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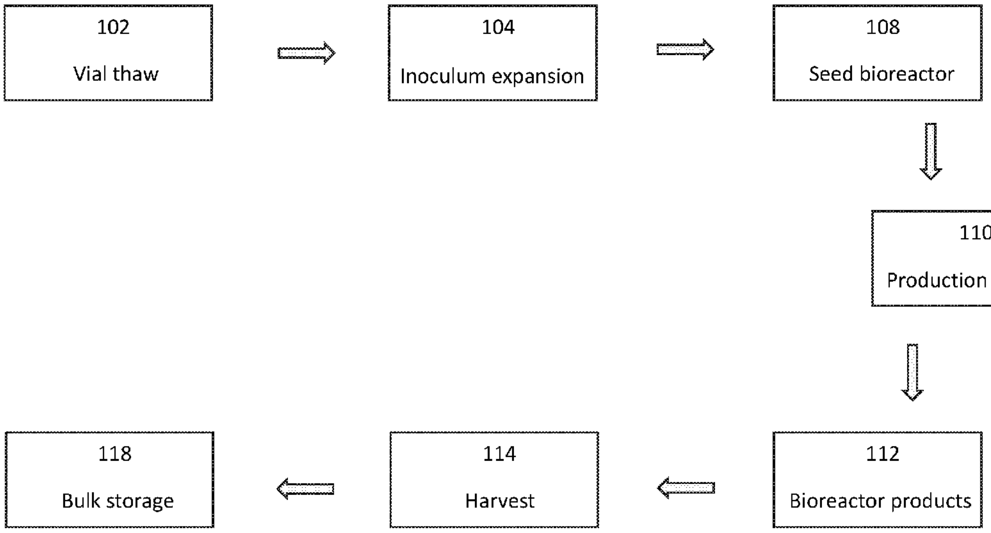

FIG. 1

(57) **Abstract:** Disclosed herein are methods for improving a parameter of a protein-related process comprising providing a viscosity-reducing excipient compound selected from the group consisting of hindered amines, aromatics and anionic aromatics, functionalized amino acids, oligopeptides, short-chain organic acids, low molecular weight aliphatic polyacids, diones and sulfones, zwitterionic excipients, and crowding agents with hydrogen bonding elements, and adding a viscosity-reducing amount of the viscosity-reducing excipient compound to a carrier solution for the protein-related process, wherein the carrier solution contains a protein of interest, and carrier solutions comprising a liquid medium in which is dissolved a protein of interest, and a viscosity-reducing excipient, wherein the viscosity of the carrier solution has a lower viscosity that that of a control solution that is substantially similar to the carrier solution except for the presence of the viscosity-reducing excipient.

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EXCIPIENT COMPOUNDS FOR PROTEIN PROCESSING

RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application 62/773,018 filed November 29, 2018. The entire contents of the above application are incorporated by reference herein.

FIELD OF APPLICATION

[0002] This application relates generally to formulations for delivering and processing biopolymers.

BACKGROUND

[0003] Biopolymers may be used for therapeutic or non-therapeutic purposes. Biopolymer-based therapeutics, such as antibody or enzyme formulations, are widely used in treating disease. Non-therapeutic biopolymers, such as enzymes, peptides, and structural proteins, have utility in non-therapeutic applications such as household, nutrition, commercial, and industrial uses.

[0004] Biopolymers used in therapeutic applications must be formulated to permit their introduction into the body for treatment of disease. For example, it is advantageous to deliver antibody and protein/peptide biopolymer formulations by subcutaneous (SC) or intramuscular (IM) routes under certain circumstances, instead of administering these formulations by intravenous (IV) injections. In order to achieve better patient compliance and comfort with SC or IM injection though, the liquid volume in the syringe is typically limited to 2 to 3 ccs and the viscosity of the formulation is typically lower than about 20 centipoise (cP) so that the formulation can be delivered using conventional medical devices and small-bore needles. These delivery parameters do not always fit well with the dosage requirements for the formulations being delivered.

[0005] Antibodies, for example, may need to be delivered at high dose levels to exert their intended therapeutic effect. Using a restricted liquid volume to deliver a high dose level of an antibody formulation can require a high concentration of the antibody in the delivery vehicle, sometimes exceeding a level of 150 mg/mL. At this dosage level, the viscosity-versus-concentration plots of protein solutions lie beyond their linear-nonlinear transition, such that the viscosity of the formulation rises dramatically with increasing concentration. Increased viscosity, however, is not compatible with standard SC or IM delivery systems. The solutions of biopolymer-based therapeutics are also prone to stability problems, such as precipitation, hazing, opalescence, denaturing, liquid-liquid phase separation, gel formation, and reversible or irreversible aggregation. The stability problems limit the shelf life of the solutions or require special handling.

[0006] One approach to producing protein formulations for injection is to transform the therapeutic protein solution into a powder that can be reconstituted to form a suspension suitable for SC or IM delivery. Lyophilization is a standard technique to produce protein powders. Freeze-drying, spray drying and even precipitation followed by super-critical-fluid extraction have been used to generate protein powders for subsequent reconstitution. Powdered suspensions are low in viscosity before re-dissolution (compared to solutions at the same overall dose) and thus may be suitable for SC or IM injection, provided the particles are sufficiently small to fit through the needle. However, protein crystals that are present in the powder have the inherent risk of triggering immune response. The uncertain protein stability/activity following re-dissolution poses further concerns. There remains a need in the art for techniques to produce low viscosity protein formulations for therapeutic purposes while avoiding the limitations introduced by protein powder suspensions.

[0007] In addition to the therapeutic applications of proteins described above, biopolymers such as enzymes, peptides, and structural proteins can be used in non-therapeutic applications. These non-therapeutic biopolymers can be produced from a number of different pathways, for example, derived from plant sources, animal sources, or produced from cell cultures.

[0008] The non-therapeutic proteins can be produced, transported, stored, and handled as a granular or powdered material or as a solution or dispersion, usually in water. The biopolymers for non-therapeutic applications can be globular or fibrous proteins, and certain forms of these materials can have limited solubility in water or exhibit high viscosity upon dissolution. These solution properties can present challenges to the formulation, handling, storage, pumping, and performance of the non-therapeutic materials, so there is a need for methods to reduce viscosity and improve solubility and stability of non-therapeutic protein solutions.

[0009] Proteins are complex biopolymers, each with a uniquely folded 3-D structure and surface energy map (hydrophobic/hydrophilic regions and charges). In concentrated protein solutions, these macromolecules may strongly interact and even inter-lock in complicated ways, depending on their exact shape and surface energy distribution. “Hot-spots” for strong specific interactions lead to protein clustering, increasing solution viscosity. To address these concerns, a number of excipient compounds are used in biotherapeutic formulations, aiming to reduce solution viscosity by impeding localized interactions and clustering. These efforts are individually tailored, often empirically, sometimes guided by *in silico* simulations. Combinations of excipient compounds may be provided, but optimizing such combinations again must progress empirically and on a case-by-case basis.

[0010] There remains a need in the art for a truly universal approach to reducing viscosity in protein formulations at a given concentration under nonlinear conditions. There is an additional need in the art to achieve this viscosity reduction while preserving the activity of the protein. It would be further desirable to adapt the viscosity-reduction system to use with formulations having tunable and sustained release profiles, and to use with formulations adapted for depot injection. In addition, it is desirable to improve processes for producing proteins and other biopolymers.

SUMMARY

[0011] Disclosed herein, in embodiments, are liquid formulations comprising a protein and an excipient compound selected from the group consisting of hindered amines, anionic aromatics, functionalized amino acids, oligopeptides, short-chain organic acids, low molecular weight aliphatic polyacids, diones and sulfones, zwitterionic excipients, and crowding agents with hydrogen bonding elements, wherein the excipient compound is added in a viscosity-reducing amount. In embodiments, the protein is a PEGylated protein and the excipient is a low molecular weight aliphatic polyacid. In embodiments, the formulation is a pharmaceutical composition, and the therapeutic formulation comprises a therapeutic protein, wherein the excipient compound is a pharmaceutically acceptable excipient compound. In embodiments, the formulation is a non-therapeutic formulation, and the non-therapeutic formulation comprises a non-therapeutic protein. In embodiments, the viscosity-reducing amount reduces viscosity of the formulation to a viscosity less than the viscosity of a control formulation. In embodiments, the viscosity of the formulation is at least about 10% less than the viscosity of the control formulation or is at least about 30% less than the viscosity of the control formulation, or is at least about 50% less than the viscosity of the control formulation, or is at least about 70% less than the viscosity of the control formulation, or is at least about 90% less than the viscosity of the control formulation. In embodiments, the viscosity is less than about 100 cP, or is less than about 50 cP, or is less than about 20 cP, or is less than about 10 cP. In embodiments, the excipient compound has a molecular weight of <5000 Da, or <1500 Da, or <500 Da. In embodiments, the formulation contains at least about 25 mg/mL of the protein, or at least about 100 mg/mL of the protein, or at least about 200 mg/mL of the protein, or at least about 300 mg/mL of the protein. In embodiments, the formulation comprises between about 5 mg/mL to about 300 mg/mL of the excipient compound or comprises between about 10 mg/mL to about 200 mg/mL of the excipient compound or comprises between about 20 mg/mL to about 100 mg/mL, or comprises between about 25 mg/mL to about 75 mg/mL of the excipient compound. In embodiments, the formulation has an improved stability when compared to the control formulation. In embodiments, the excipient compound is a hindered amine. In embodiments, the hindered amine is selected from the group consisting of caffeine, theophylline, tyramine, procaine, lidocaine, imidazole, aspartame, saccharin, and acesulfame potassium. In embodiments, the hindered amine is caffeine. In embodiments, the hindered amine is a local injectable anesthetic compound. The hindered amine can possess an independent pharmacological property, and the hindered amine can be present in the formulation in an amount that has an independent pharmacological effect. In embodiments the hindered amine can be present in the formulation in an amount that is less than a therapeutically effective amount. The independent pharmacological activity can be a local anesthetic activity. In embodiments, the hindered amine possessing the independent pharmacological activity is combined with a second excipient compound that further decreases the viscosity of the formulation. The second excipient compound can be selected from the group consisting of caffeine, theophylline, tyramine, procaine, lidocaine, imidazole, aspartame, saccharin, and acesulfame potassium. In embodiments, the formulation can comprise an additional agent selected from the group consisting of preservatives, surfactants, sugars, polysaccharides, arginine, proline, hyaluronidase, stabilizers, and buffers.

[0012] Further disclosed herein are methods of treating a disease or disorder in a mammal, comprising administering to said mammal a liquid therapeutic formulation, wherein the therapeutic formulation comprises a therapeutically effective amount of a therapeutic protein, and wherein the formulation further comprises an pharmaceutically acceptable excipient compound selected from the group consisting of hindered amines, anionic aromatics, functionalized amino acids, oligopeptides, short-chain organic acids, low molecular weight aliphatic polyacids, diones and sulfones, zwitterionic excipients, and crowding agents with hydrogen bonding elements; and wherein the therapeutic formulation is effective for the treatment of the disease or disorder. In embodiments, the therapeutic protein is a PEGylated protein, and the excipient compound is a low molecular weight aliphatic polyacid. In embodiments, the excipient is a hindered amine. In embodiments, the hindered amine is a local anesthetic compound. In embodiments, the formulation is administered by subcutaneous injection, or an intramuscular injection, or an intravenous injection. In embodiments, the excipient compound is present in the therapeutic formulation in a viscosity-reducing amount, and the viscosity-reducing amount reduces viscosity of the therapeutic formulation to a viscosity less than the viscosity of a control formulation. In embodiments, the therapeutic formulation has an improved stability when compared to the control formulation. In embodiments, the excipient compound is essentially pure.

[0013] Further disclosed herein are methods of reducing pain at an injection site of a therapeutic protein in a mammal in need thereof, comprising: administering a liquid therapeutic formulation by injection, wherein the therapeutic formulation comprises a therapeutically effective amount of the therapeutic protein, wherein the formulation further comprises an pharmaceutically acceptable excipient compound selected from the group consisting of local injectable anesthetic compounds, wherein the pharmaceutically acceptable excipient compound is added to the formulation in a viscosity-reducing amount; and wherein the mammal experiences less pain with administration of the therapeutic formulation comprising the excipient compound than that with administration of a control therapeutic formulation, wherein the control therapeutic formulation does not contain the excipient compound and is otherwise identical to the therapeutic formulation.

[0014] Disclosed herein, in embodiments, are methods of improving stability of a liquid protein formulation, comprising: preparing a liquid protein formulation comprising a therapeutic protein and an excipient compound selected from the group selected from the group consisting of hindered amines, anionic aromatics, functionalized amino acids, oligopeptides, short-chain organic acids, low molecular weight aliphatic polyacids, diones and sulfones, zwitterionic excipients, and crowding agents with hydrogen bonding elements, wherein the liquid protein formulation demonstrates improved stability compared to a control liquid protein formulation, wherein the control liquid protein formulation does not contain the excipient compound and is otherwise identical to the liquid protein formulation. The stability of the liquid formulation can be a cold storage conditions stability, a room temperature stability or an elevated temperature stability.

[0015] Also disclosed herein, in embodiments, are liquid formulations comprising a protein and an excipient compound selected from the group consisting of hindered amines, anionic aromatics, functionalized amino acids, oligopeptides, short-chain organic acids, low molecular weight aliphatic polyacids, diones and sulfones, zwitterionic excipients, and crowding agents with hydrogen bonding elements, wherein the presence of the excipient compound in the formulation results in improved protein-protein interaction characteristics as measured by the protein diffusion interaction parameter k_D, or the second virial coefficient B_{22}. In embodiments, the formulation is a therapeutic formulation, and comprises a therapeutic protein. In embodiments, the formulation is a non-therapeutic formulation, and comprises a non-therapeutic protein.

[0016] Further disclosed herein, in embodiments, are methods of improving a protein-related process comprising providing the liquid formulation described above, and employing it in a processing method. In embodiments, the processing method includes filtration, (e.g., tangential flow filtration, sterile filtration, microfiltration, ultrafiltration, diafiltration, centrifugal concentration, and in-line filtration), pumping, mixing, centrifugation, membrane separation, lyophilization, or chromatography. In embodiments, the processing method is selected from the group consisting of cell culture harvest, chromatography, such as Protein A chromatography, hydrophobic interaction chromatography, anion exchange chromatography and cation exchange chromatography, viral inactivation, and filtration. In embodiments, the processing method is a chromatography process or a filtration process. In embodiments, the filtration process is a virus filtration process or an ultrafiltration/diafiltration process.

[0017] Also disclosed herein are methods of improving a parameter of a protein-related process, comprising providing a viscosity-reducing excipient additive comprising at least one excipient compound selected from the group consisting of hindered amines, aromatics and anionic aromatics, functionalized amino acids, oligopeptides, short-chain organic acids, low molecular weight aliphatic polyacids, diones and sulfones, zwitterionic excipients, and crowding agents with hydrogen bonding elements, and adding a viscosity-reducing amount of the at least one excipient compound to a carrier solution for the protein-related process, wherein the carrier solution contains a protein of interest, thereby improving the parameter. In embodiments, the viscosity-reducing excipient additive in the liquid formulation comprises at least one excipient compound selected from the group consisting of pyrimidines, methyl-substituted pyrimidines, and phenethylamines. In embodiments, the parameter can be selected from the group consisting of cost of protein production, amount of protein production, rate of protein production, purity of protein produced, and efficiency of protein production. In embodiments, the parameter can be selected from the group consisting of cost of protein purification, amount of protein purification, rate of protein purification, purity of protein purified, and efficiency of protein purification. The parameter can be a proxy parameter, and the proxy parameter can be a reduced protein-protein interaction The reduced protein interaction can be determined by a technique selected from the group consisting of biolayer interferometry, surface plasmon resonance, intrinsic fluorescence measurement, extrinsic fluorescence measurement, dynamic light scattering, kD value, static light scattering, B22 value, isothermal titration calorimetry, and *in silico* simulation. In embodiments, the protein-related process is an upstream processing process. The carrier solution for the upstream processing process can be a cell culture medium. In embodiments, if the carrier solution is a cell culture medium, the step of adding the excipient additive to the carrier solution comprises a first substep of adding the excipient additive to a supplemental medium to form an excipient-containing supplemental medium, and a second substep of adding the excipient-containing supplemental medium to the cell culture medium. In other embodiments, the protein-related process is a downstream processing process. The downstream process can be a chromatography process, and the chromatography process can be a Protein-A chromatography process. In embodiments, the chromatography process recovers the protein of interest, wherein the protein of interest is characterized by an improved protein-related parameter selected from the group consisting of improved purity, improved yield, fewer particles, less misfolding, improved biological activity, increased percentage recovered in a monomeric form, or less aggregation, as compared to a control solution. In embodiments, the improved protein-related parameter is improved yield of the protein of interest from the chromatography process. In other embodiments, the protein-related process is a process selected from the group consisting of filtration, tangential flow filtration, sterile filtration, microfiltration, diafiltration, centrifugal concentration, in-line filtration, injection, syringing, pumping, mixing, centrifugation, membrane separation, and lyophilization, and the selected process can require less force than a process-specific control process, wherein the process-specific control process is the protein-related process performed in the absence of a viscosity-reducing excipient additive. In embodiments, the protein-related process is selected from the group consisting of a cell culture process, a cell culture harvesting process, a chromatography process, a viral inactivation process, and a filtration process. In embodiments, the protein-related process is the viral inactivation process, and the viral inactivation process is conducted at a pH level of about 2.5 to about 5.0, or the viral inactivation process is conducted at a higher pH than a viral-inactivation-specific control process, wherein the viral-inactivation-specific control process is a viral inactivation process performed in the absence of a viscosity-reducing excipient additive. In other embodiments, the protein-related process is the filtration process. The filtration process can be a virus removal filtration process, a sterile filtration process, or an ultrafiltration/diafiltration process. The filtration process can be characterized by an improved filtration-related parameter. The improved filtration-related parameter can be a faster filtration rate than the filtration rate of the control solution, wherein the control solution is a solution that does not contain a viscosity-reducing excipient additive. The improved filtration-related parameter can be a production of a smaller amount of aggregated protein than the amount of aggregated protein produced by a control filtration process, wherein the control filtration process is a filtration process performed in the absence of a viscosity-reducing excipient additive. The improved filtration-related parameter can be a higher mass transfer efficiency than the mass transfer efficiency of the control filtration process. The improved filtration-related parameter can be a higher concentration or a higher yield of the target protein than a concentration or yield of the target protein produced by the control filtration process.

