(s). In particular, the invention relates to such pharmaceutical compositions that include one or more amino acid molecules, particularly arginine, alanine, glycine, lysine or proline, or derivatives and salts thereof, or mixtures thereof, as stabilizing compounds. The inclusion of such stabilizing compounds decreases reconstitution time whilst improving and/or maintaining the long-term stability of the protein biomolecule, so as to facilitate the treatment, management, amelioration and/or prevention of a disease or condition by the pharmaceutical composition. The invention particularly pertains to such pharmaceutical compositions that lack, or substantially lack, a sugar stabilizing agent.

I. The Pharmaceutical Compositions of the Present Invention

[0043] As used herein, the term “pharmaceutical composition” is intended to refer to a “therapeutic” medicament (*i.e.*, a medicament formulated to treat an existing disease or condition of a recipient subject) or a “prophylactic” medicament (*i.e.*, a medicament formulated to prevent or ameliorate the symptoms of a potential or threatened disease or condition of a recipient subject) containing one or more protein biomolecules as its active therapeutic or prophylactic agent or component. The pharmaceutical compositions of the present invention comprise one or more protein biomolecule(s) that serve(s) as an active agent or component of the composition. For therapeutic use, the pharmaceutical composition will contain and provide

a “therapeutically effective” amount of the protein biomolecule(s), which is an amount that reduces or ameliorates the progression, severity, and/or duration of a disease or condition, and/or ameliorates one or more symptoms associated with such disease or condition. For prophylactic use, the pharmaceutical composition will contain and provide a “prophylactically effective” amount of the protein biomolecule(s), which is an amount that is sufficient to result in the prevention of the development, recurrence, onset or progression of a disease or condition. The recipient subject is an animal, preferably a mammal including a non-primate (*e.g.*, a cow, pig, horse, cat, dog, rat, or mouse), or a primate (*e.g.*, a chimpanzee, a monkey such as a cynomolgus monkey, and a human), and is more preferably a human.

II. The Stabilizing Compounds of the Pharmaceutical Compositions of the Present Invention

[0044] The stabilizing compounds of the present invention are “lyoprotectants” (and as such serve to protect the protein biomolecule of the pharmaceutical composition from denaturation during freeze-drying and subsequent storage) and/or “cryoprotectants” (and as such serve to protect the protein biomolecule of the pharmaceutical composition from denaturation caused by freezing). A “stabilizing” compound is said to “stabilize” or “protect” a protein biomolecule of a pharmaceutical compositions of the present invention, if it serves to preserve the structure and functionality of the protein biomolecule that is the active agent or component of the composition, relative to changes in such structure and functionality observed in the absence of such formulation. A stabilizing compound is one that serves to prevent or decrease the extent of freezing or melting of a composition at that composition’s normal melt temperature (T_m).

[0045] The “protection” provided to the protein biomolecule may be assessed using high performance size-exclusion chromatography (“HPSEC”), which is an industry standard technique for the detection and quantification of pharmaceutical protein aggregates (US Patent Publication No. 2015/0005475; Gabrielson, J.P. *et al.* (2006) “*Quantitation Of Aggregate Levels In A Recombinant Humanized Monoclonal Antibody Formulation By Size-Exclusion Chromatography, Asymmetrical Flow Field Flow Fractionation, And Sedimentation Velocity*,” J. Pharm. Sci. 96(2):268-279; Liu, H. *et al.* (2009) “*Analysis Of Reduced Monoclonal Antibodies Using Size Exclusion Chromatography Coupled With Mass Spectrometry*,” J. Amer. Soc. Mass Spectrom. 20:2258-2264; Mahler, H. C. *et al.* (2008) “*Protein Aggregation: Pathways, Induction Factors And Analysis*,” J. Pharm. Sci. 98(9):2909-2934). Such protection permits the protein biomolecule to exhibit “low to undetectable levels” of fragmentation (*i.e.*,

such that, in a sample of the pharmaceutical composition, more than 80%, 85%, 90% 95%, 98%, or 99% of the protein biomolecule migrates in a single peak as determined by HPSEC and/or “low to undetectable levels” of loss of the biological activity/ies associated (*i.e.*, such that, in a sample of the pharmaceutical composition, more than 80%, 85%, 90% 95%, 98%, or 99% of the protein biomolecule present exhibits its initial biological activity/ies as measured by HPSEC, and/or low to undetectable levels” of aggregation (*i.e.*, such that, in a sample of the pharmaceutical composition, no more than 5%, no more than 4%, no more than 3%, no more than 2%, no more than 1%, and most preferably no more than 0.5%, aggregation by weight protein as measured by HPSEC. The “long-term” stability provided by the pharmaceutical compositions of the present permit such compositions to be stored for more than 3 months, more than 6 months, more than 9 months, more than 1 year, more than 18 months, more than 2 years, or more than 30 months.

[0046] The preferred “stabilizing compounds” of the present invention achieve shorter reconstitution times for lyophilized pharmaceutical compositions that contain high concentrations of one or more protein biomolecule(s). Most preferably, such stabilizing compounds are amino acid molecules, and more preferably, the amino acids: alanine, arginine, glycine, lysine and/or proline, or derivatives and salts thereof, or mixtures thereof, and even more preferably, the amino acids: alanine, arginine, and/or glycine, or derivatives and salts thereof, or mixtures thereof. Such amino acid molecules will preferably be L-amino acid molecules, but may be D-amino acid molecules or any combination of D- and L-amino acid molecules, including a racemic mixture thereof. Preferably, the presence of such stabilizing compound(s) of the present invention will be sufficient to cause the reconstitution time of a lyophilisate of the pharmaceutical composition to be less than 20 mins, less than 15 mins, less than 10 mins, less than 8 mins, less than 5 mins, or less than 2 mins, and to enhance a stability characteristic (*e.g.*, a lyoprotective or cryoprotective property, such as single dosage reconstitution time, mean shelf life, percent activity remaining at a designated time interval at a set temperature (*e.g.*, a subzero temperature, room temperature or an elevated temperature), *etc.*) of the pharmaceutical composition by more than 400%, by more than 200%, by more than 100%, by more than 50%, or by more than 10%, relative to such stability characteristic as observed in the complete absence of amino acid stabilizing compound(s).

[0047] With respect to such amino acids molecules, the term “derivatives and salts thereof” denotes any pharmaceutically acceptable salt or amino acid derivative, such as those disclosed

in REMINGTON: THE SCIENCE AND PRACTICE OF PHARMACY, 21th Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA, 2005. Such derivatives include substituted amines, amino alcohols, aldehydes, lactones, esters, hydrates, *etc.* Exemplary derivatives of alanine include: 2-allyl-glycine, 2-aminobutyric acid, cis-amcilenomycin, adamantane, *etc.* Exemplary derivatives of arginine include: 2-amino-3-guanidinopropionic acid, 2-amino-4-guanidinobutyric acid, 5-methyl-arginine, arginine methyl ester, arginine-O-tBu, canavanine, citrulline, ϵ -hydroxy arginine, homoarginine, N-tosyl-arginine, N_ω-nitro-arginine, thio-citrulline, *etc.* Exemplary derivatives of lysine include: diaminobutyric acid, 2,3-diaminopropanoic acid, (2s)-2,8-diaminoactanoic acid, ornithine, thialysine, *etc.* Exemplary derivatives of proline include: trans-1-acetyl-4-hydroxyproline, 3,4-dehydroproline, cis-3-hydroxyproline, cis-4-hydroxyproline, trans-3-hydroxyproline, trans-4-hydroxyproline, α -methylproline, pipecolic acid, *etc.*

[0048] Salts of such amino acids molecules and their derivatives include addition salts of such molecules such as those derived from an appropriate acid, *e.g.*, hydrochloric, sulphuric, phosphoric, maleic, fumaric, citric, tartaric, lactic, acetic or p-toluenesulphonic acid. Particularly preferred are hydrochloride salts.

[0049] Such stabilizing compounds can be used individually or in combination in the pharmaceutical compositions of the present invention (*e.g.*, any two stabilizing compounds, any three stabilizing compounds, any four stabilizing compounds, any five stabilizing compounds, or any combination of more than five of such stabilizing compounds).

[0050] As discussed above, sugars such as dextran, sucrose, trehalose dihydrate are typically used as stabilizing compounds in lyophilized therapeutic protein formulations. In a highly preferred embodiment of the present invention, the pharmaceutical compositions of the present invention will substantially lack (*i.e.*, be substantially free of) a sugar stabilizing compound, and in a more highly preferred embodiment of the present invention, the pharmaceutical compositions of the present invention will completely lack (*i.e.*, be completely free of) a sugar stabilizing compound. As used herein, a pharmaceutical composition of the present invention is said to “substantially lack sugar stabilizing compound(s)” if the presence of such compounds does not enhance a stability characteristic (*e.g.*, a lyoprotective or cryoprotective property) of the pharmaceutical composition by more than 50%, by more than 20%, by more than 10%, by more than 5%, or by more than 1%, relative to such stability characteristic as observed in the complete absence of such sugar stabilizing compound(s). As used herein, a pharmaceutical

composition of the present invention is said to “completely lack sugar stabilizing compound(s)” if the presence of such compound(s) is not detectable. It is preferred that the pharmaceutical compositions of the present invention will completely lack any sugar stabilizing compound.

[0051] Sugars such as sucrose and trehalose dihydrate are typically used as excipients in lyophilized therapeutic protein formulations to improve drug product stability, *e.g.*, for storage at 2-8 °C (US Patents No. 8,617,576 and 8,754,195). Trehalose, in particular, has been widely used as a stabilizing agent; it is used in a variety of research applications and is contained in several commercially available therapeutic products, including HERCEPTIN®, AVASTIN®, LUCENTIS®, and ADVATE® (Ohtake, S. *et al.* (2011) “*Trehalose: Current Use and Future Applications*,” J. Pharm. Sci. 100(6):2020-2053).

III. The Protein Biomolecules Of The Pharmaceutical Compositions Of The Present Invention

[0052] The stabilizing compounds of the present invention are particularly suitable for use in pharmaceutical compositions that contain high concentrations of one or more protein biomolecule(s) as their active agents or components. As used herein, the term “high concentration” denotes a concentration of the protein biomolecule(s) that is greater than 10 mg/mL, greater than 20 mg/mL, greater than 30 mg/mL, greater than 40 mg/mL, greater than 50 mg/mL, greater than 60 mg/mL, greater than 70 mg/mL, greater than 80 mg/mL, greater than 90 mg/mL, greater than 100 mg/mL, greater than 120 mg/mL, greater than 150 mg/mL, greater than 200 mg/mL, greater than 250 mg/mL, greater than 300 mg/mL, greater than 350 mg/mL, greater than 400 mg/mL, greater than 450 mg/mL, or greater than 500 mg/mL.