[0018] Further disclosed herein are methods as described above, wherein the viscosity-reducing excipient additive comprises two or more excipient compounds. In embodiments, the at least one excipient compound is a hindered amine. In embodiments, the at least one excipient compound is a pyrimidine, a methyl-substituted pyrimidine, or a phenethylamine. In embodiments, the hindered amine is a pyrimidine compound. In other embodiments, the hindered amine is a phenethylamine compound, which can be a non-psychoactive phenethylamine. In embodiments, the at least one excipient compound is a crowding agent with hydrogen bonding elements, which can be selected from the group consisting of raffinose, inulin, pullulan, and sinistrins, or which can be raffinose. In embodiments, the at least one excipient compound is selected from the group consisting of caffeine, saccharin, acesulfame potassium, aspartame, theophylline, taurine, 1-methyl-2-pyrrolidone, 2-pyrrolidinone, niacinamide, and imidazole. In embodiments, the at least one excipient compound is selected from the group consisting of caffeine, taurine, niacinamide, and imidazole. In embodiments, the at least one excipient compound is selected from the group consisting of uracil, 1-methyluracil, 6-methyluracil, 5-methyluracil, 1,3-dimethyluracil, cytosine, 5-methylcytosine, 3-methylcytosine, thymine, 1-methylthymine, O-4-methylthymine, 1,3-dimethylthymine, and dimethylthymine dimer. In embodiments, the at least one excipient compound is selected from the group consisting of diphenhydramine, phenethylamine, N-methylphenethylamine, N,N-dimethylphenethylamine, beta-3-dihydroxyphenethylamine, beta-3-dihydroxy-N-methylphenethylamine, 3-hydroxyphenethylamine, 4-hydroxyphenethylamine, tyrosinol, tyramine, N-methyltyramine, and hordenine. In embodiments, the at least one excipient compound is selected from the group consisting of caffeine, nicotinamide, nicotinamide mononucleotide, diethylnicotinamide, taurine, imidazole, ornithine, iminodiacetic acid, nicotinic acid, and sulfanilic acid, or is selected from the group consisting of caffeine, nicotinamide, taurine, and imidazole, or is caffeine. In embodiments, the at least one excipient compound is selected from the group consisting of calcium propionate and potassium sorbate. In embodiments, the at least one excipient compound is an aromatic or anionic aromatic excipient, and, in some embodiments, the anionic aromatic excipient can be 4-hydroxybenzenesulfonic acid. In embodiments, the viscosity-reducing amount is between about 1 mg/mL to about 100 mg/mL of the at least one excipient compound, or the viscosity-reducing amount is between about 1 mM to about 400 mM of the at least one excipient compound, or the viscosity-reducing amount is between about 1 mM to about 1000 mM of the at least one excipient compound, or the viscosity-reducing amount is an amount from about 2 mM to about 150 mM. In embodiments, the carrier solution comprises an additional agent selected from the group consisting of preservatives, sugars, polyols, polysaccharides, arginine, proline, surfactants, stabilizers, and buffers. In embodiments, the protein of interest is a therapeutic protein, and the therapeutic protein can be a recombinant protein, or can be selected from the group consisting of a monoclonal antibody, a polyclonal antibody, an antibody fragment, a fusion protein, a PEGylated protein, an antibody-drug conjugate, a synthetic polypeptide, a protein fragment, a lipoprotein, an enzyme, and a structural peptide. In embodiments, the methods further comprise a step of adding a second viscosity-reducing excipient to the carrier solution, wherein the step of adding the second viscosity-reducing compound adds an additional improvement to the parameter.

[0019] In addition, carrier solutions are disclosed herein, comprising a liquid medium in which is dissolved a protein of interest, and a viscosity-reducing additive, wherein the carrier solution has a lower viscosity that that of a control solution. The carrier solution can further comprise an additional agent selected from the group consisting of preservatives, sugars, polyols, polysaccharides, arginine, proline, surfactants, stabilizers, and buffers.

BRIEF DESCRIPTION OF THE FIGURES

[0020] FIG. 1 presents a block diagram showing the steps in a fermentation process (an "upstream processing") for producing therapeutic proteins, for example monoclonal antibodies.

[0021] FIG. 2 presents a block diagram showing the steps in a purification process (a "downstream processing") for producing therapeutic proteins, for example monoclonal antibodies.

[0022] FIGs. 3A and 3B shows graphs of the amount of antibody in the retentate after timed intervals of centrifugation.

[0023] FIG. 4 shows a graph of estimated antibody concentration in the retentate after timed intervals of centrifugation.

DETAILED DESCRIPTION

[0024] Disclosed herein are formulations and methods for their production that permit the delivery of concentrated protein solutions. In embodiments, the approaches disclosed herein can yield a lower viscosity liquid formulation or a higher concentration of therapeutic or nontherapeutic proteins in the liquid formulation, as compared to traditional protein solutions. In embodiments, the approaches disclosed herein can yield a liquid formulation having improved stability when compared to a traditional protein solution. A stable formulation is one in which the protein contained therein substantially retains its physical and chemical stability and its therapeutic or nontherapeutic efficacy upon storage under storage conditions, whether cold storage conditions, room temperature conditions, or elevated temperature storage conditions. Advantageously, a stable formulation can also offer protection against aggregation or precipitation of the proteins dissolved therein. For example, the cold storage conditions can entail storage in a refrigerator or freezer. In some examples, cold storage conditions can entail storage at a temperature of 10° or less. In additional examples, the cold storage conditions entail storage at a temperature from about 2° to about 10°C. In other examples, the cold storage conditions entail storage at a temperature of about 4°C. In additional examples, the cold storage conditions entail storage at freezing temperature such as about -20°C or lower. In another example, cold storage conditions entail storage at a temperature of about -20°C to about 0°C. The room temperature storage conditions can entail storage at ambient temperatures, for example, from about 10°C to about 30°C. Elevated storage conditions can entail storage at a temperature greater. Elevated temperature stability, for example at temperatures from about 30°C to about 50°C, can be used as part an accelerated aging study to predict the long-term storage at typical ambient (10-30°C) conditions.

[0025] It is well known to those skilled in the art of polymer science and engineering that proteins in solution tend to form entanglements, which can limit the translational mobility of the entangled chains and interfere with the protein’s therapeutic or nontherapeutic efficacy. In embodiments, excipient compounds as disclosed herein can suppress protein clustering due to specific interactions between the excipient compound and the target protein in solution.

Excipient compounds as disclosed herein can be natural or synthetic, and desirably are substances that the FDA generally recognizes as safe ("GRAS").

1. Definitions

[0026] For the purpose of this disclosure, the term "protein" refers to a sequence of amino acids having a chain length long enough to produce a discrete tertiary structure, typically having a molecular weight between 1-3000 kDa. In some embodiments, the molecular weight of the protein is between about 50-200 kDa; in other embodiments, the molecular weight of the protein is between about 20-1000 kDa or between about 20-2000 kDa. In contrast to the term "protein," the term "peptide" refers to a sequence of amino acids that does not have a discrete tertiary structure. A wide variety of biopolymers are included within the scope of the term "protein." For example, the term "protein" can refer to therapeutic or non-therapeutic proteins, including antibodies, aptamers, fusion proteins, PEGylated proteins, synthetic polypeptides, protein fragments, lipoproteins, enzymes, structural peptides, and the like.

[0027] As non-limiting examples, therapeutic proteins can include mammalian proteins such as hormones and prohormones (e.g., insulin and proinsulin, glucagon, calcitonin, thyroid hormones (T3 or T4 or thyroid-stimulating hormone), parathyroid hormone, follicle-stimulating hormone, luteinizing hormone, growth hormone, growth hormone releasing factor, and the like); clotting and anti-clotting factors (e.g., tissue factor, von Willebrand's factor, Factor VIIIC, Factor IX, protein C, plasminogen activators (urokinase, tissue-type plasminogen activators), thrombin); cytokines, chemokines, and inflammatory mediators; interferons; colony-stimulating factors; interleukins (e.g., IL-1 through IL-10); growth factors (e.g., vascular endothelial growth factors, fibroblast growth factor, platelet-derived growth factor, transforming growth factor, neurotrophic growth factors, insulin-like growth factor, and the like); albumins; collagens and elastins; fibrin sealants; hematopoietic factors (e.g., erythropoietin, thrombopoietin, and the like); osteoinductive factors (e.g., bone morphogenetic protein); receptors (e.g., integrins, cadherins, and the like); surface membrane proteins; transport proteins; regulatory proteins; antigenic proteins (e.g., a viral component that acts as an antigen); and antibodies. The term "antibody" is used herein in its broadest sense, to include as non-limiting examples monoclonal antibodies (including, for example, full-length antibodies with an immunoglobulin Fc region), single-chain molecules, bi-specific and multi-specific antibodies, diabodies, antibody-drug conjugates, antibody compositions having polyepitopic specificity, polyclonal antibodies (such as polyclonal immunoglobulins used as therapies for immune-compromised patients), and fragments of antibodies (including, for example, Fc, Fab, Fv, and $F(ab')_2$). Antibodies can also be termed "immunoglobulins."

An antibody is understood to be directed against a specific protein or non-protein "antigen," which is a biologically important material; the administration of a therapeutically effective amount of an antibody to a patient can complex with the antigen, thereby altering its biological properties so that the patient experiences a therapeutic effect.

[0028] In embodiments, the proteins are PEGylated, meaning that they comprise poly(ethylene glycol) ("PEG") and/or poly(propylene glycol) ("PPG") units. PEGylated proteins, or PEG-protein conjugates, have found utility in therapeutic applications due to their beneficial properties such as solubility, pharmacokinetics, pharmacodynamics, immunogenicity, renal clearance, and stability. Non-limiting examples of PEGylated proteins are PEGylated interferons (PEG-IFN), PEGylated anti-VEGF, PEG protein conjugate drugs, Adagen, Pegaspargase, Pegfilgrastim, Pegloticase, Pegvisomant, PEGylated epoetin-β, and Certolizumab pegol.

[0029] PEGylated proteins can be synthesized by a variety of methods such as a reaction of protein with a PEG reagent having one or more reactive functional groups. The reactive functional groups on the PEG reagent can form a linkage with the protein at targeted protein sites such as lysine, histidine, cysteine, and the N-terminus. Typical PEGylation reagents have reactive functional groups such as aldehyde, maleimide, or succinimide groups that have specific reactivity with targeted amino acid residues on proteins. The PEGylation reagents can have a PEG chain length from about 1 to about 1000 PEG and/or PPG repeating units. Other methods of PEGylation include glyco-PEGylation, where the protein is first glycosylated and then the glycosylated residues are PEGylated in a second step. Certain PEGylation processes are assisted by enzymes like sialyltransferase and transglutaminase.

[0030] While the PEGylated proteins can offer therapeutic advantages over native, non-PEGylated proteins, these materials can have physical or chemical properties that make them difficult to purify, dissolve, filter, concentrate, and administer. The PEGylation of a protein can lead to a higher solution viscosity compared to the native protein, and this generally requires the formulation of PEGylated protein solutions at lower concentrations.

[0031] It is desirable to formulate protein therapeutics in stable, low viscosity solutions so they can be administered to patients in a minimal injection volume. For example, the subcutaneous (SC) or intramuscular (IM) injection of drugs generally requires a small injection volume, preferably less than 2 mL. The SC and IM injection routes are well suited to self-administered care, and this is a less costly and more accessible form of treatment compared with intravenous (IV) injection which is only conducted under direct medical supervision. Formulations for SC or IM injection require a low solution viscosity, generally below 30 cP, and preferably below 20 cP, to allow easy flow of the therapeutic solution through a narrow-gauge needle. This combination of small injection volume and low viscosity requirements present a challenge to the use of PEGylated protein therapeutics in SC or IM injection routes.

[0032] Those proteins having therapeutic effects may be termed “therapeutic proteins”; formulations containing therapeutic proteins in therapeutically effective amounts may be termed “therapeutic formulations.” The therapeutic protein contained in a therapeutic formulation may also be termed its “protein active ingredient.” Typically, a therapeutic formulation comprises a therapeutically effective amount of a protein active ingredient and an excipient, with or without other optional components. As used herein, the term “therapeutic” includes both treatments of existing disorders and preventions of disorders. Therapeutic proteins include, for example, proteins such as bevacizumab, trastuzumab, adalimumab, infliximab, etanercept, darbepoetin alfa, epoetin alfa, cetuximab, filgrastim, and rituximab. Other therapeutic proteins will be familiar to those having ordinary skill in the art.

[0033] A “treatment” includes any measure intended to cure, heal, alleviate, improve, remedy, or otherwise beneficially affect the disorder, including preventing or delaying the onset of symptoms and/or alleviating or ameliorating symptoms of the disorder. Those patients in need of a treatment include both those who already have a specific disorder, and those for whom the prevention of a disorder is desirable. A disorder is any condition that alters the homeostatic wellbeing of a mammal, including acute or chronic diseases, or pathological conditions that predispose the mammal to an acute or chronic disease. Non-limiting examples of disorders include cancers, metabolic disorders (e.g., diabetes), allergic disorders (e.g., asthma), dermatological disorders, cardiovascular disorders, respiratory disorders, hematological disorders, musculoskeletal disorders, inflammatory or rheumatological disorders, autoimmune disorders, gastrointestinal disorders, urological disorders, sexual and reproductive disorders, neurological disorders, and the like. The term “mammal” for the purposes of treatment can refer to any animal classified as a mammal, including humans, domestic animals, pet animals, farm animals, sporting animals, working animals, and the like. A “treatment” can therefore include both veterinary and human treatments. For convenience, the mammal undergoing such “treatment” can be referred to as a “patient.” In certain embodiments, the patient can be of any age, including fetal animals *in utero*.

[0034] In embodiments, a treatment involves providing a therapeutically effective amount of a therapeutic formulation to a mammal in need thereof. A “therapeutically effective amount" is at least the minimum concentration of the therapeutic protein administered to the mammal in need thereof, to effect a treatment of an existing disorder or a prevention of an anticipated disorder (either such treatment or such prevention being a "therapeutic intervention"). Therapeutically effective amounts of various therapeutic proteins that may be included as active ingredients in the therapeutic formulation may be familiar in the art; or, for therapeutic proteins discovered or applied to therapeutic interventions hereinafter, the therapeutically effective amount can be determined by standard techniques carried out by those having ordinary skill in the art, using no more than routine experimentation.

[0035] Those proteins used for non-therapeutic purposes (i.e., purposes not involving treatments), such as household, nutrition, commercial, and industrial applications, may be termed "non-therapeutic proteins." Formulations containing non-therapeutic proteins may be termed "non-therapeutic formulations". The non-therapeutic proteins can be derived from plant sources, animal sources, or produced from cell cultures; they also can be enzymes or structural proteins. The non-therapeutic proteins can be used in household, nutrition, commercial, and industrial applications such as catalysts, human and animal nutrition, processing aids, cleaners, and waste treatment.

[0036] An important category of non-therapeutic biopolymers is enzymes. Enzymes have a number of non-therapeutic applications, for example, as catalysts, human and animal nutritional ingredients, processing aids, cleaners, and waste treatment agents. Enzyme catalysts are used to accelerate a variety of chemical reactions. Examples of enzyme catalysts for non-therapeutic uses include catalases, oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. Human and animal nutritional uses of enzymes include nutraceuticals, nutritive sources of protein, chelation or controlled delivery of micronutrients, digestion aids, and supplements; these can be derived from amylase, protease, trypsin, lactase, and the like. Enzymatic processing aids are used to improve the production of food and beverage products in operations like baking, brewing, fermenting, juice processing, and winemaking. Examples of these food and beverage processing aids include amylases, cellulases, pectinases, glucanases, lipases, and lactases. Enzymes can also be used in the production of biofuels. Ethanol for biofuels, for example, can be aided by the enzymatic degradation of biomass feedstocks such as cellulosic and lignocellulosic materials. The treatment of such feedstock materials with cellulases and ligninases transforms the biomass into a substrate that can be fermented into fuels. In other commercial applications, enzymes are used as detergents, cleaners, and stain lifting aids for laundry, dish washing, surface cleaning, and equipment cleaning applications. Typical enzymes for this purpose include proteases, cellulases, amylases, and lipases. In addition, non-therapeutic enzymes are used in a variety of commercial and industrial processes such as textile softening with cellulases, leather processing, waste treatment, contaminated sediment treatment, water treatment, pulp bleaching, and pulp softening and debonding. Typical enzymes for these purposes are amylases, xylanases, cellulases, and ligninases.

[0037] Other examples of non-therapeutic biopolymers include fibrous or structural proteins such as keratins, collagen, gelatin, elastin, fibroin, actin, tubulin, or the hydrolyzed, degraded, or derivatized forms thereof. These materials are used in the preparation and formulation of food ingredients such as gelatin, ice cream, yogurt, and confections; they are also added to foods as thickeners, rheology modifiers, mouthfeel improvers, and as a source of nutritional protein. In the cosmetics and personal care industry, collagen, elastin, keratin, and hydrolyzed keratin are widely used as ingredients in skin care and hair care formulations. Still other examples of non-therapeutic biopolymers are whey proteins such as beta-lactoglobulin, alpha-lactalbumin, and serum albumin. These whey proteins are produced in mass scale as a byproduct from dairy operations and have been used for a variety of non-therapeutic applications.

2. Measurements

[0038] In embodiments, the protein-containing formulations described herein are resistant to monomer loss as measured by size exclusion chromatography (SEC) analysis. In SEC analysis as used herein, the main analyte peak is generally associated with the target protein contained in the formulation, and this main peak of the protein is referred to as the monomer peak. The monomer peak represents the amount of target protein, e.g., a protein active ingredient, in the monomeric state, as opposed to aggregated (dimeric, trimeric, oligomeric, etc.) or fragmented states. The monomer peak area can be compared with the total area of the monomer, aggregate, and fragment peaks associated with the target protein. Thus, the stability of a protein-containing formulation can be observed by the relative amount of monomer after an elapsed time; an improvement in stability of a protein-containing formulation of the invention can therefore be measured as a higher percent monomer after a certain elapsed time, as compared to the percent monomer in a control formulation that does not contain the excipient.

[0039] In embodiments, an ideal stability result is to have from 98 to 100% monomer peak as determined by SEC analysis. In embodiments, an improvement in stability of a protein-containing formulation of the invention can be measured as a higher percent monomer after exposure to a stress condition, as compared to the percent monomer in a control formulation that does not contain the excipient when such control formulation is exposed to the same stress condition. In embodiments, the stress conditions can be a low temperature storage, high temperature storage, exposure to air, exposure to gas bubbles, exposure to shear conditions, or exposure to freeze/thaw cycles.

[0040] In embodiments, the protein-containing formulations as described herein are resistant to an increase in protein particle size as measured by dynamic light scattering (DLS) analysis. In DLS analysis as used herein, the particle size of the protein in the protein-containing formulation can be observed as a median particle diameter. Ideally, the median particle diameter of the target protein should be relatively unchanged when subjected to DLS analysis, since the particle diameter represents the active component in the monomeric state, as opposed to aggregated (dimeric, trimeric, oligomeric, etc.) or fragmented states. An increase of the median particle diameter could represent an aggregated protein. Thus, the stability of a protein-containing formulation can be observed by the relative change in median particle diameter after an elapsed time.

[0041] In embodiments, the protein-containing formulations as described herein are resistant to forming a polydisperse particle size distribution as measured by dynamic light scattering (DLS) analysis. In embodiments, a protein-containing formulation can contain a monodisperse particle size distribution of colloidal protein particles. In embodiments, an ideal stability result is to have less than a 10% change in the median particle diameter compared to the initial median particle diameter of the formulation. In embodiments, an improvement in stability of a protein-containing formulation of the invention can be measured as a lower percent change of the median particle diameter after a certain elapsed time, as compared to the median particle diameter in a control formulation that does not contain the excipient. In embodiments, an improvement in stability of a protein-containing formulation of the invention can be measured as a lower percent change of the median particle diameter after exposure to a stress condition, as compared to the percent change of the median particle diameter in a control formulation that does not contain the excipient when such control formulation is exposed to the same stress condition. In embodiments, the stress conditions can be a low temperature storage, high temperature storage, exposure to air, exposure to gas bubbles, exposure to shear conditions, or exposure to freeze/thaw cycles. In embodiments, an improvement in stability of a protein-containing formulation therapeutic formulation of the invention can be measured as a less polydisperse particle size distribution as measured by DLS, as compared to the polydispersity of the particle size distribution in a control formulation that does not contain the excipient when such control formulation is exposed to the same stress condition.

[0042] In embodiments, the protein-containing formulations of the invention are resistant to precipitation as measured by turbidity, light scattering, and/or particle counting analysis. In turbidity, light scattering, or particle counting analysis, a lower value generally represents a lower number of suspended particles in a formulation. An increase of turbidity, light scattering, or particle counting can indicate that the solution of the target protein is not stable. Thus, the stability of a protein-containing formulation can be observed by the relative amount of turbidity, light scattering, or particle counting after an elapsed time. In embodiments, an ideal stability result is to have a low and relatively constant turbidity, light scattering, or particle counting value. In embodiments, an improvement in stability of a protein-containing formulation of the invention can be measured as a lower turbidity, lower light scattering, or lower particle count after a certain elapsed time, as compared to the turbidity, light scattering, or particle count values in a control formulation that does not contain the excipient. In embodiments, an improvement in stability of a protein-containing formulation as described herein can be measured as a lower turbidity, lower light scattering, or lower particle count after exposure to a stress condition, as compared to the turbidity, light scattering, or particle count in a control formulation that does not contain the excipient when such control formulation is exposed to the same stress condition. In embodiments, the stress conditions can be a low temperature storage, high temperature storage, exposure to air, exposure to gas bubbles, exposure to shear conditions, or exposure to freeze/thaw cycles.

3. Therapeutic Formulations

[0043] In one aspect, the formulations and methods disclosed herein provide stable liquid formulations of improved or reduced viscosity, comprising a therapeutic protein in a therapeutically effective amount and an excipient compound. In embodiments, the formulation can improve the stability while providing an acceptable concentration of active ingredients and an acceptable viscosity. In embodiments, the formulation provides an improvement in stability when compared to a control formulation; for the purposes of this disclosure, a control formulation is a formulation containing the protein active ingredient that is identical on a dry weight basis in every way to the therapeutic formulation except that it lacks the excipient compound. In embodiments, improved stability of the protein containing formulation is in the form of lower percentage of soluble aggregates, particulates, subvisible particles, or gel formation, compared to a control formulation.

[0044] It is understood that the viscosity of a liquid protein formulation can be affected by a variety of factors, including, but not limited to: the nature of the protein itself (e.g., enzyme, antibody, receptor, fusion protein, etc.); its size, three-dimensional structure, chemical composition, and molecular weight; its concentration in the formulation; the components of the formulation besides the protein; the desired pH range; the storage conditions for the formulation; and the method of administering the formulation to the patient. Therapeutic proteins most suitable for use with the excipient compounds described herein are preferably essentially pure, i.e., free from contaminating proteins. In embodiments, an "essentially pure" therapeutic protein is a protein composition comprising at least 90% by weight of the therapeutic protein, or preferably at least 95% by weight, or more preferably, at least 99% by weight, all based on the total weight of therapeutic proteins and contaminating proteins in the composition. For the purposes of clarity, a protein added as an excipient is not intended to be included in this definition. The therapeutic formulations described herein are intended for use as pharmaceutical-grade formulations, i.e., formulations intended for use in treating a mammal, in such a form that the desired therapeutic efficacy of the protein active ingredient can be achieved, and without containing components that are toxic to the mammal to whom the formulation is to be administered.

[0045] In embodiments, the therapeutic formulation contains at least 25 mg/mL of protein active ingredient. In other embodiments, the therapeutic formulation contains at least 100 mg/mL of protein active ingredient. In other embodiments, the therapeutic formulation contains at least 200 mg/mL of protein active ingredient. In yet other embodiments, the therapeutic formulation solution contains at least 300 mg/mL of protein active ingredient. Generally, the excipient compounds disclosed herein are added to the therapeutic formulation in an amount between about 5 to about 300 mg/mL. In embodiments, the excipient compound can be added in an amount of about 10 to about 200 mg/mL. In embodiments, the excipient compound can be added in an amount of about 20 to about 100 mg/mL. In embodiments, the excipient can be added in an amount of about 25 to about 75 mg/mL.

[0046] Excipient compounds of various molecular weights are selected for specific advantageous properties when combined with the protein active ingredient in a formulation. Examples of therapeutic formulations comprising excipient compounds are provided below. In embodiments, the excipient compound has a molecular weight of <5000 Da. In embodiments, the excipient compound has a molecular weight of <1000 Da. In embodiments, the excipient compound has a molecular weight of <500 Da.

[0047] In embodiments, the excipient compounds disclosed herein is added to the therapeutic formulation in a viscosity-reducing amount. In embodiments, a viscosity-reducing amount is the amount of an excipient compound that reduces the viscosity of the formulation at least 10% when compared to a control formulation; for the purposes of this disclosure, a control formulation is a formulation containing the protein active ingredient that is identical on a dry weight basis in every way to the therapeutic formulation except that it lacks the excipient compound. In embodiments, the viscosity-reducing amount is the amount of an excipient compound that reduces the viscosity of the formulation at least 30% when compared to the control formulation. In embodiments, the viscosity-reducing amount is the amount of an excipient compound that reduces the viscosity of the formulation at least 50% when compared to the control formulation. In embodiments, the viscosity-reducing amount is the amount of an excipient compound that reduces the viscosity of the formulation at least 70% when compared to the control formulation. In embodiments, the viscosity-reducing amount is the amount of an excipient compound that reduces the viscosity of the formulation at least 90% when compared to the control formulation.

[0048] In embodiments, the viscosity-reducing amount yields a therapeutic formulation having a viscosity of less than 100 cP. In other embodiments, the therapeutic formulation has a viscosity of less than 50 cP. In other embodiments, the therapeutic formulation has a viscosity of less than 20 cP. In yet other embodiments, the therapeutic formulation has a viscosity of less than 10 cP. The term “viscosity” as used herein refers to a dynamic viscosity value when measured by the methods disclosed herein.

[0049] Therapeutic formulations in accordance with this disclosure have certain advantageous properties. In embodiments, the therapeutic formulations are resistant to shear degradation, phase separation, clouding out, oxidation, deamidation, aggregation, precipitation, and denaturing. In embodiments, the therapeutic formulations are processed, purified, stored, syringed, dosed, filtered, and centrifuged more effectively, compared with a control formulation. In embodiments, the therapeutic formulations are administered to a patient at high concentration of therapeutic protein. In embodiments, the therapeutic formulations are administered to patients with less discomfort than would be experienced with a similar formulation lacking the therapeutic excipient. In embodiments, the therapeutic formulations are administered as a depot injection. In embodiments, the therapeutic formulations extend the half-life of the therapeutic protein in the body.

[0050] These features of therapeutic formulations as disclosed herein would permit the administration of such formulations by intramuscular or subcutaneous injection in a clinical situation, i.e., a situation where patient acceptance of an intramuscular injection would include the use of small-bore needles typical for IM/SC purposes and the use of a tolerable (for example, 2-3 cc) injected volume, and where these conditions result in the administration of an effective amount of the formulation in a single injection at a single injection site. By contrast, injection of a comparable dosage amount of the therapeutic protein using conventional formulation techniques would be limited by the higher viscosity of the conventional formulation, so that a SC/IM injection of the conventional formulation would not be suitable for a clinical situation. High concentration solutions of therapeutic proteins formulated with the excipient compounds described herein can be administered to patients using syringes or pre-filled syringes.

[0051] In embodiments, the therapeutic excipient has antioxidant properties that stabilize the therapeutic protein against oxidative damage. In embodiments, the therapeutic formulation is stored at ambient temperatures, or for extended time at refrigerator conditions without appreciable loss of potency for the therapeutic protein. In embodiments, the therapeutic formulation is dried down for storage until it is needed; then it is reconstituted with an appropriate solvent, e.g., water. Advantageously, the formulations prepared as described herein can be stable over a prolonged period of time, from months to years. When exceptionally long periods of storage are desired, the formulations can be preserved in a freezer (and later reactivated) without fear of protein denaturation. In embodiments, formulations can be prepared for long-term storage that do not require refrigeration.

[0052] Methods for preparing therapeutic formulations may be familiar to skilled artisans. The therapeutic formulations of the present invention can be prepared, for example, by adding the excipient compound to the formulation before or after the therapeutic protein is added to the solution. The therapeutic formulation can, for example, be produced by combining the therapeutic protein and the excipient at a first (lower) concentration and then processed by filtration or centrifugation to produce a second (higher) concentration of the therapeutic protein. Therapeutic formulations can be made with one or more of the excipient compounds with chaotropes, kosmotropes, hydrotropes, and salts. Therapeutic formulations can be made with one or more of the excipient compounds using techniques such as encapsulation, dispersion, liposome, vesicle formation, and the like. Methods for preparing therapeutic formulations comprising the excipient compounds disclosed herein can include combinations of the excipient compounds. In embodiments, combinations of excipients can produce benefits in lower viscosity, improved stability, or reduced injection site pain. Other additives may be introduced into the therapeutic formulations during their manufacture, including preservatives, surfactants, sugars, sucrose, trehalose, polysaccharides, arginine, proline, hyaluronidase, stabilizers, buffers, and the like. As used herein, a pharmaceutically acceptable excipient compound is one that is non-toxic and suitable for animal and/or human administration.

4. Non-Therapeutic Formulations

[0053] In one aspect, the formulations and methods disclosed herein provide stable liquid formulations of improved or reduced viscosity, comprising a non-therapeutic protein in an effective amount and an excipient compound. In embodiments, the formulation improves the stability while providing an acceptable concentration of active ingredients and an acceptable viscosity. In embodiments, the formulation provides an improvement in stability when compared to a control formulation; for the purposes of this disclosure, a control formulation is a formulation containing the protein active ingredient that is identical on a dry weight basis in every way to the non-therapeutic formulation except that it lacks the excipient compound.

[0054] It is understood that the viscosity of a liquid protein formulation can be affected by a variety of factors, including but not limited to: the nature of the protein itself (e.g., enzyme, structural protein, degree of hydrolysis, etc.); its size, three-dimensional structure, chemical composition, and molecular weight; its concentration in the formulation; the components of the formulation besides the protein; the desired pH range; and the storage conditions for the formulation.

[0055] In embodiments, the non-therapeutic formulation contains at least 25 mg/mL of protein active ingredient. In other embodiments, the non-therapeutic formulation contains at least 100 mg/mL of protein active ingredient. In other embodiments, the non-therapeutic formulation contains at least 200 mg/mL of protein active ingredient. In yet other embodiments, the non-therapeutic formulation solution contains at least 300 mg/mL of protein active ingredient. Generally, the excipient compounds disclosed herein are added to the non-therapeutic formulation in an amount between about 5 to about 300 mg/mL. In embodiments, the excipient compound is added in an amount of about 10 to about 200 mg/mL. In embodiments, the excipient compound is added in an amount of about 20 to about 100 mg/mL. In embodiments, the excipient is added in an amount of about 25 to about 75 mg/mL.

[0056] Excipient compounds of various molecular weights are selected for specific advantageous properties when combined with the protein active ingredient in a formulation. Examples of non-therapeutic formulations comprising excipient compounds are provided below. In embodiments, the excipient compound has a molecular weight of <5000 Da. In embodiments, the excipient compound has a molecular weight of <1000 Da. In embodiments, the excipient compound has a molecular weight of <500 Da.

[0057] In embodiments, the excipient compounds disclosed herein is added to the non-therapeutic formulation in a viscosity-reducing amount. In embodiments, a viscosity-reducing amount is the amount of an excipient compound that reduces the viscosity of the formulation at least 10% when compared to a control formulation; for the purposes of this disclosure, a control formulation is a formulation containing the protein active ingredient that is identical on a dry weight basis in every way to the therapeutic formulation except that it lacks the excipient compound. In embodiments, the viscosity-reducing amount is the amount of an excipient compound that reduces the viscosity of the formulation at least 30% when compared to the control formulation. In embodiments, the viscosity-reducing amount is the amount of an excipient compound that reduces the viscosity of the formulation at least 50% when compared to the control formulation. In embodiments, the viscosity-reducing amount is the amount of an excipient compound that reduces the viscosity of the formulation at least 70% when compared to the control formulation. In embodiments, the viscosity-reducing amount is the amount of an excipient compound that reduces the viscosity of the formulation at least 90% when compared to the control formulation.

[0058] In embodiments, the viscosity-reducing amount yields a non-therapeutic formulation having a viscosity of less than 100 cP. In other embodiments, the non-therapeutic formulation has a viscosity of less than 50 cP. In other embodiments, the non-therapeutic formulation has a viscosity of less than 20 cP. In yet other embodiments, the non-therapeutic formulation has a viscosity of less than 10 cP. The term “viscosity” as used herein refers to a dynamic viscosity value.

[0059] Non-therapeutic formulations in accordance with this disclosure can have certain advantageous properties. In embodiments, the non-therapeutic formulations are resistant to shear degradation, phase separation, clouding out, oxidation, deamidation, aggregation, precipitation, and denaturing. In embodiments, the therapeutic formulations can be processed, purified, stored, pumped, filtered, and centrifuged more effectively, compared with a control formulation.

[0060] In embodiments, the non-therapeutic excipient has antioxidant properties that stabilize the non-therapeutic protein against oxidative damage. In embodiments, the non-therapeutic formulation is stored at ambient temperatures, or for extended time at refrigerator conditions without appreciable loss of potency for the non-therapeutic protein. In embodiments, the non-therapeutic formulation is dried down for storage until it is needed;

then it can be reconstituted with an appropriate solvent, e.g., water. Advantageously, the formulations prepared as described herein is stable over a prolonged period of time, from months to years. When exceptionally long periods of storage are desired, the formulations are preserved in a freezer (and later reactivated) without fear of protein denaturation. In embodiments, formulations are prepared for long-term storage that do not require refrigeration.

[0061] Methods for preparing non-therapeutic formulations comprising the excipient compounds disclosed herein may be familiar to skilled artisans. For example, the excipient compound can be added to the formulation before or after the non-therapeutic protein is added to the solution. The non-therapeutic formulation can be produced at a first (lower) concentration and then processed by filtration or centrifugation to produce a second (higher) concentration. Non-therapeutic formulations can be made with one or more of the excipient compounds with chaotropes, kosmotropes, hydrotropes, and salts. Non-therapeutic formulations can be made with one or more of the excipient compounds using techniques such as encapsulation, dispersion, liposome, vesicle formation, and the like. Other additives can be introduced into the non-therapeutic formulations during their manufacture, including preservatives, surfactants, stabilizers, and the like.

5. Excipient Compounds

[0062] Several excipient compounds are described herein, each suitable for use with one or more therapeutic or non-therapeutic proteins, and each allowing the formulation to be composed so that it contains the protein(s) at a high concentration. Some of the categories of excipient compounds described below are: (1) hindered amines; (2) anionic aromatics; (3) functionalized amino acids; (4) oligopeptides; (5) short-chain organic acids; (6) low-molecular-weight aliphatic polyacids; (7) diones and sulfones, (8) zwitterionic excipients, and (9) crowding agents with hydrogen bonding elements. Without being bound by theory, the excipient compounds described herein are thought to associate with certain fragments, sequences, structures, or sections of a therapeutic protein that otherwise would be involved in inter-particle (i.e., protein-protein) interactions. The association of these excipient compounds with the therapeutic or non-therapeutic protein can mask the inter-protein interactions such that the proteins can be formulated in high concentration without causing excessive solution viscosity. Excipient compounds advantageously can be water-soluble, therefore suitable for use with aqueous vehicles. In embodiments, the excipient compounds have a water solubility of >10 mg/mL. In embodiments, the excipient compounds have a water solubility of >100 mg/mL. In embodiments, the excipient compounds have a water solubility of >500 mg/mL. Advantageously for therapeutic proteins, the excipient compounds can be derived from materials that are biologically acceptable and are non-immunogenic, and are thus suitable for pharmaceutical use. In therapeutic embodiments, the excipient compounds can be metabolized in the body to yield biologically compatible and non-immunogenic byproducts.

a. Excipient Compound Category 1: Hindered Amines

[0063] High concentration solutions of therapeutic or non-therapeutic proteins can be formulated with hindered amine small molecules as excipient compounds. As used herein, the term "hindered amine" refers to a small molecule containing at least one bulky or sterically hindered group, consistent with the examples below. Hindered amines can be used in the free base form, in the protonated form, or a combination of the two. In protonated forms, the hindered amines can be associated with an anionic counterion such as chloride, hydroxide, bromide, iodide, fluoride, acetate, formate, phosphate, sulfate, or carboxylate. Hindered amine compounds useful as excipient compounds can contain secondary amine, tertiary amine, quaternary ammonium, pyridinium, pyrrolidone, pyrrolidine, piperidine, morpholine, or guanidinium groups, such that the excipient compound has a cationic charge in aqueous solution at neutral pH. The hindered amine compounds also contain at least one bulky or sterically hindered group, such as cyclic aromatic, cycloaliphatic, cyclohexyl, or alkyl groups. In embodiments, the sterically hindered group can itself be an amine group such as a dialkylamine, trialkylamine, guanidinium, pyridinium, or quaternary ammonium group. Without being bound by theory, the hindered amine compounds are thought to associate with aromatic sections of the proteins such as phenylalanine, tryptophan, and tyrosine, by a cation pi interaction. In embodiments, the cationic group of the hindered amine can have an affinity for the electron rich pi structure of the aromatic amino acid residues in the protein, so that they can shield these sections of the protein, thereby decreasing the tendency of such shielded proteins to associate and agglomerate.

[0064] In embodiments, the hindered amine excipient compounds has a chemical structure comprising imidazole, imidazoline, or imidazolidine groups, or salts thereof, such as imidazole, 1-methylimidazole, 4-methylimidazole, 1-hexyl-3-methylimidazolium chloride, 1-ethylimidazole, 4-ethylimidazole, 1-hexyl-3-ethylimidazolium chloride, imidazoline, 2-imidazoline, imidazolidone, 2-imidazolidone, histamine, 4-methylhistamine, alpha-methylhistamine, betahistine, beta-alanine, 2-methyl-2-imidazoline, 1-butyl-3-methylimidazolium chloride, butyl imidazole, uric acid, potassium urate, betazole, carnosine, spermine, spermidine, aspartame, saccharin, acesulfame potassium, xanthine, theophylline, theobromine, caffeine, and anserine. In embodiments, the hindered amine excipient compound is a pyrimidine derivative selected from the group consisting of 1,3-dimethyluracil, 1-methyluracil, 3-methyluracil, 1,3-diethyluracil, 6-methyluracil, uracil, 1,3-dimethyl-tetrahydro pyrimidinone, 1-methyl-2-pyrridinone, phenylserine, DL-3-phenylserine, cycloserine, dicyclomine, thymine, 1-methylthymine, O-4-methylthymine, 1,3-dimethylthymine, dimethylthymine dimer, cytosine, cysteamine, 5-methylcytosine, and 3-methylcytosine. In embodiments, the hindered amine excipient compounds is selected from the group consisting of dimethylethanolamine, ethanolamine, dimethylaminoethanol, dimethylaminopropylamine, triethanolamine, 1,3-diaminopropane, 1,2-diaminopropane, polyetheramines, Jeffamine® brand polyetheramines, polyether-monoamines, polyether-diamines, polyether-triamines, 1-(1-adamantyl)ethylamine, hordenine, benzylamine, dimethylbenzylamine, dimethylcyclohexylamine, diethylcyclohexylamine, dicyclohexylmethylamine, hexamethylene biguanide, poly(hexamethylene biguanide), imidazole, dimethylglycine, meglumine, agmatine, agmatine sulfate, diazabicyclo[2.2.2]octane, tetramethylethylenediamine, N,N-dimethylethanolamine, ethanolamine phosphate, glucosamine, choline chloride, phosphocholine, niacinamide, isonicotinamide, N,N-diethyl nicotinamide, nicotinic acid, nicotinic acid sodium salt, isonicotinic acid, tyramine, N-methyltyramine, 3-aminopyridine, 4-aminopyridine, 2,4,6-trimethylpyridine, 3-pyridine methanol, dipyridamole, nicotinamide adenosine dinucleotide, biotin, folic acid, folinic acid, folinic acid calcium salt, morpholine, N-methylpyrrolidone, 2-pyrrolidinone, procaine, lidocaine, dicyandiamide-taurine adduct, 2-pyridylethylamine, dicyandiamide-benzyl amine adduct, dicyandiamide-alkylamine adduct, dicyandiamide-cycloalkylamine adduct, and dicyandiamide-aminomethanephosphonic acid adducts. In embodiments, a hindered amine compound consistent with this disclosure is formulated as a protonated ammonium salt. In embodiments, a hindered amine compound consistent with this disclosure is formulated as a salt with an inorganic anion or organic anion as the counterion. In embodiments, high concentration solutions of therapeutic or non-therapeutic proteins are formulated with a combination of caffeine with a benzoic acid, a hydroxybenzoic acid, or a benzenesulfonic acid as excipient compounds. In embodiments, the hindered amine excipient compounds are metabolized in the body to yield biologically compatible byproducts. In some embodiments, the hindered amine excipient compound is present in the formulation at a concentration of about 250 mg/mL or less. In additional embodiments, the hindered amine excipient compound is present in the formulation at a concentration of about

10 mg/mL to about 200 mg/mL. In yet additional aspects, the hindered amine excipient compound is present in the formulation at a concentration of about 20 to about 120 mg/mL.