[0053] Without limitation, the “protein biomolecules” contained in such pharmaceutical compositions may be any kind of protein molecule, including single polypeptide chain proteins or multiple polypeptide chain proteins. As used herein the term protein biomolecule does not connote that the molecule is of any particular size and is intended to include protein biomolecules that comprise fewer than 5, fewer than 10, fewer than 20 fewer than 30, fewer than 40 or fewer than 50 amino acid residues, as well as protein biomolecules that comprise more than 50, more than 100, more than 200 more than 300, more than 400, or more than 500 amino acid residues.

[0054] Examples of protein biomolecules that may be present in the pharmaceutical compositions of the present invention are provided in Tables 1 and 2, and include antibody or

antibody-based immunotherapeutics (for example, palivizumab which is directed to an epitope in the A antigenic site of the F protein of respiratory syncytial virus (RSV) (SYNAGIS®; US Patents No. 8,460,663 and 8,986,686), antibody directed against angiopoietin-2 (US Patents No. 8,507,656 and 8,834,880); antibody directed against Delta-like Protein Precursor 4 (DLL4) (US Patent No. 8,663,636; US Patent Publication No. 2015/0005475; PCT Publication No. WO 2013/113898); antibody directed against Platelet-Derived Growth Factor- α (PDGRF- α) (US Patent No. 8,697,664); antibody directed against alpha-V-beta-6 integrin (α V β 6) (US Patent No. 8,894,998; antibody directed against Growth and Differentiation Factor (GDF-8) (US Patent No. 8,697,664), enzymes, hormones and factors, and antigenic proteins for use in vaccines (for example, insulin, erythropoietin, growth hormone, *etc.*).

Table 1		
Antibody and Immunotherapeutic Molecules		
Antibody Name	Disease-Associated Antigen	Therapeutic Target Application
3F8	Gd2	Neuroblastoma
8H9	B7-H3	Neuroblastoma, Sarcoma, Metastatic Brain Cancers
Abagovomab	CA-125	Ovarian Cancer
Abciximab	CD41	Platelet Aggregation Inhibitor
<u>Actoxumab</u>	<i>Clostridium Difficile</i>	<i>Clostridium Difficile</i> Infection
Adalimumab	TNF-A	Rheumatoid Arthritis, Crohn's Disease, Plaque Psoriasis, Psoriatic Arthritis, Ankylosing Spondylitis, Juvenile Idiopathic Arthritis, Hemolytic Disease Of The Newborn
Adecatumumab	Epcam	Prostate And Breast Cancer
Aducanumab	Beta-Amyloid	Alzheimer's Disease
Afelimomab	TNF-A	Sepsis
Afutuzumab	CD20	Lymphoma
Alacizumab	VEGFR2	Cancer
Ald518	Il-6	Rheumatoid Arthritis
Alemtuzumab	CD52	Multiple Sclerosis
Alirocumab	NARP-1	Hypercholesterolemia
Altumomab	CEA	Colorectal Cancer
Amatuximab	Mesothelin	Cancer
Anatumomab Mafenatox	TAG-72	Non-Small Cell Lung Carcinoma
Anifrolumab	Interferon A/B Receptor	Systemic Lupus Erythematosus
Anrukinzumab	IL-13	Cancer
Apolizumab	HLA-DR	Hematological Cancers

Table 1		
Antibody and Immunotherapeutic Molecules		
Antibody Name	Disease-Associated Antigen	Therapeutic Target Application
Arcitumomab	CEA	Gastrointestinal Cancer
Aselizumab	L-Selectin (CD62L)	Severely Injured Patients
Atinumab	RTN4	Cancer
Atlizumab	IL-6 Receptor	Rheumatoid Arthritis
Atorolimumab	Rhesus Factor	Hemolytic Disease Of The Newborn
Bapineuzumab	Beta-Amyloid	Alzheimer's Disease
Basiliximab	CD25	Prevention Of Organ Transplant Rejections
Bavituximab	Phosphatidylserine	Cancer, Viral Infections
Bectumomab	CD22	Non-Hodgkin's Lymphoma (Detection)
Belimumab	BAFF	Non-Hodgkin Lymphoma
Benralizumab	CD125	Asthma
Bertilimumab	CCL11 (Eotaxin-1)	Severe Allergic Disorders
Besilesomab	CEA-Related Antigen	Inflammatory Lesions And Metastases (Detection)
Bevacizumab	VEGF-A	Metastatic Cancer, Retinopathy Of Prematurity
Bezlotoxumab	<i>Clostridium difficile</i>	<i>Clostridium difficile</i> Infection
Biciromab	Fibrin II, Beta Chain	Thromboembolism (Diagnosis)
Bimagrumab	ACVR2B	Myostatin Inhibitor
Bivatuzumab	CD44 V6	Squamous Cell Carcinoma
Blinatumomab	CD19	Cancer
Blosozumab	SOST	Osteoporosis
Brentuximab	CD30 (TNFRSF8)	Hematologic Cancers
Briakinumab	IL-12, IL-23	Psoriasis, Rheumatoid Arthritis, Inflammatory Bowel Diseases, Multiple Sclerosis
Brodalumab	IL-17	Inflammatory Diseases
Canakinumab	IL-1	Rheumatoid Arthritis
Cantuzumab	MUC1	Cancers
Cantuzumab Mertansine	Mucin Canag	Colorectal Cancer
Caplacizumab	VWF	Cancers
Capromab	Prostatic Carcinoma Cells	Prostate Cancer (Detection)
Carlumab	MCP-1	Oncology/Immune Indications
Catumaxomab	Epcam, CD3	Ovarian Cancer, Malignant Ascites, Gastric Cancer
Cc49	Tag-72	Tumor Detection
Certolizumab	TNF-A	Crohn's Disease
Cetuximab	EGFR	Metastatic Colorectal Cancer And Head And Neck Cancer
Ch.14.18	Undetermined	Neuroblastoma

Table 1		
Antibody and Immunotherapeutic Molecules		
Antibody Name	Disease-Associated Antigen	Therapeutic Target Application
Citatumumab	Epcam	Ovarian Cancer And Other Solid Tumors
Cixutumumab	IGF-1 Receptor	Solid Tumors
Clazakizumab	<i>Oryctolagus Cuniculus</i>	Rheumatoid Arthritis
Clivatuzumab	MUC1	Pancreatic Cancer
Conatumumab	TRAIL-R2	Cancer
Concizumab	TFPI	Bleeding
Cr6261	Influenza A Hemagglutinin	Infectious Disease/Influenza A
Crenezumab	1-40-B-Amyloid	Alzheimer's Disease
Dacetuzumab	CD40	Hematologic Cancers
Daclizumab	CD25	Prevention Of Organ Transplant Rejections
Dalotuzumab	Insulin-Like Growth Factor I Receptor	Cancer
Daratumumab	CD38	Cancer
Demcizumab	DLL4	Cancer
Denosumab	RANKL	Osteoporosis, Bone Metastases
Detumomab	B-Lymphoma Cell	Lymphoma
Dorlimomab Aritox	Undetermined	Cancer
Drozitumab	DR5	Cancer
Duligotumab	HER3	Cancer
Dupilumab	IL4	Atopic Diseases
Dusigitumab	ILGF2	Cancer
Ecromeximab	GD3 Ganglioside	Malignant Melanoma
Eculizumab	C5	Paroxysmal Nocturnal Hemoglobinuria
Edobacomab	Endotoxin	Sepsis Caused By Gram-Negative Bacteria
Edrecolomab	Epcam	Colorectal Carcinoma
Efalizumab	LFA-1 (CD11a)	Psoriasis (Blocks T Cell Migration)
Efungumab	Hsp90	Invasive Candida Infection
Eldelumab	Interferon-Gamma-Induced Protein	Crohn's Disease, Ulcerative Colitis
Elotuzumab	SLAMF7	Multiple Myeloma
Elsilimomab	IL-6	Cancer
Enavatuzumab	TWEAK Receptor	Cancer
Enlimomab	ICAM-1 (CD54)	Cancer
Enokizumab	IL9	Asthma
Enoticumab	DLL4	Cancer
Ensituximab	5AC	Cancer
Epitumomab Cituxetan	Episialin	Cancer
Epratuzumab	CD22	Cancer, SLE

Table 1		
Antibody and Immunotherapeutic Molecules		
Antibody Name	Disease-Associated Antigen	Therapeutic Target Application
Erlizumab	ITGB2 (CD18)	Heart Attack, Stroke, Traumatic Shock
Ertumaxomab	HER2/Neu, CD3	Breast Cancer
Etaracizumab	Integrin $A_v\beta_3$	Melanoma, Prostate Cancer, Ovarian Cancer
Etrolizumab	Integrin $A_7 B_7$	Inflammatory Bowel Disease
Evolocumab	PCSK9	Hypocholesterolemia
Exbivirumab	Hepatitis B Surface Antigen	Hepatitis B
Fanolesomab	CD15	Appendicitis (Diagnosis)
Faralimomab	Interferon Receptor	Cancer
Farletuzumab	Folate Receptor 1	Ovarian Cancer
Fasinumab ^[51]	HNGF	Cancer
Fbta05	CD20	Chronic Lymphocytic Leukaemia
Felvizumab	Respiratory Syncytial Virus	Respiratory Syncytial Virus Infection
Fezakinumab	IL-22	Rheumatoid Arthritis, Psoriasis
Ficlatuzumab	HGF	Cancer
Figitumumab	IGF-1 Receptor	Adrenocortical Carcinoma, Non-Small Cell Lung Carcinoma
Flanvotumab	TYRP1 (Glycoprotein 75)	Melanoma
Fontolizumab	IFN- γ	Crohn's Disease
<u>Foravirumab</u>	Rabies Virus Glycoprotein	Rabies (Prophylaxis)
Fresolimumab	TGF-B	Idiopathic Pulmonary Fibrosis, Focal Segmental Glomerulosclerosis, Cancer
Fulranumab	NGF	Pain
Futuximab	EGFR	Cancer
Galiximab	CD80	B Cell Lymphoma
Ganitumab	IGF-I	Cancer
Gantenerumab	Beta-Amyloid	Alzheimer's Disease
Gavilimomab	CD147 (Basigin)	Graft-Versus-Host Disease
Gemtuzumab Ozogamicin	CD33	Acute Myelogenous Leukemia
Gevokizumab	IL-1 β	Diabetes
Girentuximab	Carbonic Anhydrase 9 (CA-IX)	Clear Cell Renal Cell Carcinoma
Glembatumumab Vedotin	GPNMB	Melanoma, Breast Cancer
Golimumab	TNF-A	Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis
Gomiliximab	CD23 (Ige Receptor)	Allergic Asthma
Guselkumab	IL13	Psoriasis