[0065] In embodiments, certain hindered amine excipient compounds can possess other pharmacological properties. As examples, xanthines are a category of hindered amines having independent pharmacological properties, including stimulant properties and bronchodilator properties when systemically absorbed. Representative xanthines include caffeine, aminophylline, 3-isobutyl-1-methylxanthine, paraxanthine, pentoxifylline, theobromine, theophylline, and the like. Methylated xanthines are understood to affect force of cardiac contraction, heart rate, and bronchodilation. In some embodiments, the xanthine excipient compound is present in the formulation at a concentration of about 30 mg/mL or less.

[0066] Another category of hindered amines having independent pharmacological properties are the local injectable anesthetic compounds. Local injectable anesthetic compounds are hindered amines that have a three-component molecular structure of (a) a lipophilic aromatic ring, (b) an intermediate ester or amide linkage, and (c) a secondary or tertiary amine. This category of hindered amines is understood to interrupt neural conduction by inhibiting the influx of sodium ions, thereby inducing local anesthesia. The lipophilic aromatic ring for a local anesthetic compound may be formed of carbon atoms (e.g., a benzene ring) or it may comprise heteroatoms (e.g., a thiophene ring). Representative local injectable anesthetic compounds include, but are not limited to, amylocaine, articaine, bupivicaine, butacaine, butanilicaine, chlorprocaine, cocaine, cyclomethycaine, dimethocaine, editocaine, hexylcaine, isobucaine, levobupivacaine, lidocaine, metabutethamine, metabutoxycaine, mepivacaine, meprylcaine, propoxycaine, prilocaine, procaine, piperocaine, tetracaine, trimecaine, and the like. The local injectable anesthetic compounds can have multiple benefits in protein therapeutic formulations, such as reduced viscosity, improved stability, and reduced pain upon injection. In some embodiments, the local anesthetic compound is present in the formulation in a concentration of about 50 mg/mL or less.

[0067] In embodiments, a hindered amine having independent pharmacological properties is used as an excipient compound in accordance with the formulations and methods described herein. In some embodiments, the excipient compounds possessing independent pharmacological properties are present in an amount that does not have a pharmacological effect and/or that is not therapeutically effective. In other embodiments, the excipient compounds possessing independent pharmacological properties are present in an amount that does have a pharmacological effect and/or that is therapeutically effective. In certain embodiments, a hindered amine having independent pharmacological properties is used in combination with another excipient compound that has been selected to decrease formulation viscosity, where the hindered amine having independent pharmacological properties is used to impart the benefits of its pharmacological activity. For example, a local injectable anesthetic compound can be used to decrease formulation viscosity and also to reduce pain upon injection of the formulation. The reduction of injection pain can be caused by anesthetic properties; also, a lower injection force can be required when the viscosity is reduced by the excipients. Alternatively, a local injectable anesthetic compound can be used to impart the desirable pharmacological benefit of decreased local sensation during formulation injection, while being combined with another excipient compound that reduces the viscosity of the formulation.

b. Excipient Compound Category 2: Anionic Aromatics

[0068] High concentration solutions of therapeutic or non-therapeutic proteins can be formulated with anionic aromatic small molecule compounds as excipient compounds. The anionic aromatic excipient compounds can contain an aromatic functional group such as phenyl, benzyl, aryl, alkylbenzyl, hydroxybenzyl, phenolic, hydroxyaryl, heteroaromatic group, or a fused aromatic group. The anionic aromatic excipient compounds also can contain an anionic functional group such as carboxylate, oxide, phenoxide, sulfonate, sulfate, phosphonate, phosphate, or sulfide. While the anionic aromatic excipients might be described as an acid, a sodium salt, or other, it is understood that the excipient can be used in a variety of salt forms. Without being bound by theory, an anionic aromatic excipient compound is thought to be a bulky, sterically hindered molecule that can associate with cationic segments of a protein, so that they can shield these sections of the protein, thereby decreasing the interactions between protein molecules that render the protein-containing formulation viscous.

[0069] In embodiments, examples of anionic aromatic excipient compounds include compounds such as salicylic acid, aminosalicylic acid, hydroxybenzoic acid, aminobenzoic acid, para-aminobenzoic acid, benzenesulfonic acid, hydroxybenzenesulfonic acid, naphthalenesulfonic acid, naphthalenedisulfonic acid, hydroquinone sulfonic acid, sulfanilic acid, vanillic acid, vanillin, vanillin-taurine adduct, aminophenol, anthranilic acid, cinnamic acid, coumaric acid, caffeic acid, isonicotinic acid, folic acid, folinic acid, folinic acid calcium salt, phenylserine, DL-3-phenylserine, adenosine monophosphate, indole acetic acid, potassium urate, furan dicarboxylic acid, furan-2-acrylic acid, 2-furanpropionic acid, sodium phenylpyruvate, sodium hydroxyphenylpyruvate, dihydroxybenzoic acid, trihydroxybenzoic acid, pyrogallol, benzoic acid, and the salts of the foregoing acids. In embodiments, the anionic aromatic excipient compounds are formulated in the ionized salt form. In embodiments, an anionic aromatic compound is formulated as the salt of a hindered amine, such as dimethylcyclohexylammonium hydroxybenzoate. In embodiments, the anionic aromatic excipient compounds are formulated with various counterions such as organic cations. In embodiments, high concentration solutions of therapeutic or non-therapeutic proteins is formulated with anionic aromatic excipient compounds and caffeine. In embodiments, the anionic aromatic excipient compounds are metabolized in the body to yield biologically compatible byproducts.

c. Excipient Compound Category 3: Functionalized Amino Acids

[0070] High concentration solutions of therapeutic or non-therapeutic proteins can be formulated with one or more functionalized amino acids, where a single functionalized amino acid or an oligopeptide comprising one or more functionalized amino acids may be used as the excipient compound. In embodiments, the functionalized amino acid compounds comprise molecules (“amino acid precursors”) that can be hydrolyzed or metabolized to yield amino acids. In embodiments, the functionalized amino acids can contain an aromatic functional group such as phenyl, benzyl, aryl, alkylbenzyl, hydroxybenzyl, hydroxyaryl, heteroaromatic group, or a fused aromatic group. In embodiments, the functionalized amino acid compounds can contain esterified amino acids, such as methyl, ethyl, propyl, butyl, benzyl, cycloalkyl, glyceryl, hydroxyethyl, hydroxypropyl, PEG, and PPG esters. In embodiments, the functionalized amino acid compounds are selected from the group consisting of arginine ethyl ester, arginine methyl ester, arginine hydroxyethyl ester, and arginine hydroxypropyl ester. In embodiments, the functionalized amino acid compound is a charged ionic compound in aqueous solution at neutral pH. For example, a single amino acid can be derivatized by forming an ester, like an acetate or a benzoate, and the hydrolysis products would be acetic acid or benzoic acid, both natural materials, plus the amino acid. In embodiments, the functionalized amino acid excipient compounds are metabolized in the body to yield biologically compatible byproducts.

d. Excipient Compound Category 4: Oligopeptides

[0071] High concentration solutions of therapeutic or non-therapeutic proteins can be formulated with oligopeptides as excipient compounds. In embodiments, the oligopeptide is designed such that the structure has a charged section and a bulky section. In embodiments, the oligopeptides consist of between 2 and 10 peptide subunits. The oligopeptide can be bifunctional, for example a cationic amino acid coupled to a non-polar one, or an anionic one coupled to a non-polar one. In embodiments, the oligopeptides consist of between 2 and 5 peptide subunits. In embodiments, the oligopeptides are homopeptides such as polyglutamic acid, polyaspartic acid, poly-lysine, poly-arginine, and poly-histidine. In embodiments, the oligopeptides have a net cationic charge. In other embodiments, the oligopeptides are heteropeptides, such as Trp2Lys3. In embodiments, the oligopeptide can have an alternating structure such as an ABA repeating pattern. In embodiments, the oligopeptide can contain both anionic and cationic amino acids, for example, Arg-Glu. Without being bound by theory, the oligopeptides comprise structures that can associate with proteins in such a way that it reduces the intermolecular interactions that lead to high viscosity solutions; for example, the oligopeptide-protein association can be a charge-charge interaction, leaving a somewhat non-polar amino acid to disrupt hydrogen bonding of the hydration layer around the protein, thus lowering viscosity. In some embodiments, the oligopeptide excipient is present in the composition in a concentration of about 50 mg/mL or less.

e. Excipient Compound Category 5: Short-Chain Organic Acids

[0072] As used herein, the term “short-chain organic acids” refers to C_2-C_6 organic acid compounds and the salts, esters, or lactones thereof. This category includes saturated and unsaturated carboxylic acids, hydroxy functionalized carboxylic acids, and linear, branched, or cyclic carboxylic acids. In embodiments, the acid group in the short-chain organic acid is a carboxylic acid, sulfonic acid, phosphonic acid, or a salt thereof.

[0073] In addition to the four excipient categories above, high concentration solutions of therapeutic or non-therapeutic proteins can be formulated with short-chain organic acids, for example, the acid or salt forms of sorbic acid, valeric acid, propionic acid, glucuronic acid, caproic acid, and ascorbic acid as excipient compounds. Examples of excipient compounds in this category include potassium sorbate, taurine, sodium propionate, calcium propionate, magnesium propionate, and sodium ascorbate.

f. Excipient Compound Category 6: Low Molecular Weight Aliphatic Polyacids

[0074] High concentration solutions of therapeutic or non-therapeutic PEGylated proteins can be formulated with certain excipient compounds that enable lower solution viscosity, where such excipient compounds are low molecular weight aliphatic polyacids. As used herein, the term “low molecular weight aliphatic polyacids” refers to organic aliphatic polyacids having a molecular weight < about 1500, and having at least two acidic groups, where an acidic group is understood to be a proton-donating moiety. The acidic groups can be in the protonated acid form, the salt form, or a combination thereof. Non-limiting examples of acidic groups include carboxylate, phosphonate, phosphate, sulfonate, sulfate, nitrate, and nitrite groups. Acidic groups on the low molecular weight aliphatic polyacid can be in the anionic salt form such as carboxylate, phosphonate, phosphate, sulfonate, sulfate, nitrate, and nitrite; their counterions can be sodium, potassium, lithium, and ammonium. Specific examples of low molecular weight aliphatic polyacids useful for interacting with PEGylated proteins as described herein include maleic acid, tartaric acid, glutaric acid, malonic acid, itaconic acid, citric acid, ethylenediaminetetraacetic acid (EDTA), aspartic acid, glutamic acid, alendronic acid, etidronic acid and salts thereof. Further examples of low molecular weight aliphatic polyacids in their anionic salt form include phosphate (PO_4^{3-}), hydrogen phosphate (HPO_4^{3-}), dihydrogen phosphate ($H_2PO_4^-$), sulfate (SO_4^{2-}), bisulfate (HSO_4^-), pyrophosphate ($P_2O_7^{4-}$), hexametaphosphate, carbonate (CO_3^{2-}), and bicarbonate (HCO_3^-). The counterion for the anionic salts can be Na, Li, K, or ammonium ion. These excipients can also be used in combination with excipients. As used herein, the low molecular weight aliphatic polyacid can also be an alpha hydroxy acid, where there is a hydroxyl group adjacent to a first acidic group, for example glycolic acid, lactic acid, and gluconic acid and salts thereof. In embodiments, the low molecular weight aliphatic polyacid is an oligomeric form that bears more than two acidic groups, for example polyacrylic acid, polyphosphates, polypeptides and salts thereof. In some embodiments, the low molecular weight aliphatic polyacid excipient is present in the composition in a concentration of about 50 mg/mL or less.

g. Excipient Compound Category 7: Diones and Sulfones

[0075] An effective viscosity-reducing excipient can be a molecule containing a sulfone, sulfonamide, or dione functional group that is soluble in pure water to at least 1 g/L at 298K and having a net neutral charge at pH 7. Preferably, the molecule has a molecular weight of less than 1000 g/mol and more preferably less than 500 g/mol. The diones and sulfones effective in reducing viscosity have multiple double bonds, are water soluble, have no net charge at pH 7, and are not strong hydrogen bonding donors. Not to be bound by theory, the double bond character can allow for weak pi-stacking interactions with protein. In embodiments, at high protein concentrations and in proteins that only develop high viscosity at high concentration, charged excipients are not effective because electrostatic interaction is a longer-range interaction. Solvated protein surfaces are predominantly hydrophilic, making them water soluble. The hydrophobic regions of proteins are generally shielded within the 3-dimensional structure, but the structure is constantly evolving, unfolding, and re-folding (sometimes called “breathing”) and the hydrophobic regions of adjacent proteins can come into contact with each other, leading to aggregation by hydrophobic interactions. The pi-stacking feature of dione and sulfone excipients can mask hydrophobic patches that may be exposed during such "breathing." Another other important role of the excipient can be to disrupt hydrophobic interactions and hydrogen bonding between proteins in close proximity, which will effectively reduce solution viscosity. Dione and sulfone compounds that fit this description include dimethylsulfone, ethyl methyl sulfone, ethyl methyl sulfonyl acetate, ethyl isopropyl sulfone, bis(methylsulfonyl)methane, methane sulfonamide, methionine sulfone, sodium bisulfite, menadione sodium bisulfite, 1,2-cyclopentanedione, 1,3-cyclopentanedione, 1,4-cyclopentanedione, and butane-2,3-dione.

h. Excipient Compound Category 8: Zwitterionic Excipients

[0076] Solutions of therapeutic or non-therapeutic proteins can be formulated with certain zwitterionic compounds as excipients to improve stability or reduce viscosity. As used herein, the term "zwitterionic" refers to a compound that has a cationic charged section and an anionic charged section. In embodiments, the zwitterionic excipient compounds are amine oxides. In embodiments, the opposing charges are separated from each other by 2-8 chemical bonds. In embodiments, the zwitterionic excipient compounds can be small molecules, such as those with a molecular weight of about 50 to about 500 g/mol, or can be medium molecular weight molecules, such as those with a molecular weight of about 500 to about 2000 g/mol, or can be high molecular weight molecules, such as polymers having a molecular weight of about 2000 to about 100,000 g/mol.

[0077] Examples of the zwitterionic excipient compounds include (3-carboxypropyl) trimethylammonium chloride, 1-aminocyclohexane carboxylic acid, homocycloleucine, 1-methyl-4-imidazoleacetic acid, 3-(1-pyridinio)-1-propanesulfonate, 4-aminobenzoic acid, alendronate, aminoethyl sulfonic acid, aminohippuric acid, aspartame, aminotris (methylenephosphonic acid) (ATMP), calcobutrol, calteridol, cocamidopropyl betaine, cocamidopropyl hydroxysultaine, creatine, cytidine, cytidine monophosphate, diaminopimelic acid, diethylenetriaminepentaacetic acid, dimethyl phenylalanine, methylglycine, sarcosine, dimethylglycine, zwitterionic dipeptides (e.g., Arg-Glu, Lys-Glu, His-Glu, Arg-Asp, Lys-Asp, His-Asp, Glu-Arg, Glu-Lys, Glu-His, Asp-Arg, Asp-Lys, Asp-His), diethylenetriamine penta(methylene phosphonic acid) (DTPMP), dipalmitoyl phosphatidylcholine, ectoine, ethylenediamine tetra(methylenephosphonic acid) (EDTMP), folate benzoate mixture, folate niacinamide mixture, gelatin, hydroxyproline, iminodiacetic acid, isoguvacine, lecithin, myristamine oxide, nicotinamide adenine dinucleotide (NAD), aspartic acid, N-methyl aspartic acid, N-methylproline, lysine, N-trimethyl lysine, ornithine, oxolinic acid, risendronate, allyl cysteine, S-allyl-L-cysteine, somapacitan, taurine, theanine, trigonelline, vigabatrin, ectoine, 4-(2-hydroxyethyl)-1-piperazineethanesulfonate, o-octylphosphoryl choline, nicotinamide mononucleotide, triglycine, tetraglycine, β-guanidinopropionic acid, 5-aminolevulinic acid hydrochloride, picolinic acid, lidofenin, phosphocholine, 1-(5-carboxypentyl)-4-methylpyridin-1-ium bromide, L-anserine nitrate, L-glutathione reduced, N-ethyl-L-glutamine, N-methyl proline, (*Z*)-1-[N-(2-aminoethyl)-N-(2-ammonioethyl) amino]diazen-1-ium-1,2-diolate (DETA-NONOate), (*Z*)-1-[N-(3-aminopropyl)-N-(3-ammoniopropyl)amino]diazen-1-ium-1,2-diolate (DPTA-NONate), and zoledronic acid.

[0078] Not to be bound by theory, the zwitterionic excipient compounds can exert viscosity reducing or stabilizing effects by interacting with the protein, for example, by charge interactions, hydrophobic interactions, and steric interactions, causing the proteins to be more resistant to aggregation, or by affecting the bulk properties of the water in the protein formulation, such as an electrolyte contribution, a surface tension reduction, a change in the amount of unbound water available, or a change in dielectric constant.

i. Excipient Compound Category 9: Crowding Agents with Hydrogen Bonding Elements

[0079] Solutions of therapeutic or non-therapeutic proteins can be formulated with crowding agents with hydrogen bonding elements as excipients to improve stability or reduce viscosity. As used herein, the term "crowding agent" refers to a formulation additive that reduces the amount of water available for dissolving a protein in solution, increasing the effective protein concentration. In embodiments, crowding agents can decrease protein particle size or reduce the amount of protein unfolding in solution. In embodiments, the crowding agents can act as solvent modifiers that cause structuring of the water by hydrogen bonding and hydration effects. In embodiments, the crowding agents can reduce the amount of intermolecular interactions between proteins in solution. In embodiments, the crowding agents have a structure containing at least one hydrogen bond donor element such as hydrogen attached to an oxygen, sulfur, or nitrogen atom. In embodiments, the crowding agents have a structure containing at least one weakly acidic hydrogen bond donor element having a pKa of about 6 to about 11. In embodiments, the crowding agents have a structure containing between about 2 and about 50 hydrogen bond donor elements. In embodiments, the crowding agents have a structure containing at least one hydrogen bond acceptor element such as a Lewis base. In embodiments, the crowding agents have a structure containing between about 2 and about 50 hydrogen bond acceptor elements. In embodiments, the crowding agents have a molecular weight between about 50 and 500 g/mol. In embodiments, the crowding agents have a molecular weight between about 100 and 350 g/mol. In other embodiments, the crowding agents can have a molecular weight above 500 g/mol, such as raffinose, inulin, pullulan, or sinistrins.

[0080] Examples of the crowding agent excipients with hydrogen bonding elements include 1,3-Dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidione, 15-crown-5, 18-crown-6, 2-butanol, 2-butanone, 2-phenoxyethanol, acetaminophen, allantoin, arabinose, meglumine, arabitol, benzyl acetoacetate, benzyl alcohol, chlorobutanol, cholestanoltetraacetyl-b-glucoside, cinnamaldehyde, cyclohexanone, deoxyribose, diethyl carbonate, dimethyl carbonate, dimethyl isosorbide, dimethylacetamide, dimethylformamide, dimethylol ethylene urea, dimethyluracil, epilactose, erythritol, erythrose, ethyl lactate, ethyl maltol, ethylene carbonate, formamide, fucose, galactose, genistein, gentisic acid ethanolamide, gluconolactone, glyceraldehyde, glycerol, glycerol carbonate, glycerol formal, glycerol urethane, glycyrrhizic acid, gossypin, harpagoside, hederacoside C, icodextrin, iditol, imidazolidone, inositol, inulins, isomaltitol, kojic acid, lactitol, lactobionic acid, lactose, lactulose, lyxose, madecassoside, maltotriose, mangiferin, mannose, melzitose, methyl lactate, methylpyrrolidone, mogroside V, N-acetylgalactosamine, N-acetylglucosamine, N-acetylneuraminic acid, N-methyl acetamide, N-methyl formamide, N-methyl propionamide, pentaerythritol, pinoresinol diglucoside, glucuronic acid, piracetam, propyl gallate, propylene carbonate, psicose, pullulan, pyrogallol, quinic acid, raffinose, rebaudioside A, rhamnose, ribitol, ribose, ribulose, saccharin, sedoheptulose, sinistrins, solketal, stachyose, sucralose, tagatose, t-butanol, tetraglycol, triacetin, N-acetyl-d-mannosamine, nystose, kestose, turanose, acarbose, D-saccharic acid 1,4-lactone, thiodigalactoside, fucoidan, hydroxysafflor yellow A, shikimic acid, diosmin, pravastatin sodium salt, D-altrose, L-gulonic gamma-lactone, neomycin, rubusoside dihydroartemisinin, phloroglucinol, naringin, baicalein, hesperidin, apigenin, pyrogallol, morin, salsalate, kaempferol, myricetin, 3',4',7-trihydroxyisoflavone, (±)-taxifolin, silybin, perseitol diformal, 4-hydroxyphenylpyruvic acid, sulfacetamide, isopropyl β-D-1-thiogalactopyranoside, ethyl 2,5-dihydroxybenzoate, spectinomycin, resveratrol, quercetin, kanamycin sulfate, 1-(2-Pyrimidyl)piperazine, 2-(2-pyridyl)ethylamine, 2-imidazolidone, DL-1,2-isopropylideneglycerol, metformin, m-xylylenediamine, x-xylylenediamine, demeclocycline, tripropylene glycol, tubeimoside I, verbenaloside, xylitol, and xylose.

6. Protein/Excipient Solutions: Properties and Processes

[0081] In certain embodiments, solutions of therapeutic or non-therapeutic proteins formulated with above-identified excipient compounds or combinations thereof (hereinafter, "excipient additives"), such as hindered amines, anionic aromatics, functionalized amino acids, oligopeptides, or short-chain organic acids, low molecular weight aliphatic polyacids, diones and sulfones, zwitterionic excipients, and crowding agents with hydrogen bonding elements result in improved protein-protein interaction characteristics or protein self-interactions as measured by the protein diffusion interaction parameter, k_D, by biolayer interferometry, by surface plasmon resonance, or by determining the second virial coefficient, B_{22}, or similar method. As used herein, an "improvement" in one or more protein-protein interaction parameters achieved by test formulations using the above-identified excipient compounds or combinations thereof can refer to a decrease in attractive protein-protein interactions when a test formulation is compared under comparable conditions with a comparable formulation that does not contain the excipient compounds or excipient additives. Such improvements can be identified by measuring certain parameters that apply to the overall process or an aspect thereof, where a parameter is any metric pertaining to the process where an alteration can be can be quantified and compared to a previous state or to a control. A parameter can pertain to the process itself, such as its efficiency, cost, yield, or rate.

[0082] A parameter can also be a proxy parameter that pertains to a feature or an aspect of the larger process. As an example, parameters such as the k_D or B_{22} parameters can be termed proxy parameters. Measurements of k_D and B_{22} can be made using standard techniques in the industry, and can be an indicator of process-related parameters such as improved solution properties or stability of the protein in solution. Not to be bound by theory, it is understood that a highly negative k_D value can indicate that the protein has strong attractive interactions, and this can lead to aggregation, instability, and rheology problems. When formulated in the presence of certain of the above-identified excipient compounds or combinations thereof, the same protein can have an improved proxy parameter of a less negative k_D value, or a k_D value near or above zero, with this improved proxy parameter being associated with an improvement in a process-related parameter.

[0083] In embodiments, certain of the above-described excipient compounds or combinations thereof, such as hindered amines, anionic aromatics, functionalized amino acids, oligopeptides, short-chain organic acids, low molecular weight aliphatic polyacids, diones and sulfones, zwitterionic excipients, and/or crowding agents with hydrogen bonding elements are used to improve a protein-related process, such as the manufacture, processing, sterile filling, purification, and analysis of protein-containing solutions, using processing methods such as filtration, sterile filtration, depth filtration, syringing, transferring, pumping, mixing, heating or cooling by heat transfer, gas transfer, centrifugation, chromatography, membrane separation, centrifugal concentration, tangential flow filtration, radial flow filtration, axial flow filtration, lyophilization, and gel electrophoresis. In these and related protein-related processes, the protein of interest is dissolved in a solution that conveys it through the processing apparatus. Such solutions, referred to herein as "carrier solutions," can include cell culture media (containing, for example, secreted proteins of interest), lysate solutions following the lysis of host cells (where the protein of interest resides in the lysate), elution solutions (which contain the protein of interest following chromatographic separations), electrophoresis solutions, transport solutions for carrying the protein of interest through conduits in a processing apparatus, and the like. A carrier solution containing the protein of interest may also be termed a protein-containing solution or a protein solution. As described in more detail below, one or more viscosity-reducing excipients can be added to the protein-containing solution to improve various aspects of processing. As used herein, the terms "improve," "improvements," and the like refer to an advantageous change in a parameter of interest in a carrier solution when that parameter is compared to the same parameter as measured in a control solution. As used herein, a "control solution" means a solution that lacks the viscosity-reducing excipient but otherwise substantially similar to the carrier solution. As used herein, a "control process," for example a control filtration process, a control chromatographic process, and the like, is a protein-related process that is substantially similar to the protein-related process of interest and is performed with a control solution instead of a carrier solution.

[0084] For example, in processes where a protein-containing solution is pumped through conduits (e.g., flow chambers, piping or tubing), adding a viscosity-lowering excipient to the protein solution, as described above, before or during the pumping process can substantially reduce the force and the power required to pump the solution. It is understood that fluids generally exhibit a resistance to flow, i.e., a viscosity, and that a force must be applied to the fluid to overcome this viscosity in order to induce and propagate flow. The power, P, required for pumping scales with the head, H, and capacity, Q, as shown in the following equation:

$$P \sim HQ \qquad \text{(Eq. 1)}$$

[0085] Viscous fluids tend to increase the power requirements for pumps, to lower pump efficiency, to decrease pump head and capacity, and to increase frictional resistance in piping. Adding the viscosity-lowering excipients described above to a protein solution prior to or during pumping can substantially lower processing costs by decreasing either the head (H, Eq. 1) or the capacity (Q, Eq. 1) or both. The benefits of reduced viscosity can be manifested, for example, by improved throughput, increased yield, or decreased processing time. Moreover, frictional losses from the transmission of a fluid through a conduit can account for a significant fraction of the costs associated with conveying such fluids. Adding a viscosity-lowering excipient as described above to a protein solution prior to or during pumping can substantially lower processing costs by decreasing the friction accompanying the pumping process. Measurement of processing costs represents a processing parameter that can be improved by using a viscosity-reducing excipient.

[0086] These processes and processing methods for protein solutions can have improved efficiency due to the lower viscosity, improved solubility, or improved stability of the proteins in the solution during manufacture, processing, purification, and analysis steps. Measurement of processing efficiency or measurement of proxy parameters such as viscosity, solubility or stability of the proteins in solution represent processing parameters that can be improved by using a viscosity-reducing excipient. Several different factors are understood to adversely affect protein viscosity, solubility, and stability during processing. For example, protein-containing solutions are subject to a variety of physical stressors during manufacturing and purification, including significant shear stresses induced by manipulating protein solutions through typical processing operations, including, but not limited to, pumping, mixing, centrifugation, and filtration. In addition, during these processing steps, air bubbles can become entrained within the fluid to which proteins can adsorb. Such interfacial tension forces, coupled with typical shear stresses encountered during processing, can cause adsorbed protein molecules to unfold and aggregate. Additionally, significant protein unfolding can occur during pump cavitation events and during exposure to solid surfaces during manufacturing, such as ultrafiltration and diafiltration membranes. Such events can impair protein folding and product quality.

[0087] For Newtonian fluids, the stress, τ, imposed by a given process scales with the shear rate, $\dot{\gamma}$, and viscosity of the fluid, η, as shown in the following equation:

$$\tau = \dot{\gamma}\eta \qquad \text{(Eq. 2)}$$

[0088] By formulating a protein solution with one or more of the above-described excipient compounds or combinations thereof, solution viscosity is decreased, thus decreasing the shear stress encountered by the protein solution. The decreased shear stress can improve the stability of the formulation being processed, as manifested, for example, by a better or more desirable measurement of a processing parameter. Such improved processing parameters can include metrics such as reduced levels of protein aggregates, particles, or subvisible particles (manifested macroscopically as turbidity), reduced product losses, or improved overall yield. As another example of an improved processing parameter, reducing viscosity of a protein-containing solution can decrease the processing time for the solution. The processing time for a given unit operation generally scales inversely with the shear rate. Therefore, for a given characteristic stress, a decrease in protein solution viscosity by the addition of the above-described excipient compounds or combinations thereof is associated with an increase in shear rate ($\dot{\gamma}$, see Eq. 2), and therefore a decrease in the processing time.

[0089] During processing, it is understood that a protein in a solution may be a desired protein active ingredient, for example a therapeutic or non-therapeutic protein. Facilitating the processing of such a protein active ingredient using the excipients described herein can increase the yield or the rate of production of the protein active ingredient, or improve the efficiency of the particular process, or decrease the energy use, or the like, any of which outcomes represent processing parameters that have been improved by the use of the viscosity-reducing excipient. It is also understood that protein contaminants can be formed during certain processing technologies, for example during the fermentation and purification steps of bioprocessing. Removing the contaminants more quickly, more thoroughly, or more efficiently can also improve the processing of the desired protein, i.e., the protein active ingredient; these outcomes represent processing parameters that have been improved by the use of the viscosity-reducing excipient compound or additive. As described herein, certain excipients as described herein, by lowering solution viscosity, improving protein stability, and/or increasing protein solubility, can improve the transport of desired protein active ingredients, and can improve the removal of undesirable protein contaminants; both effects, which represent processing parameters that have been improved by the use of the viscosity-reducing excipient or additive, show that these excipients or additives improve the overall process of protein manufacture. Advantageously, a viscosity-reducing excipient used in processing is selected based on its physiological impact or lack thereof on a potential patient. For example, while certain substituted phenethylamines are understood to modulate various neurotransmitters, such as the monoamine neurotransmitter systems, and these may have various psychotropic effects (e.g., stimulant, hallucinogenic, or entactogenic effects) because of their impact on the central nervous system, it can be desirable to employ a viscosity-reducing phenethylamine excipient in a viscosity-reducing amount that does not produce psychotropic effects, or that does not produce clinically problematic psychotropic effects, or that may produce psychotropic effects in a dose-related manner, but does not produce psychotropic effects at the dosage to be found in a formulation that has been produced by the processes described herein for improving a processing parameter by adding the viscosity-reducing excipient to a step of the process. Similarly, it can be desirable to employ other viscosity-reducing excipients that do not produce other physiological effects (e.g., cardiovascular, respiratory, gastrointestinal, genitourinary, and the like), or that do not produce clinically problematic physiological effects, or that may produce physiological effects in a dose-related manner, but do not produce physiological effects at the dosage to be found in a formulation that has been produced by the processes described herein for improving a processing parameter by adding the viscosity-reducing excipient to a step of the process.

[0090] Specific platform unit operation for therapeutic protein production and purification offer further examples of the advantageous uses of viscosity-reducing excipients as disclosed herein, and further examples of these excipients' or additives' improving processing parameters. For example, introducing one or more of the viscosity-reducing excipients described above into these production and purification processes, as described below, can provide substantial improvements in molecule stability and recovery, and a decrease in operation costs.

[0091] It is understood in the art that the widely practiced technology for producing and purifying therapeutic proteins like monoclonal antibodies generally consists of a fermentation process followed by a series of steps for purification processing. Fermentation, or upstream processing (USP), comprises those steps by which therapeutic proteins are grown in bioreactors, typically using bacterial or mammalian cell lines. USP may, in embodiments, include steps such as those shown in FIG. 1. Purification, or downstream processing (DSP) may, in embodiments, include steps such as those shown in FIG. 2.

[0092] As shown in FIG. 1, USP may commence with the step 102 of thawing of vials from a master cell bank (MCB). The MCB can be expanded as shown in step 104, to form a working cell bank (not shown) and/or to produce the working stock for further production. Cell culture takes place in a series of seed and production bioreactors, as shown in steps 108 and 110, to yield those bioreactor products 112 from which the desired therapeutic protein can be harvested, as shown in step 114. Following harvest 114, the products can be submitted to further purification (i.e., DSP, as described below in more detail and as depicted in FIG. 2), or these products may be stored in bulk, typically by freezing and storing at a temperature of approximately -80°C.

[0093] In embodiments, protein production by cell culture techniques can be improved by the use of the above-identified excipients, as manifested by improvements in process-related parameters. In embodiments, the desired excipient can be added during USP in an amount effective to reduce the viscosity of the cell culture medium by at least 20%. In other embodiments, the desired excipient can be added during USP in an amount effective to reduce the viscosity of the cell culture medium by at least 30%. In embodiments, the desired excipient can be added to the cell culture medium in an amount of about 1 mM to about 400 mM. In embodiments, the desired excipient can be added to the cell culture medium in an amount of about 20 mM to about 200 mM. In embodiments, the desired excipient can be added to the cell culture medium in an amount of about 25 mM to about 100 mM. The desired excipient or combination of excipients can be added directly to the cell culture medium, or it can be added as a component of a more complex supplemental medium, for example a nutrient-containing solution or “feed solution” that is formulated separately and added to the cell culture medium. In embodiments, a second viscosity-reducing compound can be added to the carrier solution, either directly or via a supplemental medium, wherein the second viscosity-reducing compound adds an additional improvement to a particular parameter of interest.

[0094] As described below, there are many process-related parameters during USP that can be improved by use of one or more viscosity-reducing excipients. For example, in embodiments, use of a viscosity-reducing excipient can improve parameters such as the rate and/or degree of cell growth during steps such as inoculum expansion 104, and cell culture 108 and 110, and/or can improve proxy parameters that are correlated with the improvement in various process parameters. For example, adding the above-identified excipients to the USP process at a step such as the production bioreactor step 110, can decrease the viscosity of the cell culture medium, which can subsequently improve heat transfer efficiency and gas transfer efficiency. Because the cell culture process requires oxygen infusion to the cells to enable protein expression, and the diffusion of oxygen into the cells can therefore be a rate-limiting step, improving the rate of oxygen uptake by improving gas transfer efficiency through decreasing solution viscosity can improve the rate or amount of protein expression and/or its efficiency. In this context, parameters such as the rate of oxygen uptake and the rate of gas transfer efficiency can be deemed proxy parameters, whose improvement is correlated with an improvement in the process parameter of improved protein expression or improved processing efficiency. As another example, the availability of viscosity-reducing excipients can improve processing, for example, during the inoculum expansion step 104 and during the cell culture steps 108 and 110, by improving a proxy parameter such as the solubility of protein growth factors that are required for protein expression; with improved growth factor solubility, these substances can become more available to the cells, thereby facilitating cell growth.

[0095] In embodiments, process parameters such as the amount of protein recovery or the rate of protein recovery during USP can be improved by reducing viscosity during USP by several mechanisms. For example, the harvest of therapeutic protein at the end of the lysis step during harvest 114 from the completed cell culture can be more efficient or can be otherwise improved with the use of the above-identified excipients. Not to be bound by theory, by reducing viscosity of the expressed protein, these viscosity-reducing excipients can increase the efficiency of diffusion of therapeutic protein away from other lysate components. In addition, the separation of membranes and other cell debris from the protein-containing supernate can be accomplished with a faster separation rate or a higher degree of supernate purity, with the use of the viscosity-reducing excipients, thereby improving the process parameter of USP efficiency. Furthermore, the protein separation steps that use centrifugation or filtration steps can be accomplished faster with the use of the viscosity-reducing excipients, since the excipients reduce the viscosity of the medium.

[0096] In embodiments, as an additional benefit, use of the above-described viscosity-reducing excipients in cell culture can increase a process parameter such as protein yield during USP because protein misfolding and aggregation are reduced. It is understood that, as the cell culture is optimized to produce a maximum yield of recombinant protein, the resulting protein is expressed in a highly concentrated manner, which can result in misfolding; adding a viscosity-reducing excipient can reduce the attractive protein-protein interactions that lead to misfolding and aggregation, thereby increasing the amount of intact recombinant protein that is available for harvest 114.

[0097] Downstream processing (DSP), depicted in FIG. 2 in an illustrative embodiment, involves a sequence of steps that results in the recovery and purification of therapeutic proteins, for example monoclonal antibodies, biopharmaceuticals, vaccines, and other biologics. At the end of USP, the therapeutic protein of interest can be dissolved in the cell culture medium, having been secreted from the host cells. The therapeutic protein can also be dissolved in a fluid medium following the lysis of the host cells at the end of the USP sequences. DSP is undertaken to retrieve the protein of interest from the solution in which it is dissolved (e.g., the culture medium or host cell lysate medium), and to purify it. During DSP, (i) various contaminants (such as insoluble cell debris and particulates) are removed from the media, (ii) the protein product is isolated through techniques such as extraction, precipitation, adsorption or ultrafiltration, (iii) the protein product is purified through techniques such as affinity chromatography, precipitation, or crystallization, and (iv) the product is further polished, and viruses are removed.

[0098] As shown in FIG. 2, a feedstock from cell culture harvest 200 (also as described in FIG. 1) is initially subjected to affinity chromatography 204, typically involving Protein-A chromatography or other analogous chromatographic steps. The virus inactivation step 208 typically entails subjecting the feedstock to a low pH hold. One or more polishing chromatography steps 210 and 212 are performed to remove impurities, such as host cell proteins (HCP), DNA, charge variants, and aggregates. Cation exchange (CEX) chromatography is commonly used as an initial polishing chromatography step 210, but it may be accompanied by a second chromatography step 212 that either precedes or follows it. The second chromatography step 212 further removes host-cell-related impurities (e.g., HCP or DNA), or product related impurities such as aggregates. Anion exchange (AEX) chromatography and hydrophobic interaction chromatography (HIC) can be employed as second chromatography steps 212. Virus filtration 214 is performed to effect virus removal. Final purification steps 218 can include ultrafiltration and diafiltration, and preparation for formulation.