Table 1		
Antibody and Immunotherapeutic Molecules		
Antibody Name	Disease-Associated Antigen	Therapeutic Target Application
Ibritumomab Tiuxetan	CD20	Non-Hodgkin's Lymphoma
Icrucumab	VEGFR-1	Cancer
Igovomab	CA-125	Ovarian Cancer (Diagnosis)
Imab362	Cldn18.2	Gastrointestinal Adenocarcinomas And Pancreatic Tumor
Imgatuzumab	EGFR	Cancer
Inclacumab	Selectin P	Cancer
Indatuximab Ravtansine	SDC1	Cancer
Infliximab	TNF-A	Rheumatoid Arthritis, Ankylosing Spondylitis, Psoriatic Arthritis, Psoriasis, Crohn's Disease, Ulcerative Colitis
Inolimomab	CD25 (A Chain Of IL-2 Receptor)	Graft-Versus-Host Disease
Inotuzumab Ozogamicin	CD22	Cancer
Intetumumab	CD51	Solid Tumors (Prostate Cancer, Melanoma)
Ipilimumab	CD152	Melanoma
Iratumumab	CD30 (TNFRSF8)	Hodgkin's Lymphoma
Itolizumab	CD6	Cancer
Ixekizumab	IL-17A	Autoimmune Diseases
Keliximab	CD4	Chronic Asthma
Labetuzumab	CEA	Colorectal Cancer
Lambrolizumab	PDCD1	Antineoplastic Agent
Lampalizumab	CFD	Cancer
Lebrikizumab	IL-13	Asthma
Lemalesomab	NCA-90 (Granulocyte Antigen)	Diagnostic Agent
Lerdelimumab	TGF Beta 2	Reduction Of Scarring After Glaucoma Surgery
Lexatumumab	TRAIL-R2	Cancer
Libivirumab	Hepatitis B Surface Antigen	Hepatitis B
Ligelizumab	IGHE	Cancer
Lintuzumab	CD33	Cancer
Lirilumab	KIR2D	Cancer
Lodelcizumab	PCSK9	Hypercholesterolemia
Lorvotuzumab	CD56	Cancer
Lucatumumab	CD40	Multiple Myeloma, Non-Hodgkin's Lymphoma, Hodgkin's Lymphoma
Lumiliximab	CD23	Chronic Lymphocytic Leukemia
Mapatumumab	TRAIL-R1	Cancer

Table 1		
Antibody and Immunotherapeutic Molecules		
Antibody Name	Disease-Associated Antigen	Therapeutic Target Application
Margetuximab	Ch4d5	Cancer
Matuzumab	EGFR	Colorectal, Lung And Stomach Cancer
Mavrilimumab	GMCSF Receptor A-Chain	Rheumatoid Arthritis
Mepolizumab	IL-5	Asthma And White Blood Cell Diseases
Metelimumab	TGF Beta 1	Systemic Scleroderma
Milatumzumab	CD74	Multiple Myeloma And Other Hematological Malignancies
Minretumomab	TAG-72	Cancer
Mitumomab	GD3 Ganglioside	Small Cell Lung Carcinoma
Mogamulizumab	CCR4	Cancer
Morolimumab	Rhesus Factor	Cancer
Motavizumab	Respiratory Syncytial Virus	Respiratory Syncytial Virus (Prevention)
Moxetumomab Pasudotox	CD22	Cancer
Muromonab-CD3	CD3	Prevention Of Organ Transplant Rejections
Nacolomab Tafenatox	C242 Antigen	Colorectal Cancer
Namilumab	CSF2	Cancer
Naptumomab Estafenatox	5T4	Non-Small Cell Lung Carcinoma, Renal Cell Carcinoma
Narnatumab	RON	Cancer
Natalizumab	Integrin A4	Multiple Sclerosis, Crohn's Disease
Nebacumab	Endotoxin	Sepsis
Necitumumab	EGFR	Non-Small Cell Lung Carcinoma
Nerelimomab	TNF-A	Cancer
Nesvacumab	Angiopoietin 2	Cancer
Nimotuzumab	EGFR	Squamous Cell Carcinoma, Head And Neck Cancer, Nasopharyngeal Cancer, Glioma
Nivolumab	PD-1	Cancer
Nofetumomab Merpentan	Undetermined	Cancer
Ocaratuzumab	CD20	Cancer
Ocrelizumab	CD20	Rheumatoid Arthritis, Lupus Erythematosus
Odulimomab	LFA-1 (CD11a)	Prevention Of Organ Transplant Rejections, Immunological Diseases
Ofatumumab	CD20	Chronic Lymphocytic Leukemia
Olaratumab	PDGF-R A	Cancer
Olokizumab	IL6	Cancer
Onartuzumab	Human Scatter Factor Receptor Kinase	Cancer
Ontuxizumab	TEM1	Cancer

Table 1 Antibody and Immunotherapeutic Molecules		
Antibody Name	Disease-Associated Antigen	Therapeutic Target Application
Oportuzumab Monatox	Epcam	Cancer
Oregovomab	CA-125	Ovarian Cancer
Orticumab	Oxldl	Cancer
Otlertuzumab	CD37	Cancer
Oxelumab	OX-40	Asthma
Ozanezumab	NOGO-A	ALS And Multiple Sclerosis
Ozoralizumab	TNF-A	Inflammation
Pagibaximab	Lipoteichoic Acid	Sepsis (<i>Staphylococcus</i>)
Palivizumab	F Protein Of Respiratory Syncytial Virus	Respiratory Syncytial Virus (Prevention)
Panitumumab	EGFR	Colorectal Cancer
Pankomab	Tumor Specific Glycosylation Of MUC1	Ovarian Cancer
Panobacumab	Pseudomonas Aeruginosa	<i>Pseudomonas Aeruginosa</i> Infection
Parsatuzumab	EGFL7	Cancer
Pascolizumab	IL-4	Asthma
Pateclizumab	LTA	TNF
Patritumab	HER3	Cancer
Pembrolizumab	PD-1	Cancer
Pemtumomab	MUC1	Cancer
Perakizumab	IL17A	Arthritis
Pertuzumab	HER2/Neu	Cancer
Pexelizumab	C5	Reduction Of Side-Effects Of Cardiac Surgery
Pidilizumab	PD-1	Cancer And Infectious Diseases
Pinatuzumab Vedotin	CD22	Cancer
Pintumomab	Adenocarcinoma Antigen	Adenocarcinoma
Placulumab	Human TNF	Cancer
Polatuzumab Vedotin	CD79B	Cancer
Ponezumab	Human Beta- Amyloid	Alzheimer's Disease
Pritoxaximab	<i>E. Coli</i> Shiga Toxin Type-1	Cancer
Pritumumab	Vimentin	Brain Cancer
Pro 140	Ccr5	HIV Infection
Quilizumab	IGHE	Cancer

Table 1 Antibody and Immunotherapeutic Molecules		
Antibody Name	Disease-Associated Antigen	Therapeutic Target Application
Racotumomab	N-Glycolylneuraminic Acid	Cancer
Radretumab	Fibronectin Extra Domain-B	Cancer
Rafivirumab	Rabies Virus Glycoprotein	Rabies (Prophylaxis)
Ramucirumab	VEGFR2	Solid Tumors
Ranibizumab	VEGF-A	Macular Degeneration (Wet Form)
Raxibacumab	Anthrax Toxin, Protective Antigen	Anthrax (Prophylaxis And Treatment)
Regavirumab	Cytomegalovirus Glycoprotein B	Cytomegalovirus Infection
Reslizumab	IL-5	Inflammations Of The Airways, Skin And Gastrointestinal Tract
Rilotumumab	HGF	Solid Tumors
Rituximab	CD20	Lymphomas, Leukemias, Some Autoimmune Disorders
Robatumumab	IGF-1 Receptor	Cancer
Roledumab	RHD	Cancer
Romosozumab	Sclerostin	Osteoporosis
Rontalizumab	IFN- α	Systemic Lupus Erythematosus
Rovelizumab	CD11, CD18	Haemorrhagic Shock
Ruplizumab	CD154 (CD40L)	Rheumatic Diseases
Samalizumab	CD200	Cancer
Sarilumab	IL6	Rheumatoid Arthritis, Ankylosing Spondylitis
Satumomab Pendetide	TAG-72	Cancer
Secukinumab	IL-17A	Uveitis, Rheumatoid Arthritis Psoriasis
Seribantumab	ERBB3	Cancer
Setoxaximab	<i>E. Coli</i> Shiga Toxin Type-1	Cancer
Sevirumab	Cytomegalovirus	Cytomegalovirus Infection
Sgn-CD19a	CD19	Acute Lymphoblastic Leukemia And B Cell Non-Hodgkin Lymphoma
Sgn-CD33a	CD33	Acute Myeloid Leukemia
Sibrotuzumab	FAP	Cancer
Sifalimumab	IFN-A	SLE, Dermatomyositis, Polymyositis
Siltuximab	IL-6	Cancer
Simtuzumab	LOXL2	Fibrosis

Table 1		
Antibody and Immunotherapeutic Molecules		
Antibody Name	Disease-Associated Antigen	Therapeutic Target Application
Siplizumab	CD2	Psoriasis, Graft-Versus-Host Disease (Prevention)
Sirukumab	IL-6	Rheumatoid Arthritis
Solanezumab	Beta-Amyloid	Alzheimer's Disease
Solitumab	Epcam	Cancer
Sonepcizumab	Sphingosine-1-Phosphate	Choroidal And Retinal Neovascularization
Sontuzumab	Episialin	Cancer
Stamulumab	Myostatin	Muscular Dystrophy
Sulesomab	NCA-90 (Granulocyte Antigen)	Osteomyelitis
Suvizumab	HIV-1	Viral Infections
Tabalumab	BAFF	B Cell Cancers
Tacatuzumab Tetraxetan	Alpha-Fetoprotein	Cancer
Tadocizumab	Integrin AIIIB β 3	Percutaneous Coronary Intervention
Tanezumab	NGF	Pain
Taplitumomab Paptox	CD19	Cancer
Tefibazumab	Clumping Factor A	<i>Staphylococcus Aureus</i> Infection
Telimomab	Undetermined	Cancer
Tenatumomab	Tenascin C	Cancer
Teneliximab	CD40	Cancer
Teprotumumab	CD221	Hematologic Tumors
Ticilimumab	CTLA-4	Cancer
Tigatuzumab	TRAIL-R2	Cancer
Tildrakizumab	IL23	Immunologically Mediated Inflammatory Disorders
Tnx-650	IL-13	Hodgkin's Lymphoma
Tocilizumab	IL-6 Receptor	Rheumatoid Arthritis
Toralizumab	CD154 (CD40L)	Rheumatoid Arthritis, Lupus Nephritis
Tositumomab	CD20	Follicular Lymphoma
Tovetumab	CD140a	Cancer
Tralokinumab	IL-13	Asthma
Trastuzumab	HER2/Neu	Breast Cancer
Trbs07	Gd2	Melanoma
Tremelimumab	CTLA-4	Cancer
Tucotuzumab Celmoleukin	Epcam	Cancer
Tuvirumab	Hepatitis B Virus	Chronic Hepatitis B
Ublituximab	MS4A1	Cancer
Urelumab	4-1BB	Cancer