[0099] As generally described above, purification processes or DSP following the fermentation process can include (1) cell culture harvest, (2) chromatography (e.g., Protein-A chromatography and chromatographic polishing steps, including ion exchange and hydrophobic interaction chromatography), (3) viral inactivation, and (4) filtration (e.g., viral filtration, sterile filtration, dialysis, and ultrafiltration and diafiltration steps to concentrate the protein and exchange the protein into the formulation buffer). Examples are provided below to illustrate the advantages from using a viscosity-reducing excipient as described herein to improve process parameters associated with these purification processes. It is understood that the viscosity-reducing excipient or combinations thereof can be introduced at any phase of DSP by adding it to a carrier solution or in any other way engineering the contact of the protein of interest with the excipient, whether in soluble or stabilized form. In embodiments, a second viscosity-reducing compound can be added to the carrier solution during DSP, wherein the second viscosity-reducing compound adds an additional improvement to a particular parameter of interest.

[00100] (1) Cell culture harvest: Cell culture harvest generally involves centrifugation and depth filtration operations in which cellular debris is physically removed from protein-containing solutions. The centrifugation step can provide a more complete separation of soluble protein from cell debris with the benefit of a viscosity-reducing excipient. Whether done by batch or continuous processing, the centrifuge separation requires the dense phase to consolidate as much as possible to maximize recovery of the target protein. In embodiments, addition of the above-identified excipients or combinations thereof can increase the process parameter of protein yield, for example, by increasing the yield of protein-containing centrate that flows away from the dense phase of the centrifuge separation process. The depth filtration step is a viscosity-limited step, and thus can be made more efficient by using an excipient that reduces solution viscosity. These processes can also introduce air bubbles into the protein solution, which can couple with shear-induced stresses to destabilize the therapeutic protein molecules being purified. Adding a viscosity-reducing excipient to the protein-containing solution, before and/or during cell culture harvest, as described above, can protect the protein from these stresses, thereby improving the process parameter of quantified product recovery.

[00101] (2) Chromatography: After cell culture harvest by centrifugation or filtration, chromatography is typically used to separate the therapeutic protein from the fermentation broth. Protein A chromatography is used when the therapeutic protein is an antibody: Protein A is selective towards IgG antibodies, which it will bind dynamically at a high flow rate and capacity. Cation exchange (CEX) chromatography can be used as a cost-effective alternative to Protein A chromatography. If CEX is used, the pH of the feed must be adjusted and its conductivity decreased prior to loading onto the column to optimize the dynamic binding capacity. Mimetic resins can also be used as an alternative to Protein A chromatography. These resins provide ligands to bind immunoglobulins, for example Ig-binding proteins like protein G or protein L, synthetic ligands, or protein A-like porous polymers.

[00102] Other chromatography processes can be employed during DSP. Ion exchange chromatography (IEC) can be used to remove impurities introduced during previous processes, for example, leached Protein A, endotoxins or viruses from the cell line, remaining host cell proteins or DNA, or media components. IEC, whether CEX or anion exchange chromatography, can be applied directly after Protein A chromatography. Hydrophobic interaction chromatography (HIC) can complement IEC, generally used as a polishing step to remove aggregates. In embodiments, the use of the above-identified excipients can increase the solubility of, and decrease the viscosity of host cell proteins during chromatography column loading steps. In embodiments, the use of the above-identified excipients can increase the solubility of, and decrease the viscosity of the therapeutic protein during chromatography column loading steps and elution steps.

[00103] Chromatographic processes during protein purification impose harsh conditions on the protein formulation, such as (a) low pH conditions during elution from Protein-A chromatography columns, (b) elevated local protein concentration (often on the order of 300-400 mg/mL) within the pore-space of chromatographic resin, (c) elevated salt concentrations during ion exchange chromatography, and (d) elevated concentrations of salting-out agents during elution from HIC columns. Adding a viscosity-reducing excipient to the protein-containing solution, before and/or during chromatography, as described above, can facilitate the transit of the proteins through the chromatography column so that they are less exposed to the potentially damaging conditions imposed by chromatographic processing steps. In addition, the elevated local protein concentration within the column pore-space can result in a highly viscous material within this space, which places significant back pressure on the column. To alleviate this back pressure, media with relatively large pores are typically used. However, the resolving power of large-pore media is lower than small-pore counterparts. The incorporation of viscosity-modifying excipients as described above can enable the use of smaller pores in the chromatographic media. In embodiments, the elution steps from Protein-A chromatography expose the therapeutic protein to a low pH condition that can reduce solubility and increase aggregation of the target protein; addition of the excipients can increase the solubility of the target protein such that recovery yield from the Protein-A chromatography step is improved. In other embodiments, use of the excipient can enable elution of the target protein from Protein-A resin at a higher pH, and this can reduce chemical stresses on the target protein, resulting in improving a process parameter of protein yield by reducing the amount of protein degradation during processing.

[00104] (3) Viral inactivation: Viral inactivation processes typically involve holding the protein solution at a low pH, e.g., pH lower than 4, for an extended period of time. This environment, though, can destabilize therapeutic proteins. Formulating the protein in the presence of a viscosity-reducing excipient, for example, by adding a viscosity-reducing excipient before and/or during a viral inactivation process, can improve process parameters such as the stability or solubility of the protein, or its net yield. Also, formulating the protein in the presence of a stabilizing excipient, for example, by adding a stabilizing excipient before and/or during a viral inactivation process, can improve process parameters such as the stability or solubility of the protein, its structural integrity in the monomeric form, its resistance to aggregation, or its net yield.

[00105] (4) Filtration: Filtration processes include viral filtration processes (nanofiltration) to remove virus particles, microfiltration to remove micron-scale impurities, and ultrafiltration/diafiltration processes to concentrate protein solutions and to exchange buffer systems.

[00106] (a) Viral filtration purifies the protein solution by removing virus particles, which can be on the order of twice the size of a recombinant human monoclonal antibodies. Thus, the filtration membrane for viral filtration can require nano-sized pores. As a result of the small pore size through which the proteins must pass, this filtration step can introduce stress to the protein, and is accompanied by significant levels of membrane fouling from protein aggregate particles. The addition of a viscosity-reducing excipient, for example, before and/or during filtration, as described above, can reduce a measurable parameter such as back pressure in the filtration system by increasing collective diffusivity, and can decrease the tendency for membrane fouling by mitigating the protein-protein interactions that give rise to it. The end result is improvement in those parameters indicting improved performance of the viral filtration unit during protein purification.

[00107] (b) Ultrafiltration and diafiltration (UF/DF) processes concentrate protein solutions and exchange buffer systems by passing the protein-containing solution through a filter membrane with a characteristic molecular weight cutoff that is smaller than the protein of interest. In this step, the protein solution faces high shear stresses within the filter units, elevated protein concentrations, and adsorption of the protein to the hydrophobic membranes typically used during UF/DF processes, all of which can increase protein aggregation. The addition of a viscosity-reducing excipient, for example, before and/or during a UF/DF process, as described above, can reduce back pressure in the filtration system by increasing collective diffusivity (measured, for example, by an increase in k_D). This not only reduces shear stress across the membrane, but also promotes back-diffusion away from the filter membrane, thus lowering the effective protein concentration at the membrane interface and increasing the permeate flux. As a result, the use of viscosity-reducing excipients can improve parameters associated with higher throughput during these filtration processes, with reduced product losses and increased net yield. Additionally, passing viscous fluids through ultra- and diafilters can produce a large pressure drop across the filter device, making the separation inefficient. Formulating the protein solution in the presence of viscosity-reducing excipients as described above can substantially reduce the pressure drop across the filter device, thereby improving the process parameters of operation, costs and processing time by decreasing them both.

[00108] In more detail, UF/DF is an operation of DSP during which the biologic molecule of interest is retained while buffer and other analytes pass through the filter membrane. In

UF/DF, the target protein can be retained by the filter membrane itself, leading to the formation of a gel layer at the filter membrane surface. This gel layer can effectively limit the efficiency of the processing step due to increased protein-protein interactions (PPIs) in the region of high local protein concentration at the filter membrane surface, thereby reducing filter throughput and/or resulting in aggregation of the protein of interest. Incorporating a viscosity-reducing excipient in the protein-containing solution can have the effect of reducing PPIs and/or increasing the solubility of the target protein during UF/DF. Incorporating a viscosity-reducing excipient can also improve filtration efficiency, decrease operation time, and/or increase yield of the target protein. The viscosity reducing excipients may also provide benefit during other filtration processes such as viral filtration and sterile filtration.

[00109] A preferred structure for a viscosity-reducing excipient useful in UF/DF is a small molecule having a net charge of 0 at physiological pH, and comprising a saturated or unsaturated five-membered or six-membered carbocycle or heterocycle ring. In embodiments, the ring structure is a heterocycle such as a lactam, a furan, a tetrahydrofuran, a pyrrole, a pyrrolidine, a pyran, a pyridine, a piperidine, an imidazole, a dioxane, a morpholine, a pyrimidine, a sulfimide, a sulfonamide, or combinations thereof. In embodiments, the ring structure is part of a polycyclic ring system in which the component rings can be saturated or unsaturated, with optional substitutions that include short-chain (for example, C_1-C_6) aliphatic or cyclic saturated or unsaturated molecules containing functional groups such as hydroxyl, carbonyl, carboxylic acid, amide, and the like.

[00110] Another preferred structure for a viscosity-reducing excipient useful in UF/DF is a small molecule having a net positive charge at physiological pH and comprising an aromatic ring structure with or without a heteroatom. Other preferred structures include short-chain (for example, C_3-C_6) aliphatic or cyclic saturated or unsaturated molecules optionally substituted with functional groups such as hydroxyl, carbonyl, carboxylic acid, amide, and the like. Desirable excipients are soluble in the buffer solution used for processing, and do not contain a sugar molecule. Examples of viscosity-reducing excipients useful in UF/DF include: 1,3-dimethyluracil, 1-methyluracil, 3-methyluracil, 1,3-diethyluracil, 6-methyluracil, uracil, thymine, 1-methylthymine, O-4-methylthymine, 1,3-dimethylthymine, dimethylthymine dimer, cytosine, 5-methylcytosine, 3-methylcytosine, 2-pyrrolidinone, N-methylpyrrolidone, dimethylisosorbide, dimethylphenylalanine, nicotinamide, isonicotinamide, diethylnicotinamide, 2-butanol, 2-butanone, imidazole, aspartame, saccharin, acesulfame potassium, caffeine, theacrine, cyclohexanone, dimethylsulfone, piracetam, 1,3-dimethyl-2-oxohexahydropyrimidine, trigonelline, sulfolane, hordenine, diphenhydramine, phenethylamine, N-methylphenethylamine, N,N-dimethylphenethylamine, β,3-dihydroxyphenethylamine, β,3-dihydroxy-N-methylphenethylamine, 3-hydroxyphenethylamine, 4-hydroxyphenethylamine, tyrosinol, tyramine, N-methyltyramine, pyridoxine, dicyclomine, 2-pyridylethylamine. Advantageously, the viscosity-reducing excipients can be used alone or in combinations thereof.

[00111] In embodiments, the viscosity-reducing excipient can be dissolved in the processing buffer solution for UF/DF at an effective concentration from about 25 mM to about 1000 mM, or at an effective concentration from about 50mM to about 500 mM, or at an effective concentration from about 75 mM to about 300 mM, or at an effective concentration from about 25 mM to about 500 mM, or at an effective concentration from about 50 mM to about 300 mM. In an exemplary embodiment, the viscosity-reducing excipient is 1,3-dimethyluracil, added to the processing buffer solution for UF/DF at a concentration from about 25 mM to about 1000 mM, or from about 50 mM to about 500 mM, or from about 75 mM to about 300 mM. In another exemplary embodiment, the viscosity-reducing excipient is hordenine HCl, added to the processing buffer solution at a concentration from about 25 mM to about 500 mM, or from about 50 mM to about 300 mM, or from about 75 mM to about 200 mM.

[00112] After the upstream protein processing or downstream purification have been completed with the added excipient, the excipient can remain as a part of the drug substance mixture or it can be separated from the protein active ingredient. Typical small molecule separation methods can be used to separate the excipient from the protein active ingredient, such as buffer exchange, ion exchange, ultrafiltration, and dialysis. In addition to the beneficial effects on the protein purification processes as outlined above, the use of the above-identified excipients can protect and preserve equipment used in protein manufacture, processing, and purification. For example, equipment-related processes such as the cleanup, sterilization, and maintenance of protein processing equipment can be facilitated by the use of the above-identified excipients due to decreased fouling, decreased denaturing, lower viscosity, and improved solubility of the protein, and parameters associated with the improvement of these processes are similarly improved.

[00113] The downstream process can be a chromatography process, and the chromatography process can be a Protein-A chromatography process. In embodiments, the chromatography process recovers the protein of interest, wherein the protein of interest is characterized by an improved protein-related parameter selected from the group consisting of improved purity, improved yield, fewer particles, less misfolding, improved biological activity, or less aggregation, as compared to a control solution. In embodiments, the improved protein-related parameter is improved yield of the protein of interest from the chromatography process. In embodiments, the chromatography process recovers the protein of interest, wherein the protein of interest is characterized by an improved percentage recovered in the monomeric form, i.e., with lower level of aggregation compared with the recovery process in the absence of the excipient.

[00114] While the use of an excipient compound to improve upstream and/or downstream processing has been described extensively herein, it is understood that a combination of excipients can be added together in order to achieve a desired effect, such as an improvement in a parameter of interest. The term "excipient additive" can refer to either a single excipient compound that leads to the desired effect or improved parameter, or to a combination of excipient compounds where the combination is responsible for the desired effect or the improved parameter.

EXAMPLES

Materials:

- Bovine gamma globulin (BGG), >99% purity, Catalog #G5009, Sigma Aldrich
- Human gamma globulin (HGG), Octagam 10%, Octapharma, Switzerland
- Histidine, Sigma Aldrich
- Other materials described in the examples below were from Sigma Aldrich unless otherwise specified.

Example 1: Preparation of formulations containing excipient compounds and test protein

[00115] Formulations were prepared using an excipient compound and a test protein, where the test protein was intended to simulate either a therapeutic protein that would be used in a therapeutic formulation, or a non-therapeutic protein that would be used in a non-therapeutic formulation. Such formulations were prepared in 50 mM histidine hydrochloride with different excipient compounds for viscosity measurement in the following way. Histidine hydrochloride was first prepared by dissolving 1.94 g histidine in distilled water and adjusting the pH to about 6.0 with 1 M hydrochloric acid (Sigma-Aldrich, St. Louis, MO) and then diluting to a final volume of 250 mL with distilled water in a volumetric flask. Excipient compounds were then dissolved in 50 mM histidine HCl. Lists of excipients are provided below in Examples 4, 5, 6, and 7. In some cases excipient compounds were adjusted to pH 6 prior to dissolving in 50 mM histidine HCl. In this case the excipient compounds were first dissolved in deionized water at about 5 wt% and the pH was adjusted to about 6.0 with either hydrochloric acid or sodium hydroxide. The prepared salt solution was then placed in a convection laboratory oven at about 65°C to evaporate the water and isolate the solid excipient. Once excipient solutions in 50 mM histidine HCl had been prepared, the test protein bovine gamma globulin (BGG) was dissolved at a ratio of about 0.336 g BGG per 1 mL excipient solution. This resulted in a final protein concentration of about 280 mg/mL. Solutions of BGG in 50 mM histidine HCl with excipient were formulated in 20 mL vials and allowed to shake at 100 rpm on an orbital shaker table overnight. BGG solutions were then transferred to 2 mL microcentrifuge tubes and centrifuged for ten minutes at 2300 rpm in an IEC MicroMax microcentrifuge to remove entrained air prior to viscosity measurement.

Example 2: Viscosity measurement

[00116] Viscosity measurements of formulations prepared as described in Example 1 were made with a DV-IIT LV cone and plate viscometer (Brookfield Engineering, Middleboro, MA). The viscometer was equipped with a CP-40 cone and was operated at 3 rpm and 25°C. The formulation was loaded into the viscometer at a volume of 0.5 mL and allowed to incubate at the given shear rate and temperature for 3 minutes, followed by a measurement collection period of twenty seconds. This was then followed by 2 additional steps consisting of 1 minute of shear incubation and subsequent twenty-second measurement collection period. The three data points collected were then averaged and recorded as the viscosity for the sample.

Example 3: Protein concentration measurement

[00117] The concentration of the protein in the experimental solutions was determined by measuring the optical absorbance of the protein solution at a wavelength of 280 nm in a UV/VIS Spectrometer (Perkin Elmer Lambda 35). First the instrument was calibrated to zero absorbance with a 50 mM histidine buffer at pH 6. Next the protein solutions were diluted by a factor of 300 with the same histidine buffer and the absorbance at 280 nm recorded. The final concentration of the protein in the solution was calculated by using the extinction coefficient value of 1.264 mL/(mg x cm).

Example 4: Formulations with hindered amine excipient compounds

[00118] Formulations containing 280 mg/mL BGG were prepared as described in Example 1, with some samples containing added excipient compounds. In these tests, the hydrochloride salts of dimethylcyclohexylamine (DMCHA), dicyclohexylmethylamine (DCHMA), dimethylaminopropylamine (DMAPA), triethanolamine (TEA), dimethylethanolamine (DMEA), and niacinamide were tested as examples of the hindered amine excipient compounds. Also, a hydroxybenzoic acid salt of DMCHA and a taurine-dicyandiamide adduct were tested as examples of the hindered amine excipient compounds. The viscosity of each protein solution was measured as described in Example 2, and the results are presented in Table 1 below, showing the benefit of the added excipient compounds in reducing viscosity.

TABLE 1

Test Number	Excipient Added	Excipient Concentration (mg/mL)	Viscosity (cP)	Viscosity Reduction
4.1	None	0	79	0%
4.2	DMCHA-HCl	28	50	37%
4.3	DMCHA-HCl	41	43	46%
4.4	DMCHA-HCl	50	45	43%
4.5	DMCHA-HCl	82	36	54%
4.6	DMCHA-HCl	123	35	56%
4.7	DMCHA-HCl	164	40	49%
4.8	DMAPA-HCl	87	57	28%
4.9	DMAPA-HCl	40	54	32%
4.10	DCHMA-HCl	29	51	35%
4.11	DCHMA-HCl	50	51	35%
4.14	TEA-HCl	97	51	35%
4.15	TEA-HCl	38	57	28%
4.16	DMEA-HCl	51	51	35%
4.17	DMEA-HCl	98	47	41%
4.20	DMCHA-hydroxybenzoate	67	46	42%
4.21	DMCHA-hydroxybenzoate	92	42	47%
4.22	Product of Example 8	26	58	27%
4.23	Product of Example 8	58	50	37%
4.24	Product of Example 8	76	49	38%
4.25	Product of Example 8	103	46	42%
4.26	Product of Example 8	129	47	41%
4.27	Product of Example 8	159	42	47%

Test Number	Excipient Added	Excipient Concentration (mg/mL)	Viscosity (cP)	Viscosity Reduction
4.28	Product of Example 8	163	42	47%
4.29	Niacinamide	48	39	51%
4.30	N-Methyl-2-pyrrolidone	30	45	43%
4.31	N-Methyl-2-pyrrolidone	52	52	34%

Example 5: Formulations with anionic aromatic excipient compounds

[00119] Formulations of 280 mg/mL BGG were prepared as described in Example 1, with some samples containing added excipient compounds. The viscosity of each solution was measured as described in Example 2, and the results are presented in Table 2 below, showing the benefit of the added excipient compounds in reducing viscosity.

TABLE 2

Test Number	Excipient Added	Excipient Concentration (mg/mL)	Viscosity (cP)	Viscosity Reduction
5.1	None	0	79	0%
5.2	Sodium aminobenzoate	43	48	39%
5.3	Sodium hydroxybenzoate	26	50	37%
5.4	Sodium sulfanilate	44	49	38%
5.5	Sodium sulfanilate	96	42	47%
5.6	Sodium indole acetate	52	58	27%
5.7	Sodium indole acetate	27	78	1%
5.8	Vanillic acid, sodium salt	25	56	29%
5.9	Vanillic acid, sodium salt	50	50	37%
5.10	Sodium salicylate	25	57	28%
5.11	Sodium salicylate	50	52	34%
5.12	Adenosine monophosphate	26	47	41%
5.13	Adenosine monophosphate	50	66	16%
5.14	Sodium benzoate	31	61	23%
5.15	Sodium benzoate	56	62	22%

Example 6: Formulations with oligopeptide excipient compounds

[00120] Oligopeptides (n=5) were synthesized by NeoBioLab Inc. (Woburn, MA) in >95% purity with the N terminus as a free amine and the C terminus as a free acid. Dipeptides (n=2) were synthesized by LifeTein LLC (Somerset, NJ) in 95% purity. Formulations of 280 mg/mL BGG were prepared as described in Example 1, with some samples containing the synthetic oligopeptides as added excipient compounds. The viscosity of each solution was measured as described in Example 2, and the results are presented in Table 3 below, showing the benefit of the added excipient compounds in reducing viscosity.

TABLE 3

Test Number	Excipient Added	Excipient Concentration (mg/mL)	Viscosity (cP)	Viscosity Reduction
6.1	None	0	79	0%
6.2	ArgX5	100	55	30%
6.3	ArgX5	50	54	32%
6.4	HisX5	100	62	22%
6.5	HisX5	50	51	35%
6.6	HisX5	25	60	24%
6.7	Trp2Lys3	100	59	25%
6.8	Trp2Lys3	50	60	24%
6.9	AspX5	100	102	-29%
6.10	AspX5	50	82	-4%
6.11	Dipeptide LE (Leu-Glu)	50	72	9%
6.12	Dipeptide YE (Tyr-Glu)	50	55	30%
6.13	Dipeptide RP (Arg-Pro)	50	51	35%
6.14	Dipeptide RK (Arg-Lys)	50	53	33%
6.15	Dipeptide RH (Arg-His)	50	52	34%
6.16	Dipeptide RR (Arg-Arg)	50	57	28%
6.17	Dipeptide RE (Arg-Glu)	50	50	37%
6.18	Dipeptide LE (Leu-Glu)	100	87	-10%
6.19	Dipeptide YE (Tyr-Glu)	100	68	14%
6.20	Dipeptide RP (Arg-Pro)	100	53	33%

Test Number	Excipient Added	Excipient Concentration (mg/mL)	Viscosity (cP)	Viscosity Reduction
6.21	Dipeptide RK (Arg-Lys)	100	64	19%
6.22	Dipeptide RH (Arg-His)	100	72	9%
6.23	Dipeptide RR (Arg-Arg)	100	62	22%
6.24	Dipeptide RE (Arg-Glu)	100	66	16%

Example 8: Synthesis of guanyl taurine excipient

[00121] Guanyl taurine was prepared following method described in U.S. Pat. No. 2,230,965. Taurine (Sigma-Aldrich, St. Louis, MO) 3.53 parts were mixed with 1.42 parts of dicyandiamide (Sigma-Aldrich, St. Louis, MO) and grinded in a mortar and pestle until a homogeneous mixture was obtained. Next the mixture was placed in a flask and heated at 200°C for 4 hours. The product was used without further purification.

Example 9: Protein formulations containing excipient compounds

[00122] Formulations were prepared using an excipient compound and a test protein, where the test protein was intended to simulate either a therapeutic protein that would be used in a therapeutic formulation, or a non-therapeutic protein that would be used in a non-therapeutic formulation. Such formulations were prepared in 50 mM aqueous histidine hydrochloride buffer solution with different excipient compounds for viscosity measurement in the following way. Histidine hydrochloride buffer solution was first prepared by dissolving 1.94 g histidine in distilled water and adjusting the pH to about 6.0 with 1 M hydrochloric acid (Sigma-Aldrich, St. Louis, MO) and then diluting to a final volume of 250 mL with distilled water in a volumetric flask. Excipient compounds were then dissolved in the 50 mM histidine HCl buffer solution. A list of the excipient compounds is provided in Table 4. In some cases, excipient compounds were dissolved in 50 mM histidine HCl buffer solution and the resulting solution pH was adjusted with small amounts of sodium hydroxide or hydrochloric acid to achieve pH 6 prior to dissolution of the model protein. In some cases, excipient compounds were adjusted to pH 6 prior to dissolving in 50 mM histidine HCl. In this case the excipient compounds were first dissolved in deionized water at about 5 wt% and the pH was adjusted to about 6.0 with either hydrochloric acid or sodium hydroxide. The prepared salt solution was then placed in a convection laboratory oven at about 65°C to evaporate the water and isolate the solid excipient. Once excipient solutions in 50 mM histidine HCl had been prepared, the test protein, bovine gamma globulin (BGG) was dissolved at a ratio to achieve a final protein concentration of about 280 mg/mL. Solutions of BGG in 50 mM histidine HCl with excipient were formulated in 20 mL vials and allowed to shake at 100 rpm on an orbital shaker table overnight. BGG solutions were then transferred to 2 mL microcentrifuge tubes and centrifuged for ten minutes at 2300 rpm in an IEC MicroMax microcentrifuge to remove entrained air prior to viscosity measurement.

[00123] Viscosity measurements of formulations prepared as described above were made with a DV-IIT LV cone and plate viscometer (Brookfield Engineering, Middleboro, MA). The viscometer was equipped with a CP-40 cone and was operated at 3 rpm and 25°C. The formulation was loaded into the viscometer at a volume of 0.5 mL and allowed to incubate at the given shear rate and temperature for 3 minutes, followed by a measurement collection period of twenty seconds. This was then followed by 2 additional steps consisting of 1 minute of shear incubation and subsequent twenty-second measurement collection period. The three data points collected were then averaged and recorded as the viscosity for the sample. Viscosities of solutions with excipient were normalized to the viscosity of the model protein solution without excipient. The normalized viscosity is the ratio of the viscosity of the model protein solution with excipient to the viscosity of the model protein solution with no excipient.

TABLE 4

Test Number	Excipient Added	Excipient Concentration (mg/mL)	Normalized Viscosity (cP)	Viscosity Reduction
9.1	DMCHA-HCl	120	0.44	56%
9.2	Niacinamide	50	0.51	49%
9.3	Isonicotinamide	50	0.48	52%
9.4	Tyramine HCl	70	0.41	59%
9.5	Histamine HCl	50	0.41	59%
9.6	Imidazole HCl	100	0.43	57%
9.7	2-methyl-2-imidazoline HCl	60	0.43	57%
9.8	1-butyl-3-methylimidazolium chloride	100	0.48	52%
9.9	Procaine HCl	50	0.53	47%

Test Number	**Excipient Added**	**Excipient Concentration (mg/mL)**	**Normalized Viscosity (cP)**	**Viscosity Reduction**
9.10	3-aminopyridine	50	0.51	49%
9.11	2,4,6-trimethylpyridine	50	0.49	51%
9.12	3-pyridine methanol	50	0.53	47%
9.13	Nicotinamide adenine dinucleotide	20	0.56	44%
9.15	Sodium phenylpyruvate	55	0.57	43%
9.16	2-Pyrrolidinone	60	0.68	32%
9.17	Morpholine HCl	50	0.60	40%
9.18	Agmatine sulfate	55	0.77	23%
9.19	1-butyl-3-methylimidazolium iodide	60	0.66	34%
9.21	L-Anserine nitrate	50	0.79	21%
9.22	1-hexyl-3-methylimidazolium chloride	65	0.89	11%
9.23	N,N-diethyl nicotinamide	50	0.67	33%
9.24	Nicotinic acid, sodium salt	100	0.54	46%
9.25	Biotin	20	0.69	31%

Example 10: Preparation of formulations containing excipient combinations and test protein

[00124] Formulations were prepared using a primary excipient compound, a secondary excipient compound and a test protein, where the test protein was intended to simulate either a therapeutic protein that would be used in a therapeutic formulation, or a non-therapeutic protein that would be used in a non-therapeutic formulation. The primary excipient compounds were selected from compounds having both anionic and aromatic functionality, as listed below in Table 5. The secondary excipient compounds were selected from compounds having either nonionic or cationic charge at pH 6 and either imidazoline or benzene rings, as listed below in Table 5. Formulations of these excipients were prepared in 50 mM histidine hydrochloride buffer solution for viscosity measurement in the following way. Histidine hydrochloride was first prepared by dissolving 1.94 g histidine in distilled water and adjusting the pH to about 6.0 with 1 M hydrochloric acid (Sigma-Aldrich, St.

Louis, MO) and then diluting to a final volume of 250 mL with distilled water in a volumetric flask. The individual primary or secondary excipient compounds were then dissolved in 50 mM histidine HCl. Combinations of primary and secondary excipients were dissolved in 50 mM histidine HCl and the resulting solution pH adjusted with small amounts of sodium hydroxide or hydrochloric acid to achieve pH 6 prior to dissolution of the model protein. Once excipient solutions had been prepared as described above, the test protein bovine gamma globulin (BGG) was dissolved into each test solution at a ratio to achieve a final protein concentration of about 280 mg/mL. Solutions of BGG in 50 mM histidine HCl with excipient were formulated in 20 mL vials and allowed to shake at 100 rpm on an orbital shaker table overnight. BGG solutions were then transferred to 2 mL microcentrifuge tubes and centrifuged for ten minutes at 2300 rpm in an IEC MicroMax microcentrifuge to remove entrained air prior to viscosity measurement.

[00125] Viscosity measurements of formulations prepared as described above were made with a DV-IIT LV cone and plate viscometer (Brookfield Engineering, Middleboro, MA). The viscometer was equipped with a CP-40 cone and was operated at 3 rpm and 25°C. The formulation was loaded into the viscometer at a volume of 0.5 mL and allowed to incubate at the given shear rate and temperature for 3 minutes, followed by a measurement collection period of twenty seconds. This was then followed by 2 additional steps consisting of 1 minute of shear incubation and a subsequent twenty-second measurement collection period. The three data points collected were then averaged and recorded as the viscosity for the sample. Viscosities of solutions with excipient were normalized to the viscosity of the model protein solution without excipient, and summarized in Table 5 below. The normalized viscosity is the ratio of the viscosity of the model protein solution with excipient to the viscosity of the model protein solution with no excipient. The example shows that a combination of primary and secondary excipients can give a better result than a single excipient.

TABLE 5

	Primary Excipient		**Secondary Excipient**		
Test Number	**Name**	**Concentration (mg/mL)**	**Name**	**Concentration (mg/mL)**	**Normalized Viscosity**
10.1	Salicylic Acid	30	None	0	0.79
10.2	Salicylic Acid	25	Imidazole	4	0.59
10.3	4-hydroxybenzoic	30	None	0	0.61

10.4	4-hydroxybenzoic	25	Imidazole	5	0.57
10.5	4-hydroxybenzene	31	None	0	0.59
10.6	4-hydroxybenzene	26	Imidazole	5	0.70
10.7	4-hydroxybenzene	25	Caffeine	5	0.69
10.8	None	0	Caffeine	10	0.73
10.9	None	0	Imidazole	5	0.75

Example 11: Preparation of formulations containing excipient combinations and test protein

[00126] Formulations were prepared using a primary excipient compound, a secondary excipient compound and a test protein, where the test protein was intended to simulate a therapeutic protein that would be used in a therapeutic formulation, or a non-therapeutic protein that would be used in a non-therapeutic formulation. The primary excipient compounds were selected from compounds having both anionic and aromatic functionality, as listed below in Table 6. The secondary excipient compounds were selected from compounds having either nonionic or cationic charge at pH 6 and either imidazoline or benzene rings, as listed below in Table 6. Formulations of these excipients were prepared in distilled water for viscosity measurement in the following way. Combinations of primary and secondary excipients were dissolved in distilled water and the resulting solution pH adjusted with small amounts of sodium hydroxide or hydrochloric acid to achieve pH 6 prior to dissolution of the model protein. Once excipient solutions in distilled water had been prepared, the test protein bovine gamma globulin (BGG) was dissolved at a ratio to achieve a final protein concentration of about 280 mg/mL. Solutions of BGG in distilled water with excipient were formulated in 20 mL vials and allowed to shake at 100 rpm on an orbital shaker table overnight. BGG solutions were then transferred to 2 mL microcentrifuge tubes and centrifuged for ten minutes at 2300 rpm in an IEC MicroMax microcentrifuge to remove entrained air prior to viscosity measurement.

[00127] Viscosity measurements of formulations prepared as described above were made with a DV-IIT LV cone and plate viscometer (Brookfield Engineering, Middleboro, MA). The viscometer was equipped with a CP-40 cone and was operated at 3 rpm and 25°C. The formulation was loaded into the viscometer at a volume of 0.5 mL and allowed to incubate at the given shear rate and temperature for 3 minutes, followed by a measurement collection period of twenty seconds. This was then followed by 2 additional steps consisting of 1 minute of shear incubation and a subsequent twenty-second measurement collection period. The three data points collected were then averaged and recorded as the viscosity for the sample. Viscosities of solutions with excipient were normalized to the viscosity of the model protein solution without excipient, and summarized in Table 6 below. The normalized viscosity is the ratio of the viscosity of the model protein solution with excipient to the viscosity of the model protein solution with no excipient. The example shows that a combination of primary and secondary excipients can give a better result than a single excipient.

TABLE 6

	Primary Excipient		**Secondary Excipient**		
Test Number	**Name**	**Concentration (mg/mL)**	**Name**	**Concentration (mg/mL)**	**Normalized Viscosity**
11.1	Salicylic Acid	20	None	0	0.96
11.2	Salicylic Acid	20	Caffeine	5	0.71
11.3	Salicylic Acid	20	Niacinamide	5	0.76
11.4	Salicylic Acid	20	Imidazole	5	0.73

Example 12: Preparation of formulations containing excipient compounds and PEG

[00128] Materials: All materials were purchased from Sigma-Aldrich, St. Louis, MO. Formulations were prepared using an excipient compound and PEG, where the PEG was intended to simulate a therapeutic PEGylated protein that would be used in a therapeutic formulation. Such formulations were prepared by mixing equal volumes of a solution of PEG with a solution of the excipient. Both solutions were prepared in a Tris buffer consisting of 10 mM Tris, 135 mM NaCl, 1 mM trans-cinnamic acid at pH of 7.3.

[00129] The PEG solution was prepared by mixing 3 g of poly(ethylene oxide) average Mw ~1,000,000 (Aldrich Catalog # 372781) with 97 g of the Tris buffer solution. The mixture was stirred overnight for complete dissolution.

[00130] An example of the excipient solution preparation is as follows: An approximately 80 mg/mL solution of citric acid in the Tris buffer was prepared by dissolving 0.4 g of citric acid (Aldrich cat. # 251275) in 5 mL of the Tris buffer solution and adjusted the pH to 7.3 with minimum amount of 10 M NaOH solution.

[00131] The PEG excipient solution was prepared by mixing 0.5 mL of the PEG solution with 0.5 mL of the excipient solution and mixed by using a vortex for a few seconds. A control sample was prepared by mixing 0.5 mL of the PEG solution with 0.5 mL of the Tris buffer solution.

Example 13: Viscosity measurements of formulations containing excipient compounds and PEG

[00132] Viscosity measurements of the formulations prepared were made with a DV-IIT LV cone and plate viscometer (Brookfield Engineering, Middleboro, MA). The viscometer was equipped with a CP-40 cone and was operated at 3 rpm and 25°C. The formulation was loaded into the viscometer at a volume of 0.5 mL and allowed to incubate at the given shear rate and temperature for 3 minutes, followed by a measurement collection period of twenty seconds. This was then followed by 2 additional steps consisting of 1 minute of shear incubation and subsequent twenty second measurement collection period. The three data points collected were then averaged and recorded as the viscosity for the sample.

[00133] The results presented in Table 7 show the effect of the added excipient compounds in reducing viscosity.

TABLE 7

Test Number	Excipient	Excipient Concentration (mg/mL)	Viscosity (cP)	Viscosity Reduction
13.1	None	0	104.8	0%
13.2	Citric acid Na salt	40	56.8	44%
13.3	Citric acid Na salt	20	73.3	28%
13.4	glycerol phosphate	40	71.7	30%
13.5	glycerol phosphate	20	83.9	18%
13.6	Ethylene diamine	40	84.7	17%
13.7	Ethylene diamine	20	83.9	15%
13.8	EDTA/K salt	40	67.1	36%
13.9	EDTA/K salt	20	76.9	27%
13.10	EDTA/Na salt	40	68.1	35%
13.11	EDTA/Na salt	20	77.4	26%
13.12	D-Gluconic acid/K salt	40	80.32	23%
13.13	D-Gluconic acid/K salt	20	88.4	16%
13.14	D-Gluconic acid/Na salt	40	81.24	23%
13.15	D-Gluconic acid/Na salt	20	86.6	17%
13.16	lactic acid/K salt	40	80.42	23%

Test Number	Excipient	Excipient Concentration (mg/mL)	Viscosity (cP)	Viscosity Reduction
13.17	lactic acid/K salt	20	85.1	19%
13.18	lactic acid/Na salt	40	86.55	17%
13.19	lactic acid/Na salt	20	87.2	17%
13.20	etidronic acid/K salt	24	71.91	31%
13.21	etidronic acid/K salt	12	80.5	23%
13.22	etidronic acid/Na salt	24	71.6	32%
13.23	etidronic acid/Na salt	12	79.4	24%

Example 14: Preparation of PEGylated BSA with 1 PEG chain per BSA molecule

[00134] To a beaker was added 200 mL of a phosphate buffered saline (Aldrich Cat. # P4417) and 4 g of BSA (Aldrich Cat. # A7906) and mixed with a magnetic bar. Next 400 mg of methoxy polyethylene glycol maleimide, MW=5,000, (Aldrich Cat. # 63187) was added. The reaction mixture was allowed to react overnight at room temperature. The following day, 20 drops of HCl 0.1 M were added to stop the reaction. The reaction product was characterized by SDS-Page and SEC which clearly showed the PEGylated BSA. The reaction mixture was placed in an Amicon centrifuge tube with a molecular weight cutoff (MWCO) of 30,000 and concentrated to a few milliliters. Next the sample was diluted 20 times with a histidine buffer, 50 mM at a pH of approximately 6, followed by concentrating until a high viscosity fluid was obtained. The final concentration of the protein solution was obtained by measuring the absorbance at 280 nm and using a coefficient of extinction for the BSA of 0.6678. The results indicated that the final concentration of BSA in the solution was 342 mg/mL.