Table 1		
Antibody and Immunotherapeutic Molecules		
Antibody Name	Disease-Associated Antigen	Therapeutic Target Application
Urtioxazumab	Escherichia Coli	Diarrhoea Caused By <i>E. Coli</i>
Ustekinumab	IL-12, IL-23	Multiple Sclerosis, Psoriasis, Psoriatic Arthritis
Vantictumab	Frizzled Receptor	Cancer
Vapaliximab	AOC3 (VAP-1)	Cancer
Vatelizumab	ITGA2	Cancer
Vedolizumab	Integrin A4 β 7	Crohn's Disease, Ulcerative Colitis
Veltuzumab	CD20	Non-Hodgkin's Lymphoma
Vepalimomab	AOC3 (VAP-1)	Inflammation
Vesencumab	NRP1	Cancer
Volociximab	Integrin A5 β 1	Solid Tumors
Vorsetuzumab	CD70	Cancer
Votumumab	Tumor Antigen CTAA16.88	Colorectal Tumors
Zalutumumab	EGFR	Squamous Cell Carcinoma Of The Head And Neck
Zatuximab	HER1	Cancer
Ziralimumab	CD147	Cancer
Zolimomab Aritox	CD5	Systemic Lupus Erythematosus, Graft-Versus-Host Disease

Table 2
Hormones / Factors
Alpha-Glactosidase A
Alpha-L-Iduronidase
Dornase Alfa
Erythropoietin
Factor VIII
Follicle-Stimulating Hormone
Glucocerebrosidase
Granulocyte Colony-Stimulating Factor (G-CSF)
Growth Hormone
Insulin
Insulin-Like Growth Factor 1 (IGF-1)
Interferon-B-1 α
Interferon-B-1 β
N-Acetylgalactosamine-4-Sulfatase
Tissue Plasminogen Activator (TPA)

IV. Formulation of the Pharmaceutical Compositions of the Present Invention

[0055] The pharmaceutical compositions of the present invention will typically be formulated, at least initially, as an aqueous liquid, but are most preferably then suitable for lyophilization. The pharmaceutical compositions of the present invention subsequent to such lyophilization is referred to herein as a “lyophilisate.”

[0056] The liquid formulations of the pharmaceutical compositions of the present invention preferably comprise a suitable sterile aqueous carrier, a high concentration (as defined above) of the protein biomolecule, a buffer, and a stabilizing compound of the present invention. Optionally, such liquid formulations of the pharmaceutical compositions of the present invention may contain additional components, for example, a pharmaceutically acceptable, non-toxic excipient, buffer or detergent. The pharmaceutical compositions of the present invention lack sugar, or are substantially free of sugar.

[0057] Examples of suitable sterile aqueous carriers which may be employed in the pharmaceutical compositions of the present invention include water, saline, phosphate buffered saline, ethanol, dextrose solutions, and water/polyol solutions (such as glycerol, propylene glycol, polyethylene glycol, and the like).

[0058] Any suitable buffer may be employed in accordance with the present invention. It is preferred to employ a buffer capable of buffering the liquid within a pH range of from about 3 to about 11, more preferably from about 4 to about 9, more preferably from about 5 to about 8, more preferably from about 5 to about 7.5, and more preferably at a pH of 5.0; 5.1; 5.2; 5.3; 5.4; 5.5; 5.6; 5.7; 5.8; 5.9; 6.0; 6.1; 6.2; 6.3; 6.4; 6.5; 6.6; 6.7; 6.8; 6.9; 7.0; 7.1; 7.2; 7.3; 7.4; 7.5; 7.6; 7.7; 7.8; 7.9; or 8.0.

[0059] Suitable buffers include potassium phosphate, sodium phosphate, sodium acetate, histidine, imidazole, sodium citrate, sodium succinate, ammonium bicarbonate and carbonate.

[0060] Generally, buffers are used at molarities from about 1 mM to about 2 M, from about 2 mM to about 1 M, from about 1 mM to about 100 mM, about 10 mM to about 50 mM, about 20 mM to about 30 mM, or about 23 mM to about 27 mM, and is most preferably about 5 mM, 10 mM, 15 mM, 20 mM or 25 mM. In one embodiment, the buffer can be histidine / histidine HCl. Histidine can be in the form of L-histidine, D-histidine, or a mixture thereof, but L-histidine is the most preferable. Histidine can be also in the form of a hydrate, or a

pharmaceutically acceptable salt, such as hydrochloride (*e.g.*, a monohydrochloride or a dihydrochloride), hydrobromide, sulfate, acetate, *etc.* The purity of the histidine should be at least 98%, preferably at least 99%, and most preferably at least 99.5%.

[0061] The concentration of the stabilizing compound(s) that is/are included in the composition of the present invention preferably ranges from about 1% (weight/volume (w/v)) to about 6% (w/v), more preferably from about 2% (w/v) to about 5% (w/v) or from about 2% (w/v) to about 4% (w/v)). Particularly preferred are stabilizing compositions containing 2-5% (w/v) arginine, 2-5.5% (w/v) alanine, and 2-5.5% (w/v) glycine, or mixtures thereof.

[0062] Polysorbate-80 ("PS-80") is a preferred non-ionic surfactant and emulsifier of the present invention, however, other suitable non-ionic surfactants and emulsifiers (*e.g.*, Tween-20®, Tween- 80®, Polaxamers, sodium dodecyl sulfate, *etc.*) may be alternatively or additionally employed.

[0063] Particularly preferred are liquid formulations that comprise:

- (1) about 75 mg/mL, about 25 mM histidine/histidine-HCl, about 3.5% arginine (w/v), and about 0.02% PS-80 (w/v), pH 6;
- (2) about 75 mg/mL, about 25 mM histidine/histidine-HCl, about 5% arginine (w/v), and about 0.02% PS-80 (w/v), pH 6;
- (3) about 100 mg/mL, about 25 mM histidine/histidine-HCl, about 4% alanine (w/v), about 2% arginine (w/v), and about 0.02% PS-80 (w/v), pH 6;
- (4) about 100 mg/mL, about 25 mM histidine/histidine-HCl, about 4% glycine (w/v), about 2% arginine (w/v), and about 0.02% PS-80 (w/v), pH 6;

[0064] The liquid formulation can be lyophilized to further stabilize the protein biomolecule. Any suitable lyophilization apparatus and regimen may be employed, however, it is preferred to accomplish such lyophilization as shown in Table 3, Table 5 or Table 11.

[0065] Particularly subsequent to reconstitution after such lyophilization, liquid formulations of the pharmaceutical compositions of the present invention may additionally contain non-aqueous carriers, such as mineral oil or vegetable oil (*e.g.*, olive oil, corn oil, peanut oil, cottonseed oil, and sesame oil), carboxymethyl cellulose colloidal solutions, tragacanth gum and injectable organic esters, such as ethyl oleate.

[0066] The invention provides methods of treatment, prophylaxis, and amelioration of a disease or condition or one or more symptoms thereof by administering to a subject of an effective amount of liquid formulations of the invention, either as initially formulated or subsequent to reconstitution of a lyophilisate.

[0067] Various delivery systems are known and can be used to administer such liquid compositions, including, but not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural administration, topical administration, pulmonary administration, and mucosal administration (e.g., intranasal and oral routes). In a specific embodiment, liquid formulations of the present invention are administered intramuscularly, intravenously, or subcutaneously. The formulations may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can be employed, e.g., by use of an inhaler or nebulizer.

[0068] The invention also provides that the initially formulated liquid pharmaceutical composition may be packaged in a hermetically sealed container such as an ampoule, vial, cartridge, syringe or sachette indicating the quantity of the protein biomolecule contained therein. Preferably, such initially formulated liquid pharmaceutical compositions are lyophilized while within such ampoules or sachettes, and the ampoule or sachette indicates the amount of carrier to be added in order to reconstitute the lyophilisate to contain the desired high concentration of the protein biomolecule.

[0069] The amount of the liquid formulations of the present invention which will be effective for therapeutic or prophylactic use.

[0070] The precise dose to be employed in the formulation will also depend on the route of administration, the disease or condition to be treated, the particular protein biomolecule of the pharmaceutical composition, and should be decided according to the judgment of the practitioner and each subject's circumstances. Exemplary doses include 30 mg/kg or less, 15 mg/kg or less, 5 mg/kg or less, 3 mg/kg or less, 1 mg/kg or less or 0.5 mg/kg or less.

Examples

[0071] The following examples illustrate the compositions of the present invention and their properties. The examples are intended to illustrate, but in no way limit, the scope of the invention.

Example 1 Materials & Methods

[0072] **Lyophilization** – 1.1 mL aliquots of a pharmaceutical composition were introduced into 3 cc glass vials. The vials were stoppered with 13 mm single vent lyophilization stoppers. The vials were then lyophilized using a lyophilization cycle, as described in Table 3.

Table 3 Lyophilization Parameters	
Loading	20 °C
Freezing	-5 °C at 0.3 °C/min
Annealing	-16 °C at 0.5 °C/min
Freezing	-40 °C at 0.5 °C/min
Primary Drying	-35 °C at 0.1 °C/min
Secondary Drying	40 °C at 0.3 °C/min
Unloading	5 °C

[0073] The end point of lyophilization was determined using a Pirani vacuum gauge (see, e.g., Patel, S.M. *et al.* (2009) “*Determination of End Point of Primary Drying in Freeze-Drying Process Control*,” AAPS Pharm. Sci. Tech. 11(1):73-84). Such a gauge works on the principle of measuring the thermal conductivity of the gas in the drying chamber (Nail, S.L. *et al.* (1992) “*Methodology For In-Process Determination Of Residual Water In Freeze-Dried Products*,” Dev. Biol. Stand. 74:137-151; Biol. Prod. Freeze-Drying Formulation). After completion of the lyophilization cycles, vials were vacuum stoppered and removed from the lyophilizer. The vials were then capped with West 13 mm aluminum Flip-Off overseals.

[0074] **High Performance Size-Exclusion Chromatography (HPSEC)** – HPSEC samples were diluted in 10 mg/mL phosphate buffered saline prior to HPSEC. The samples were injected onto a TSKgel G3000SWXL column, eluted isocratically with phosphate buffer containing sodium sulfate and sodium azide. The eluted protein is detected using UV absorbance at 280 nm and the results are reported as the area percent of the product monomer peak. Peaks eluting earlier than the monomer are recorded as percent aggregate and peaks eluting after the monomer are recorded as percent fragment/other.

[0075] Reconstitution Procedure – Prior to use, and generally within 6 hours prior to use, sterile water is injected into the lyophilization vial, which is then gently swirled to effect reconstitution with minimal foaming. Two reconstitution procedures were used for reconstitution: Procedure A – a 1 minute swirl followed by a 5 minute hold method until all the cake is completely dissolved in solution and Procedure B – a 1 minute hold followed by a 1 minute swirl until all the cake is completely dissolved in solution.

Example 2

Impact of Variation of Amino Acid to Sugar Ratio on Reconstitution Time and Protein Aggregation of Pharmaceutical Compositions

[0076] In order to investigate the effect of varying the ratio of amino acid to sugar concentrations in pharmaceutical compositions on the preparation, stability and storage of such compositions, a pharmaceutical composition containing an exemplary protein biomolecule (a human IgG1 monoclonal antibody) was incubated in formulations containing different amino acids and at differing amino acid to sugar ratios. More specifically, the pharmaceutical composition was formulated at 100 mg/mL in 25mM histidine/histidine-HCl, 0.02% (w/v) polysorbate-80 (PS-80), pH 6 buffer with arginine-HCl, lysine-HCl, proline, alanine or glycine at amino acid to sugar ratios as shown in Table 4 and the preparations were evaluated for their effect on reconstitution times of the lyophilized formulations.

Table 4 Composition of Amino Acid Formulations				
Description	Protein (mg/mL)	Amino Acid (mg/mL)	Sucrose (mg/mL)	Amino Acid : Sucrose Ratio
Amino Acid Formulation	100	50	0	5:0
		40	10 (1% (w/v))	4:1
		25	50 (5% (w/v))	2:1
Control	100	0	100 (10% (w/v))	N/A

[0077] The formulations were lyophilized according to the process in Table 5.

Table 5 Lyophilization Process	
Loading	20 °C
Freezing	-5 °C at 0.3 °C/min
Annealing	No Annealing
Freezing	-40 °C at 0.1 °C/min
Primary Drying	-35 °C at 0.1 °C/min
Secondary Drying	40 °C at 0.3 °C/min
Unloading	5 °C

[0078] **Figure 1** summarizes the aggregation and reconstitution time results. All formulations of the above-described pharmaceutical composition containing the exemplary protein biomolecule that contained lysine-HCl had high, and in some cases, unacceptably high reconstitution times. Formulations of the pharmaceutical composition that contained arginine, lysine-HCl or proline did not show an in-process increase in aggregation. In contrast, formulations of the pharmaceutical composition that contained alanine and glycine showed an increase in in-process aggregate level, but the addition of amorphous content (sucrose) minimized or prevented this increase, depending on the ratio employed. The results show that arginine, lysine and proline could substitute for sucrose without affecting aggregation.

[0079] The lyophilized formulations were also subjected to X-ray Powder Diffraction (XRPD) in order to determine the crystallinity of the lyophilisate. The results, as well as reconstitution times are shown in Table 6 (Reconstitution Time (RC) in minutes; n=2; XRPD, n=1; A, Amorphous; M, Mixture of Amorphous and Crystalline).

Table 6										
Impact of Amino Acid to Sucrose Ratio on Reconstitution Time and XRPD										
Amino Acid to Sugar Ratio	Arginine-HCl		Lysine-HCl		Alanine		Proline		Glycine	
	RC	XRPD	RC	XRPD	RC	XRPD	RC	XRPD	RC	XRPD
5:0	<9	(A)	<50	(A)	<5	(A)	<30	(A)	<5	M
4:1	<29	(A)	<50	(A)	<5	(A)	<30	(A)	<5	M
1:2	<29	(A)	<50	(A)	<24	(A)	<30	(A)	<24	(A)
Control	<27 (A)									

[0080] In summary, formulations of the pharmaceutical composition containing the exemplary protein biomolecule that contained arginine alone showed a significantly lower reconstitution time compared to the sucrose only formulation. The addition of even 1% (w/v) of sucrose to the arginine formulations increased the reconstitution time. All formulations with arginine were amorphous as measured by XRPD. Formulations of the pharmaceutical composition that contained alanine or glycine showed rapid reconstitution in the absence of sucrose, or in the presence of 1% (w/v) sucrose, but the addition of 5% (w/v) and higher sucrose concentrations increased reconstitution times. Formulations of the pharmaceutical composition that contained alanine or glycine and 0-1% (w/v) sucrose showed a mixture of amorphous and crystalline product by XRPD, while addition of high sucrose to these formulations resulted in an amorphous matrix as determined by XRPD. Formulations of the

pharmaceutical composition that contained lysine or proline were difficult to reconstitute and hence had longer reconstitution time. All the lysine- and proline-containing formulations were, however, amorphous as determined by XRPD. The results show that the presence of arginine, alanine or glycine could significantly reduce reconstitution time.

Example 3

Optimizing Sugar to Amino Acid Ratios in High Concentration Protein Formulations

[0081] The data presented in Example 2 indicates that both alanine and glycine have a tendency to crystallize when lyophilized alone or in the presence of low amounts of sugar. The following study was carried out to optimize ratios of sugar to amino acid (using alanine or glycine) to obtain amorphous lyophilized cakes with acceptable stability and short reconstitution times. Amino acid/sucrose formulations with various amino acid to sugar ratios were prepared for both alanine and glycine as shown in Table 7. The formulations were lyophilized according to the process shown in Table 5 with addition of annealing at -16 °C for 300 minutes. The lyophilisates were subjected to XRPD, and were then reconstituted. Reconstitution times, percent aggregate increase over pre lyophilization solutions, and osmolality were measured.

Table 7		
Amino Acid (mg/mL)	Sugar (mg/mL)	Amino Acid to Sugar Ratio
50	0	5:0
40	10	4:1
40	20	2:1
40	30	4:3
40	40	1:1
25	50	1:2

[0082] Table 8 summarizes the effect of amino acid to sugar ratios on reconstitution time, and increase in aggregate post-lyophilization. Results indicate that increasing sugar in the formulations prevents aggregate formation during the lyophilization process but increases reconstitution time. The results provide a guide to determine an acceptable balance between aggregation and reconstitution time, by adjusting the amino acid to sugar ratio.

Table 8			
Study Results			
Amino Acid	Amino Acid to Sucrose Ratio	% Aggregate Increase (Post-Lyophilization)	Reconstitution Time (n=2) in min
Alanine	5:0	0.8	4
	4:1	0.4	4
	2:1	0.1	18
	4:3	0.2	14
	1:1	0.2	18
	1:2	0.1	17
Glycine	5:0	1.5	5
	4:1	0.6	5
	2:1	0.1	13
	4:3	0.2	22
	1:1	0	24
	1:2	0.6	24

Example 4

Evaluation of High Concentration Protein/Amino Acid Formulations

[0083] As observed in Example 2, high concentration protein formulations with arginine-HCl remained amorphous during lyophilization, indicating that arginine-HCl can act as a cryoprotectant and as a lyoprotectant. Additionally, the arginine alone protein formulation exhibited a reduced reconstitution time. Because of these characteristics, arginine was evaluated in combination with alanine and/or glycine in a series of high concentration formulations of the above-described pharmaceutical composition containing the exemplary protein biomolecule. In this study, the impact of protein concentration and amino acid ratio on reconstitution time was evaluated. The formulations evaluated are shown with a check mark in Table 9 (N/A, not applicable). The formulations were lyophilized according to the process shown in Table 3. The lyophilisates were subjected to XRPD, and were then reconstituted. Reconstitution times were measured.

Table 9 Study Design						
Amino Acid (% (w/v))			Protein Concentration (mg/mL)			
Alanine	Glycine	Arginine	50	75	90	100
N/A		2	N/A			✓
		3				✓
		3.5	✓	N/A		✓
		5	✓	✓	✓	✓
2	N/A	2	✓	✓	✓	✓
4		2	✓	✓	✓	✓
N/A	2	2	✓	✓	✓	✓
	4	2	✓	✓	✓	✓

[0084] The reconstitution times of the various formulations are shown in Figure 2. These data show that protein concentration had an effect on the reconstitution time. As the protein concentration increased, reconstitution time increased. Also, the extent of increase in reconstitution time was affected by the type and quantity of the amino acid(s) present in the formulations. Both 3.5% (w/v) arginine and 5% (w/v) arginine had a similar impact on reconstitution time.

[0085] Formulations containing combinations of 4% glycine (w/v) or 4% alanine (w/v) with 2% arginine (w/v) exhibited reconstitution times reduced to about 10 minutes for the 100 mg lyophilized formulations (*i.e.*, approximately $\frac{1}{3}$ to $\frac{1}{2}$ the reconstitution time observed using other combinations of solutes). Also, the extent of reduction in reconstitution time was dependent on amino acid ratio. For example, 2:1 glycine:arginine or alanine:arginine was more effective in reducing reconstitution time than 1:1 glycine:arginine or alanine:arginine.

[0086] The XRPD results demonstrated that all of the formulations except for 2:1 glycine:arginine were amorphous (Table 10; A, Amorphous; M, Mixture of Amorphous and Crystalline; N/A, not applicable).

Table 10				
XRPD Results				
Amino Acid (% (w/v))			Nominal Protein Concentration (mg/mL)	
Alanine	Glycine	Arginine	50	100
N/A		2	N/A	A
		3.5	A	N/A
		5	A	N/A
2	N/A	2	A	A
4		2	A	A
N/A	2	2	A	A
	4	2	M	M

[0087] Based on the results in Table 10 and in Figure 2, the following lyophilized formulations of the pharmaceutical composition were evaluated for stability at 5 °C, 25 °C 60% relative humidity and 40 °C 75% relative humidity:

- (1) 75 mg/mL, 25 mM histidine/histidine-HCl, 3.5% arginine (w/v), and 0.02% PS-80 (w/v), pH 6;
- (2) 75 mg/mL, 25 mM histidine/histidine-HCl, 5% arginine (w/v), and 0.02% PS-80 (w/v), pH 6;
- (3) 100 mg/mL, 25 mM histidine/histidine-HCl, 4% alanine (w/v), 2% arginine (w/v), and 0.02% PS-80 (w/v), pH 6;
- (4) 100 mg/mL, 25 mM histidine/histidine-HCl, 4% glycine (w/v), 2% arginine (w/v), and 0.02% PS-80 (w/v), pH 6;

[0088] Prior to lyophilization, 1.1 mL of the above-described four formulations were subjected to uncontrolled 1x freeze/thaw (F/T) in 3 cc vials (freezing at -80 °C and thawing at room temperature). HPSEC was monitored pre- and post-thaw to study the impact of the freeze/thaw cycle. No significant change in purity was observed in the freeze/thaw cycle.

[0089] Figures 3A and 3B show the stability of the lyophilisates at 40 °C and 25 °C, respectively. The stability was monitored for 6 months at 25 °C and for 3 months at 40 °C. The rates of purity loss at both 25 °C and 40 °C were similar to sucrose containing formulations. Post 3 months at 40 °C, samples of the lyophilized pharmaceutical composition formulations were submitted to XRPD analysis. Based on XRPD analysis, all of the formulations except those containing glycine were amorphous. Glycine-containing formulations showed a mixture of amorphous and crystalline component, which is consistent with initial (T-0) observations. The stability was also evaluated at 5 °C for 22 months and showed no change in purity.

[0090] Ten vials of each formulation (post-lyophilization) were reconstituted and the reconstitution times were measured. The results are shown in Figure 4. The average reconstitution times of all formulations were less than 15 minutes. The 100 mg/mL formulation of the pharmaceutical composition containing the exemplary protein biomolecule containing a combination of glycine and arginine was the most effective in reducing the reconstitution time followed by the combination of alanine and arginine. For the 75 mg/mL formulation of the pharmaceutical composition containing the exemplary protein biomolecule, both the 3.5% and 5% arginine (w/v) formulations provided acceptable reconstitution times.

Example 5 **Evaluation of the Impact of Molecule Type on Reconstitution Time**

[0091] In order to understand the impact of molecule type on reconstitution time and to demonstrate the generality of the present invention with respect to any protein biomolecule, pharmaceutical compositions were prepared using alternative protein biomolecules. Specifically, pharmaceutical compositions were prepared employing a Tenascin-3-Human Serum Albumin (Tn3-HSA) fusion protein (see, *e.g.*, PCT Publication No. WO 2013/055745) or a humanized IgG4 monoclonal antibody in lieu of the human IgG1 monoclonal antibody of the above-described pharmaceutical compositions. The employed formulations were the four lead formulations noted in Example 4 (reiterated below):

- (1) 75 mg/mL, 25 mM histidine/histidine-HCl, 3.5% arginine, 0.02% PS-80, pH 6;
- (2) 75 mg/mL, 25 mM histidine/histidine-HCl, 5% arginine, 0.02% PS-80, pH 6;
- (3) 100 mg/mL, 25 mM histidine/histidine-HCl, 4% alanine, 2% arginine, 0.02% PS-80, pH 6;
- (4) 100 mg/mL, 25 mM histidine/histidine-HCl, 4% glycine, 2% arginine, 0.02% PS-80, pH 6;

[0092] The additional pharmaceutical compositions were formulated as indicated, and lyophilized according to the process in Table 11.

Table 11	
Lyophilization Process	
Loading	20 °C
Freezing	-5 °C at 0.3 °C/min
Annealing	-16 °C at 0.5 °C/min
Freezing	-40 °C at 0.5 °C/min
Primary Drying	-35 °C at 0.1 °C/min
Secondary Drying	40 °C at 0.3 °C/min
Unloading	5 °C

[0093] Lyophilisates samples are submitted for stability evaluations at various temperatures. Percent aggregation of the samples is evaluated by HPSEC. Following lyophilization, samples were reconstituted. Formulations were reconstituted using one of two alternative procedures (Procedure A was employed with the human IgG1 monoclonal antibody, and Procedure B was employed with the Tn3-HSA fusion protein and the humanized IgG4 monoclonal antibody). The reconstitution times for the IgG1 antibody are shown in Figure 5A. The reconstitution times for the Tn3-HSA fusion protein are shown in Figure 5B. The reconstitution time for the IgG4 antibody are shown in Figure 5C. The reconstitution times for the three molecules were significantly lower, compared to sucrose only formulations and were all 15 minutes or less.

[0094] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

What Is Claimed Is:

1. A pharmaceutical composition comprising a protein biomolecule as an active agent or component thereof, wherein said composition comprises:
 - (A)
 - (1) an aqueous carrier;
 - (2) a protein biomolecule;
 - (3) a buffer;
 - (4) a stabilizing compound selected from the group consisting of arginine, alanine, glycine, lysine or proline, or a derivative or salt thereof, or mixtures thereof, in a total concentration of from about 1% (w/v) to about 6% (w/v);
 - or
 - (B) a lyophilisate of (A).
2. The pharmaceutical composition of claim 1, wherein the composition substantially lacks a sugar stabilizing compound.
3. The pharmaceutical composition of any one of claims 1-2, wherein the composition comprises from about 10 mg/mL to about 200 mg/mL of a protein biomolecule.
4. The pharmaceutical composition of claim 3, wherein the composition comprises 50 mg/mL, 75 mg/mL, 100 mg/mL, 150 mg/mL or 200 mg/mL of a protein biomolecule.
5. The pharmaceutical composition of any one of claims 1-4, wherein said protein biomolecule is an antibody or an antibody-based immunotherapeutic, enzyme, or a hormone/factor.
6. The pharmaceutical composition of claim 5, wherein said protein biomolecule is an antibody or an antibody-based immunotherapeutic, and said antibody is selected from the antibodies of Table 1.
7. The pharmaceutical composition of claim 5, wherein said protein biomolecule is a hormone/factor, and said hormone/factor is selected from the hormone/factors of Table 2.

8. The pharmaceutical composition of any one of claims 1-8, wherein the composition comprises at least two protein biomolecules.
9. The pharmaceutical composition of any one of claims 1-9, wherein said stabilizing compound is arginine or a derivative or salt thereof.
10. The pharmaceutical composition of claim 10, wherein said arginine is present at a concentration from about 2.0% (w/v) to about 5.0% (w/v), preferably at a concentration of 2.0% (w/v), a concentration of 3.5% (w/v) or a concentration of 5.5% (w/v).
11. The pharmaceutical composition of any one of claims 1-9, wherein said stabilizing compound is alanine or a derivative or salt thereof.
12. The pharmaceutical composition of claim 12, wherein said alanine is present at a concentration from about 2.5% (w/v) to about 5.5% (w/v), preferably at a concentration of about 2.5% (w/v), about 3.5% (w/v), about 4.0% (w/v), or about 5.5% (w/v).
13. The pharmaceutical composition of claim 12 or 13, wherein arginine is additionally present at a concentration of about 1.25% (w/v), about 1.75% (w/v), about 2.0% (w/v) or about 2.75% (w/v).
14. The pharmaceutical composition of any one of claims 1-9, wherein said stabilizing compound is glycine or a derivative or salt thereof.
15. The pharmaceutical composition of claim 15, wherein said glycine is present at a concentration from about 2.5% (w/v) to about 5.5% (w/v), preferably at a concentration of about 2.5% (w/v), about 3.5% (w/v), about 4.0% (w/v) or about 5.5% (w/v).
16. The pharmaceutical composition of claim 15 or 16, wherein arginine is additionally present at a concentration of about 1.25% (w/v), about 1.75% (w/v), about 2.0% (w/v) or about 2.75% (w/v).
17. The pharmaceutical composition of any one of claims 1-17, wherein the composition comprises at least two stabilizing compounds.

18. The pharmaceutical composition of any one of claims 1-18, wherein the pH of said pharmaceutical composition is from about 3 to about 11, from about 4 to about 9, from about 5 to about 8, from about 5 to about 7.5, preferably 6.0 or 7.4.
19. The pharmaceutical composition of any one of claims 1-19, wherein said buffer is present in a range from about 5 mM to about 50 mM, about 20 mM to about 30 mM, or about 23 mM to about 27 mM, preferably wherein the buffer is present at 25mM.
20. The pharmaceutical composition of any one of claims 1-20, wherein said buffer comprises histidine, phosphate, acetate, citrate, succinate, Tris, or a combination thereof.
21. The pharmaceutical composition of claim 21, wherein said buffer is histidine / histidine-HCl.
22. The pharmaceutical composition of any one of claims 1-21, wherein said pharmaceutical composition additionally comprises a non-ionic detergent.
23. The pharmaceutical composition of claim 22, wherein said non-ionic detergent is polysorbate-80 (PS-80).
24. The pharmaceutical composition of claim 23, wherein said polysorbate-80 (PS-80) is present at a concentration of between 0.005 and 0.1% (w/v), preferably at a concentration of 0.02% (w/v).
25. The pharmaceutical composition of any one of claims 1-24, wherein said pharmaceutical composition is said lyophilisate.
26. The pharmaceutical composition of any one of claims 1-25, wherein the presence of said stabilizing compound(s) causes the reconstitution time of a lyophilisate of the pharmaceutical composition to be less than 20 minutes, less than 15 minutes, less than 10 minutes, less than 8 minutes, less than 5 minutes, or less than 2 minutes.

27. The pharmaceutical composition of any one of claims 1-25, wherein the presence of said stabilizing compound(s) enhances a stability characteristic of the pharmaceutical composition by more than 400%, by more than 200%, by more than 100%, by more than 50%, or by more than 10%, relative to such stability characteristic as observed in the complete absence of said amino acid stabilizing compound(s).
28. The pharmaceutical composition of any one of claims 1-25, wherein the presence of said stabilizing compound(s) enhances a stability characteristic of the pharmaceutical composition by more than 50%, by more than 20%, by more than 10%, by more than 5%, or by more than 1%, relative to such stability characteristic as observed in the complete absence of a sugar stabilizing compound.
29. The pharmaceutical composition of any one of claims 1-25, wherein the formulation comprises 75 mg/mL of a protein biomolecule, 25 mM histidine/histidine-HCl, 3.5% arginine, 0.02% PS-80 at a pH of 6.0.
30. The pharmaceutical composition of any one of claims 1-25, wherein the formulation comprises 75 mg/mL of a protein biomolecule, 25 mM histidine/histidine-HCl, 5% arginine, 0.02% PS-80 at a pH of 6.0.
31. The pharmaceutical composition of any one of claims 1-25, wherein the formulation comprises 100 mg/mL of a protein biomolecule, 25 mM histidine/histidine-HCl, 4% alanine, 2% arginine, 0.02% PS-80 at a pH of 6.0.
32. The pharmaceutical composition of any one of claims 1-25, wherein the formulation comprises 100 mg/mL of a protein biomolecule, 25 mM histidine/histidine-HCl, 4% glycine, 2% arginine, 0.02% PS-80 at a pH of 6.0.
33. An ampoule, vial, cartridge, syringe or sachette that contains the pharmaceutical composition of any one of claims 1-32.
34. A method of treating a disease or disorder by administering the pharmaceutical composition of any one of claims 1-32.
35. The pharmaceutical composition of any one of claims 1-32 for use in medicine.

36. Use of one or more amino acids, such as arginine, alanine, glycine, lysine or proline, as a replacement of one or more sugars in a pharmaceutical formulation to decrease reconstitution time.

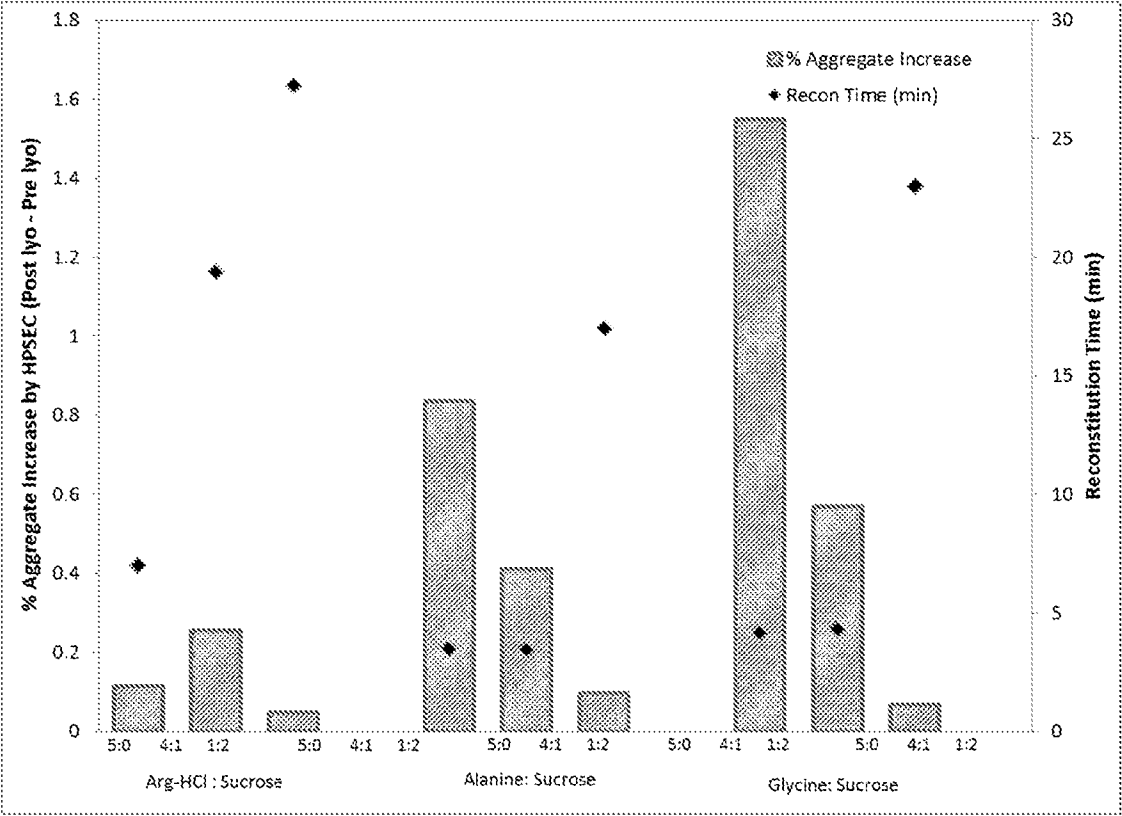


Figure 1

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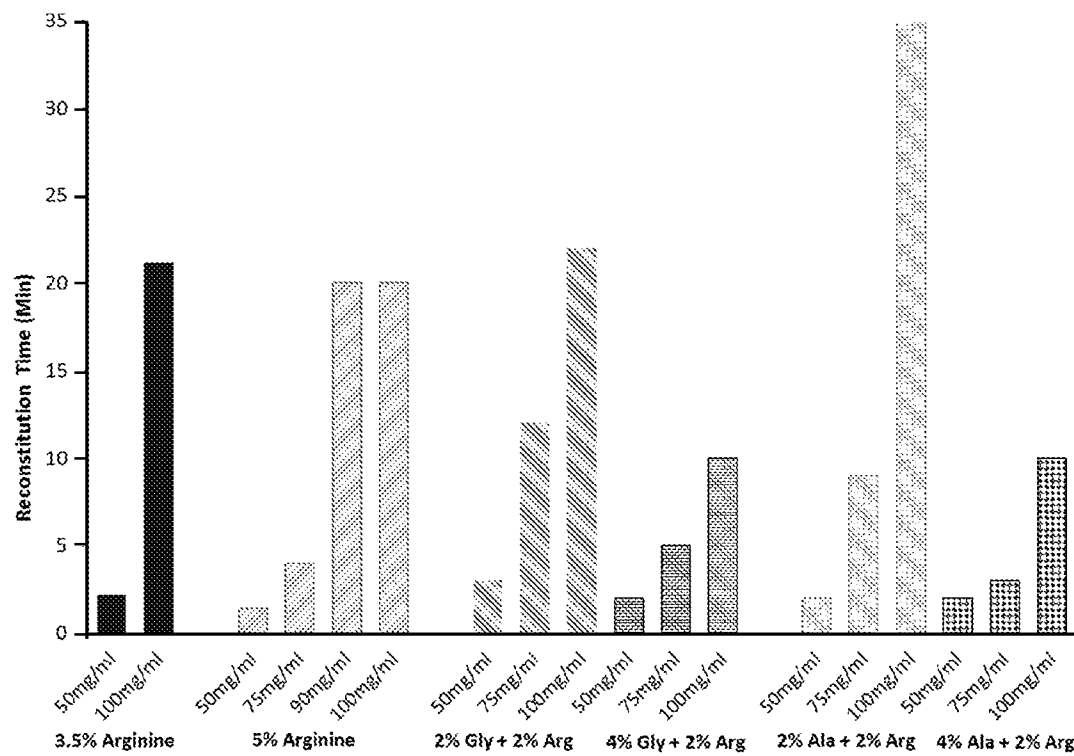


Figure 2

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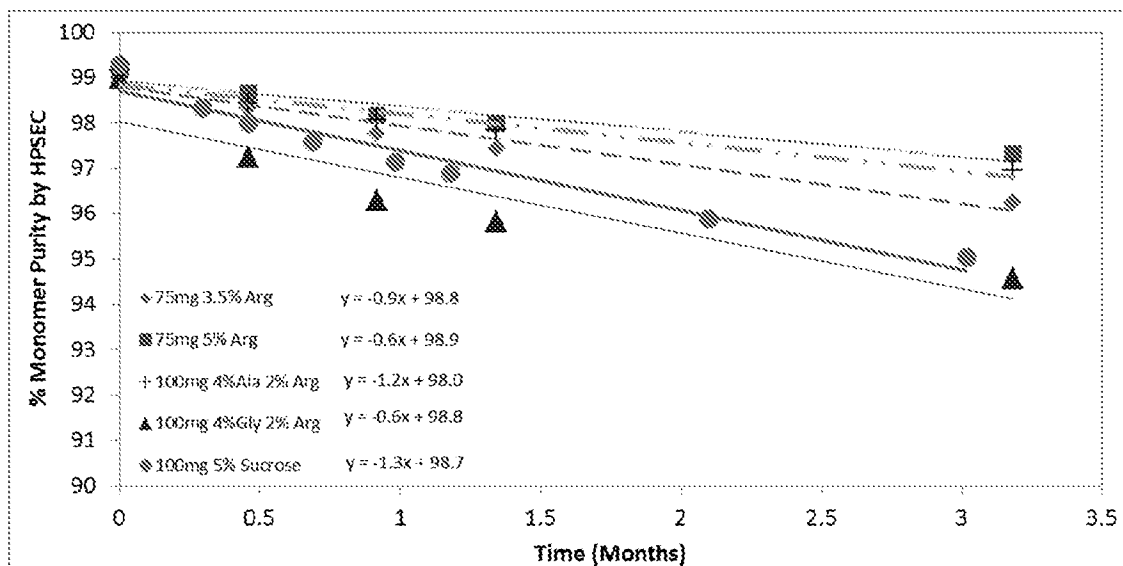


Figure 3A

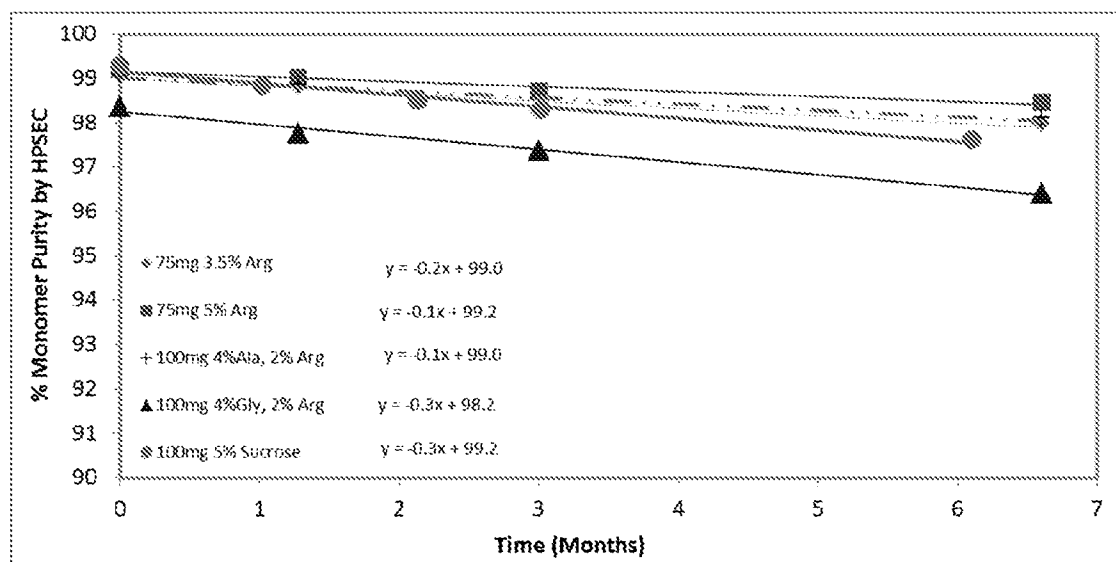


Figure 3B

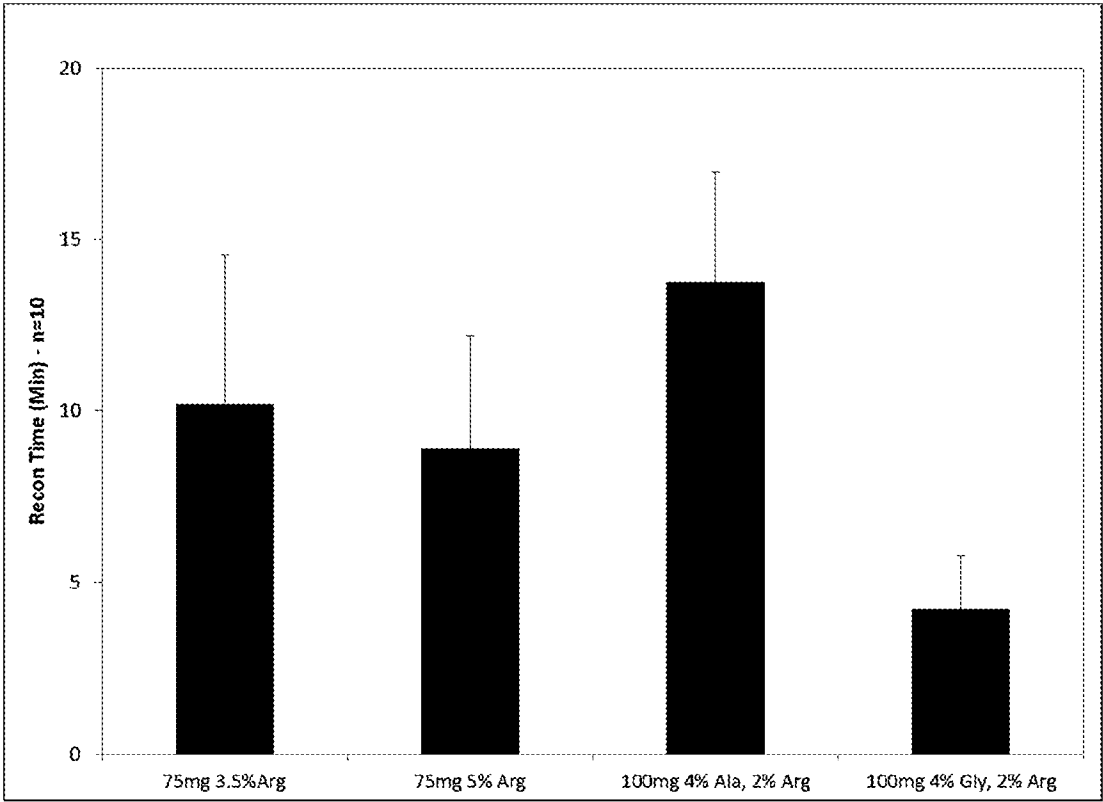


Figure 4

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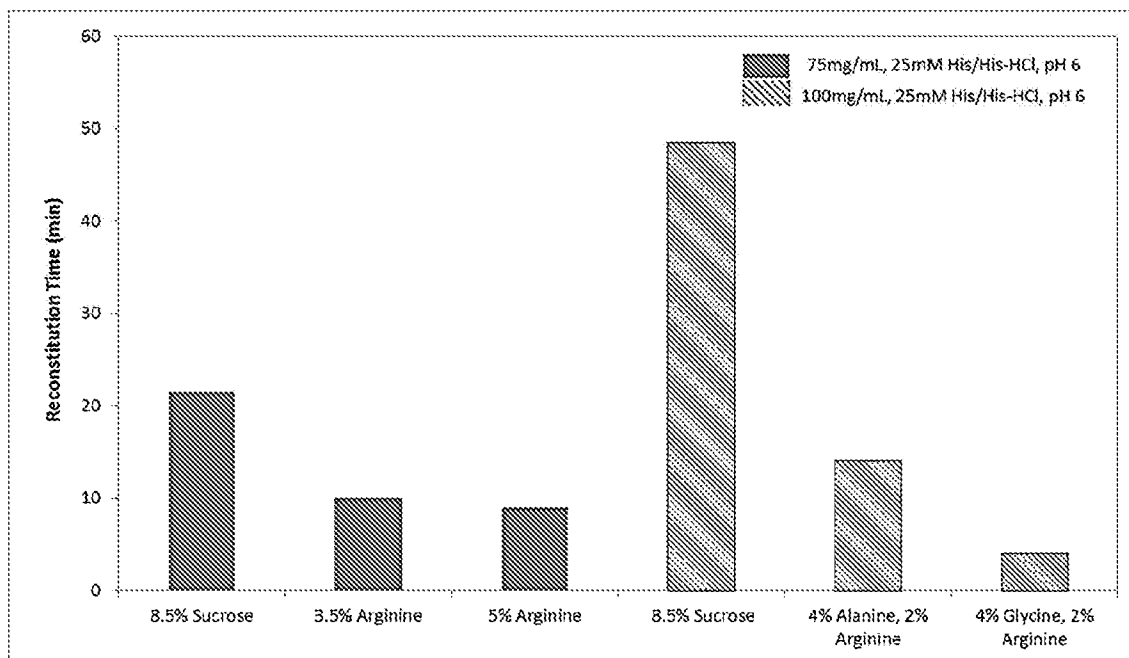


Figure 5A

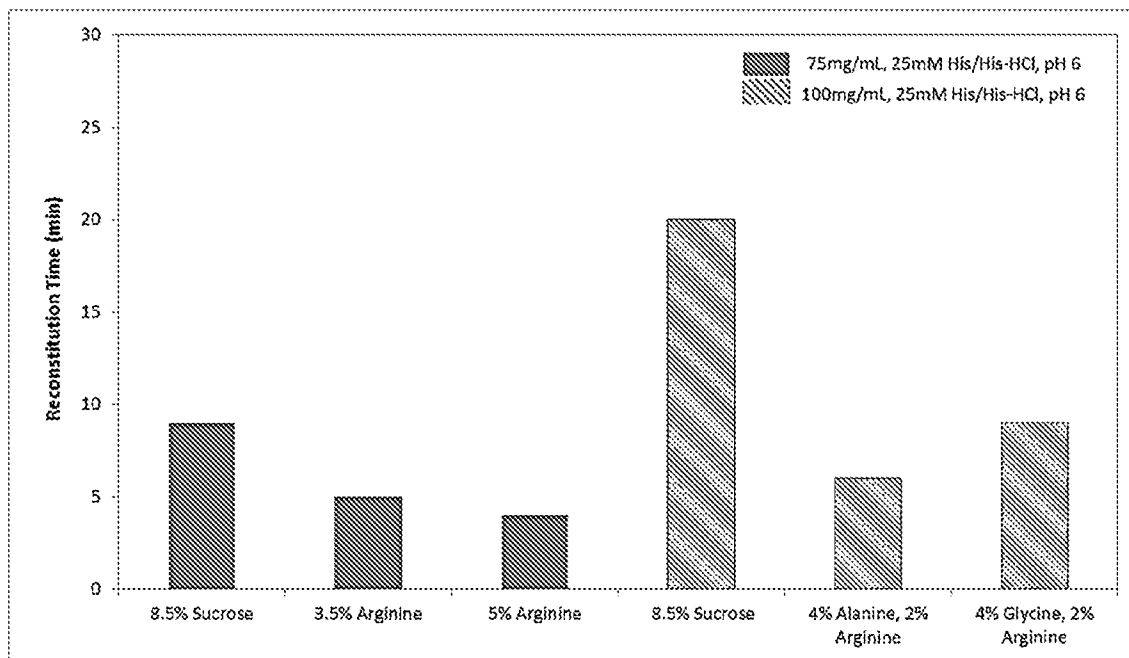


Figure 5B

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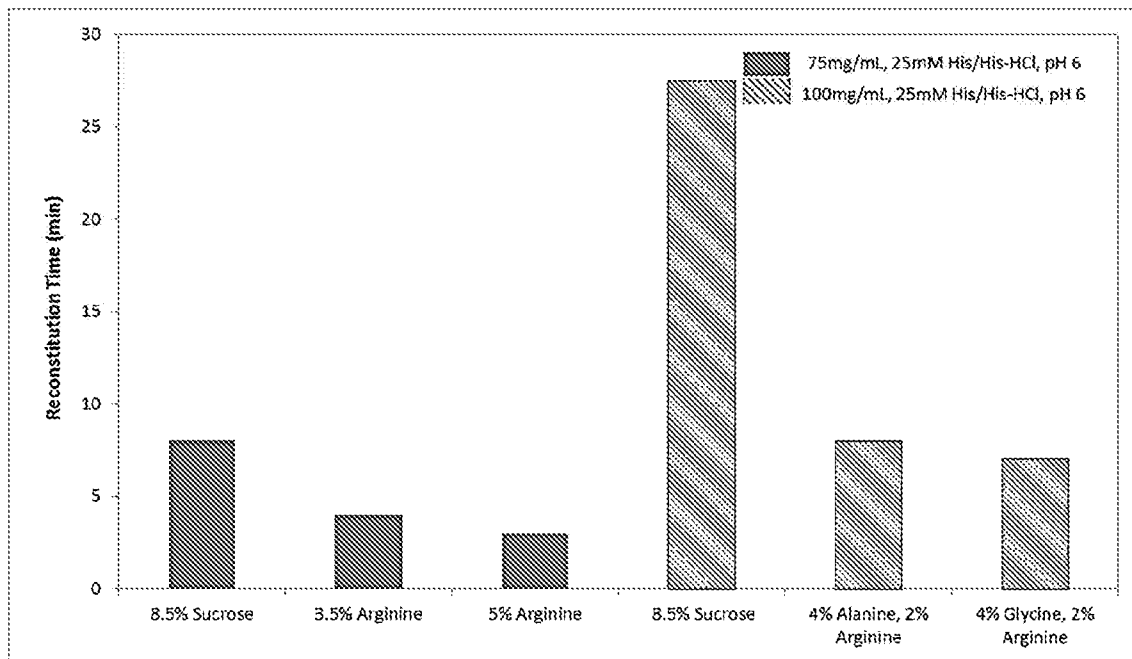


Figure 5C

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/26963

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - G01N 33/53 (2017.01)
 CPC - G01N 33/54393, G01N 33/569, A61L 2/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/0022792 A1 (Klinke et al.) 05 February 2004 (05.02.2004) para [0009], [0025], [0028], [0050]	1-4
X	US 2015/0284466 A1 (Morichika et al.) 08 October 2015 (08.10.2015) abstract, para [0009], [0085]	36
X	US 2011/0262992 A1 (Takagi et al.) 27 October 2011 (27.10.2011) para [0020], [0029]-[0030], [0040], [0049]-[0050]	1
X	US 2010/0273141 A1 (Bakaltcheva et al.) 28 October 2010 (28.10.2010) abstract	1
X	US 2013/0022625 A1 (Igawa et al.) 24 January 2013 (24.01.2013) abstract	1
X	US 2014/0186351 A1 (Britta et al.) 03 July 2014 (03.7.2014) abstract	1

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

20 June 2017

Date of mailing of the international search report

17 JUL 2017

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Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/26963

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 5-35
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.