Example 15: Preparation of PEGylated BSA with multiple PEG chains per BSA molecule

[00135] A 5 mg/mL solution of BSA (Aldrich A7906) in phosphate buffer, 25 mM at pH of 7.2, was prepared by mixing 0.5 g of the BSA with 100 mL of the buffer. Next 1 g of a methoxy PEG propionaldehyde Mw=20,000 (JenKem Technology, Plano, TX 75024) was added followed by 0.12 g of sodium cyanoborohydride (Aldrich 156159). The reaction was allowed to proceed overnight at room temperature. The following day the reaction mixture was diluted 13 times with a Tris buffer (10 mM Tris, 135 mM NaCl at pH=7.3) and concentrated using Amicon centrifuge tubes MWCO of 30,000 until a concentration of approximately 150 mg/mL was reached.

Example 16: Preparation of PEGylated lysozyme with multiple PEG chains per lysozyme molecule

[00136] A 5 mg/mL solution of lysozyme (Aldrich L6876) in phosphate buffer, 25 mM at pH of 7.2, was prepared by mixing 0.5 g of the lysozyme with 100 mL of the buffer. Next 1 g of a methoxy PEG propionaldehyde Mw=5,000 (JenKem Technology, Plano, TX 75024) was added followed by 0.12 g of sodium cyanoborohydride (Aldrich 156159). The reaction was allowed to proceed overnight at room temperature. The following day the reaction mixture was diluted 49 times with the phosphate buffer, 25 mM at pH of 7.2, and concentrated using Amicon centrifuge tubes MWCO of 30,000. The final concentration of the protein solution was obtained by measuring the absorbance at 280 nm and using a coefficient of extinction for the lysozyme of 2.63. The final concentration of lysozyme in the solution was 140 mg/mL.

Example 17: Effect of excipients on viscosity of PEGylated BSA with 1 PEG chain per BSA molecule

[00137] Formulations of PEGylated BSA (from Example 14 above) with excipients were prepared by adding 6 or 12 milligrams of the excipient salt to 0.3 mL of the PEGylated BSA solution. The solution was mixed by gently shaking and the viscosity was measured by a RheoSense microVisc equipped with an A10 channel (100-micron depth) at a shear rate of 500 sec-1. The viscometer measurements were completed at ambient temperature.

[00138] The results presented in Table 8 shows the effect of the added excipient compounds in reducing viscosity.

TABLE 8

Test Number	Excipient	Excipient Concentration (mg/mL)	Viscosity (cP)	Viscosity Reduction
17.1	None	0	228.6	0%
17.2	Alpha-Cyclodextrin sulfated Na salt	20	151.5	34%
17.3	K acetate	40	89.5	60%

Example 18: Effect of excipients on viscosity of PEGylated BSA with multiple PEG chains per BSA molecule

[00139] A formulation of PEGylated BSA (from Example 15 above) with citric acid Na salt as excipient was prepared by adding 8 milligrams of the excipient salt to 0.2 mL of the PEGylated BSA solution. The solution was mixed by gently shaking and the viscosity was measured by a RheoSense microVisc equipped with an A10 channel (100 micron depth) at a shear rate of 500 sec-1. The viscometer measurements were completed at ambient temperature. The results presented in Table 9 shows the effect of the added excipient compounds in reducing viscosity.

TABLE 9

Test Number	Excipient Added	Excipient Concentration (mg/mL)	Viscosity (cP)	Viscosity Reduction
18.1	None	0	56.8	0%
18.2	Citric acid Na salt	40	43.5	23%

Example 19: Effect of excipients on viscosity of PEGylated lysozyme with multiple PEG chains per lysozyme molecule

[00140] A formulation of PEGylated lysozyme (from Example 16 above) with potassium acetate as excipient was prepared by adding 6 milligrams of the excipient salt to 0.3 mL of the PEGylated lysozyme solution. The solution was mixed by gently shaking and the viscosity was measured by a RheoSense microVisc equipped with an A10 channel (100 micron depth) at a shear rate of 500 sec-1. The viscometer measurements were completed at ambient temperature. The results presented in the next table shows the benefit of the added excipient compounds in reducing viscosity.

TABLE 10

Test Number	Excipient	Excipient Concentration (mg/mL)	Viscosity (cP)	Viscosity Reduction
19.1	None	0	24.6	0%
19.2	K acetate	20	22.6	8%

Example 20: Protein formulations containing excipient combinations

[00141] Formulations were prepared using an excipient compound or a combination of two excipient compounds and a test protein, where the test protein was intended to simulate a therapeutic protein that would be used in a therapeutic formulation. These formulations were prepared in 20 mM histidine buffer with different excipient compounds for viscosity measurement in the following way. Excipient combinations were dissolved in 20 mM histidine and the resulting solution pH adjusted with small amounts of sodium hydroxide or hydrochloric acid to achieve pH 6 prior to dissolution of the model protein. Excipient compounds for this Example are listed below in Table 11. Once excipient solutions had been prepared, the test protein bovine gamma globulin (BGG) was dissolved at a ratio to achieve a final protein concentration of about 280 mg/mL. Solutions of BGG in the excipient solutions were formulated in 5 mL sterile polypropylene tubes and allowed to shake at 80-100 rpm on an orbital shaker table overnight. BGG solutions were then transferred to 2 mL microcentrifuge tubes and centrifuged for about ten minutes at 2300 rpm in an IEC MicroMax microcentrifuge to remove entrained air prior to viscosity measurement.

[00142] Viscosity measurements of formulations prepared as described above were made with a DV-IIT LV cone and plate viscometer (Brookfield Engineering, Middleboro, MA). The viscometer was equipped with a CP-40 cone and was operated at 3 rpm and 25°C. The formulation was loaded into the viscometer at a volume of 0.5 mL and allowed to incubate at the given shear rate and temperature for 3 minutes, followed by a measurement collection period of twenty seconds. This was then followed by 2 additional steps consisting of 1 minute of shear incubation and subsequent twenty second measurement collection period. The three data points collected were then averaged and recorded as the viscosity for the sample. Viscosities of solutions with excipient were normalized to the viscosity of the model protein solution without excipient, and the results are shown in Table 11 below. The normalized viscosity is the ratio of the viscosity of the model protein solution with excipient to the viscosity of the model protein solution with no excipient.

TABLE 11

Test #	**Excipient A**		**Excipient B**		**Normalized Viscosity**
	Name	**Conc. (mg/mL)**	**Name**	**Conc. (mg/mL)**	
20.1	None	0	None	0	1.00
20.2	Aspartame	10	None	0	0.83
20.3	Saccharin	60	None	0	0.51
20.4	Acesulfame K	80	None	0	0.44
20.5	Theophylline	10	None	0	0.84
20.6	Saccharin	30	None	0	0.58
20.7	Acesulfame K	40	None	0	0.61
20.8	Caffeine	15	Taurine	15	0.82
20.9	Caffeine	15	Tyramine	15	0.67

Example 21: Protein formulations containing excipients to reduce viscosity and injection pain

[00143] Formulations were prepared using an excipient compound, a second excipient compound, and a test protein, where the test protein was intended to simulate a therapeutic protein that would be used in a therapeutic formulation. The first excipient compound, Excipient A, was selected from a group of compounds having local anesthetic properties. The first excipient, Excipient A and the second excipient, Excipient B are listed in Table 12. These formulations were prepared in 20 mM histidine buffer using Excipient A and Excipient B in the following way, so that their viscosities could be measured. Excipients in the amounts disclosed in Table 12 were dissolved in 20 mM histidine and the resulting solutions were pH adjusted with small amounts of sodium hydroxide or hydrochloric acid to achieve pH 6 prior to dissolution of the model protein. Once excipient solutions had been prepared, the test protein bovine gamma globulin (BGG) was dissolved in the excipient solution at a ratio to achieve a final protein concentration of about 280 mg/mL. Solutions of BGG in the excipient solutions were formulated in 5 mL sterile polypropylene tubes and allowed to shake at 80-100 rpm on an orbital shaker table overnight. BGG-excipient solutions were then transferred to 2 mL microcentrifuge tubes and centrifuged for about ten minutes at 2300 rpm in an IEC MicroMax microcentrifuge to remove entrained air prior to viscosity measurement.

[00144] Viscosity measurements of the formulations prepared as described above were made with a DV-IIT LV cone and plate viscometer (Brookfield Engineering, Middleboro, MA).

The viscometer was equipped with a CP-40 cone and was operated at 3 rpm and 25°C. The formulation was loaded into the viscometer at a volume of 0.5 mL and allowed to incubate at the given shear rate and temperature for 3 minutes, followed by a measurement collection period of twenty seconds. This was then followed by 2 additional steps consisting of 1 minute of shear incubation and subsequent twenty second measurement collection period. The three data points collected were then averaged and recorded as the viscosity for the sample. Viscosities of solutions with excipient were normalized to the viscosity of the model protein solution without excipient, and the results are shown in Table 12 below. The normalized viscosity is the ratio of the viscosity of the model protein solution with excipient to the viscosity of the model protein solution with no excipient.

TABLE 12

Test #	Excipient A		Excipient B		Normalized Viscosity
	Name	Conc. (mg/mL)	Name	Conc. (mg/mL)	
21.1	None	0	None	0	1.00
21.2	Lidocaine	45	None	0	0.73
21.3	Lidocaine	23	None	0	0.74
21.4	Lidocaine	10	Caffeine	15	0.71
21.5	Procaine HCl	40	None	0	0.64
21.6	Procaine HCl	20	Caffeine	15	0.69

Example 22: Formulations containing excipient compounds and PEG

[00145] Formulations were prepared using an excipient compound and PEG, where the PEG was intended to simulate a therapeutic PEGylated protein that would be used in a therapeutic formulation, and where the excipient compounds were provided in the amounts as listed in Table 13. These formulations were prepared by mixing equal volumes of a solution of PEG with a solution of the excipient. Both solutions were prepared in deionized (DI) Water.

[00146] The PEG solution was prepared by mixing 16.5 g of poly(ethylene oxide) average Mw ~100,000 (Aldrich Catalog # 181986) with 83.5 g of DI water. The mixture was stirred overnight for complete dissolution.

[00147] The excipient solutions were prepared by this general method and as detailed in Table 13 below: An approximately 20 mg/mL solution of potassium phosphate tribasic (Aldrich Catalog # P5629) in DI water was prepared by dissolving 0.05 g of potassium phosphate in 5 mL of DI water. The PEG excipient solution was prepared by mixing 0.5 mL of the PEG solution with 0.5 mL of the excipient solution and mixed by using a vortex for a few seconds. A control sample was prepared by mixing 0.5 mL of the PEG solution with 0.5 mL of DI water. Viscosity was measured and results are recorded in Table 13 below.

TABLE 13

Test Number	Excipient	Excipient Concentration (mg/mL)	Viscosity (cP)	Viscosity Reduction (%)
22.1	None	0	79.7	0
22.2	Citric acid Na salt	10	74.9	6.0
22.3	Potassium phosphate	10	72.3	9.3
22.4	Citric acid Na salt/Potassium phosphate	10/10	69.1	13.3
22.5	Sodium sulfate	10	75.1	5.8
22.6	Citric acid Na salt/Sodium sulfate	10/10	70.4	11.7

Example 23: Improved processing of protein solutions with excipients

[00148] Two BGG solutions were prepared by mixing 0.25 g of solid BGG with 4 ml of a buffer solution. For Sample A: Buffer solution was 20 mM histidine buffer (pH=6.0). For sample B: Buffer solution was 20 mM histidine buffer containing 15 mg/ml of caffeine (pH=6). The dissolution of the solid BGG was carried out by placing the samples in an orbital shaker set at 100 rpm. The buffer sample containing caffeine excipient was observed to dissolve the protein faster. For the sample with the caffeine excipient (Sample B) complete dissolution of the BGG was achieved in 15 minutes. For the sample without the caffeine (Sample A) the dissolution needed 35 minutes.

[00149] Next the samples were placed in 2 separate Amicon Ultra 4 Centrifugal Filter Units with a 30,000 molecular weight cut off and the samples were centrifuged at 2,500 rpm at 10 minutes intervals. The filtrate volume recovered after each 10 minute centrifuge run was recorded. The results in Table 14 show the faster recovery of the filtrate for Sample B. In addition, Sample B kept concentrating with every additional run but Sample A reached a maximum concentration point and further centrifugation did not result in further sample concentration.

TABLE 14

Centrifuge time (min)	Sample A filtrate collected (mL)	Sample B filtrate collected (mL)
10	0.28	0.28
20	0.56	0.61
30	0.78	0.88
40	0.99	1.09
50	1.27	1.42
60	1.51	1.71
70	1.64	1.99
80	1.79	2.29
90	1.79	2.39
100	1.79	2.49

Example 24: Protein formulations containing multiple excipients

[00150] This example shows how the combination of caffeine and arginine as excipients has a beneficial effect on decreasing viscosity of a BGG solution. Four BGG solutions were prepared by mixing 0.18 g of solid BGG with 0.5 mL of a 20 mM Histidine buffer at pH 6. Each buffer solution contained different excipient or combination of excipients as described in the table below. The viscosity of the solutions was measured as described in previous examples. The results show that the hindered amine excipient, caffeine, can be combined with known excipients such as arginine, and the combination has better viscosity reduction properties than the individual excipients by themselves.

TABLE 15

Sample	Excipient added	Viscosity (cP)	Viscosity Reduction (%)
A	None	130.6	0
B	Caffeine (10mg/ml)	87.9	33

Sample	Excipient added	Viscosity (cP)	Viscosity Reduction (%)
C	Caffeine (10mg/ml) / Arginine (25 mg/ml)	66.1	49
D	Arginine (25 mg/ml)	76.7	41

[00151] Arginine was added to 280 mg/mL solutions of BGG in histidine buffer at pH 6. At levels above 50 mg/mL, adding more arginine did not decrease viscosity further, as shown in Table 16.

TABLE 16

Arginine added (mg/mL)	Viscosity (cP)	Viscosity reduction (%)
0	79.0	0%
53	40.9	48%
79	46.1	42%
105	47.8	40%
132	49.0	38%
158	48.0	39%
174	50.3	36%
211	51.4	35%

[00152] Caffeine was added to 280 mg/mL solutions of BGG in histidine buffer at pH 6. At levels above 10 mg/ml, adding more caffeine did not decrease viscosity further, as shown in Table 17.

TABLE 17

Caffeine added (mg/mL)	Viscosity (cP)	Viscosity reduction (%)
0	79	0%
10	60	31%
15	62	23%
22	50	45%

Example 25: Caffeine effect during TFF concentration process

[00153] In this Example, bovine gamma globulin (BGG) solutions were concentrated in the presence and absence of caffeine using tangential flow filtration (TFF). The Labscale TFF System, produced by EMD Millipore (Billerica, MA) was used to perform the experiments. The system was fitted with a Pellicon XL TFF cassette that contained an Ultracel membrane with 30 kDa molecular weight cutoff (EMD Millipore, Billerica, MA). The nominal membrane surface area was 50 cm^2. The feed pressure to the cassette was maintained at 30 psi while the retentate pressure was maintained at 10 psi. The filtrate flux was monitored over the course of the experiment by measuring its mass as a function of time. Approximately 12 grams of BGG were dissolved into 500 mL of buffer containing 15 mg/mL caffeine, 150 mM NaCl, and 20 mM histidine, adjusted to pH 6. A control sample was prepared by dissolving 12 grams of BGG into 500 mL of buffer containing 150 mM NaCl, and 20 mM histidine, adjusted to pH 6. The buffer components were purchased from Sigma-Aldrich. Both solutions were filtered through a 0.2 μm PES filter (VWR, Radnor, PA) prior to TFF processing. The performance of the test sample and control sample during TFF were measured by the mass transfer coefficient. The mass transfer coefficient was determined for each sample using the following equation (as described in J. Hung, A. U. Borwankar, B. J. Dear, T. M. Truskett, K. P. Johnston, High concentration tangential flow ultrafiltration of stable monoclonal antibody solutions with low viscosities. J. Memb. Sci. 508, 113–126 (2016)):

$$J = k_c \ln(C_w/C_b) \qquad \text{(Eq. 3)}$$

[00154] Eq. 3 describes the filtrate flux J, where k_c is the mass transfer coefficient, C_w is the protein concentration in the vicinity of the membrane, and C_b is the concentration in the liquid bulk, and Eq. 3 thereby permits calculation of the mass transfer coefficient k_c. A graph of the calculated flux J against the $\ln(C_b)$ yields a linear plot with slope of -kc. Here the flux J is calculated by taking the derivative of the filtrate mass with respect to time and C_b is calculated using a mass-balance. The best-fit mass transfer coefficients are listed in Table 18. The introduction of 15 mg/mL caffeine increased the value of the mass transfer coefficient by ~ 13%, from 22.5 to 25.4 Lm^{-2}hr^{-1} (LMH).

TABLE 18

Sample	Mass Transfer Coefficient k_c (LMH)
Control	22.5 ± 0.1
15 mg/mL caffeine	25.4 ± 0.1

Example 26: Caffeine effect during TFF concentration process

[00155] In this Example, bovine gamma globulin (BGG) solutions were concentrated in the presence and absence of caffeine using tangential flow filtration (TFF). The Labscale TFF System, produced by EMD Millipore (Billerica, MA) was used to perform the experiments. The system was fitted with a Pellicon XL TFF cassette that contained an Ultracel membrane with 30 kDa molecular weight cutoff (EMD Millipore, Billerica, MA). The nominal membrane surface area was 50 cm^2. A control sample was prepared by dissolving 14.6 grams of BGG into 582 mL of buffer containing 150 mM NaCl, and 20 mM histidine, adjusted to pH 6, such that the initial BGG concentration was nominally 25.1 mg/mL. The material was filtered through a 0.2 μm PES filter (VWR, Radnor, PA) and then processed in the TFF device. The pump speed was adjusted such that the feed pressure was initially 30 psi and the retentate valve was adjusted such that the retentate pressure was initially 10 psi. The material was concentrated without adjusting either the pump speed or retentate valve for 4.1 hours. The initial and final concentrations were determined to be 25.4 ± 0.6 and 159 ± 6 mg/mL, respectively, by a Bradford assay, as shown in Table 19 below. A caffeine-containing sample was prepared by dissolving 14.2 g of BGG into 566 mL of buffer containing 15 mg/mL caffeine, 150 mM NaCl, and 20 mM histidine, adjusted to pH 6, such that the initial BGG concentration was nominally 25.1 mg/mL. The material was filtered through a 0.2 μm PES filter (VWR, Radnor, PA) and then processed in the TFF device. The pump speed and retentate valve were set to identical levels to those previously. The feed and retentate pressures were confirmed to be 30 psi and 10 psi, respectively, as previously. The material was concentrated without adjusting either the pump speed or retentate valve for 4.1 hours. The initial and final concentrations were determined to be 24.4 ± 0.5 and 225 ±10 mg/mL, respectively, by a Bradford assay, as shown in Table 19 below. The use of caffeine during TFF processing increased the final protein concentration by approximately 42% when compared to the control, from 159 to 225 mg/mL.

TABLE 19

Sample	Initial concentration (mg/mL)	Final concentration (mg/mL)
Control	25.4 ± 0.6	159 ± 6
15 mg/mL caffeine	24.4 ± 0.5	225 ± 10

Example 27: Caffeine effect during sterile filtration of BGG solutions

[00156] Bovine gamma globulin (BGG), L-histidine, and caffeine were purchased from Sigma-Aldrich (St. Louis, MO, product numbers G5009, H6034, and C7731, respectively). Deionized (DI) water was generated from tap water with a Direct-Q 3 UV purification system from EMD Millipore (Billerica, MA). 25-mm polyethersulfone (PES) filters with 0.2-μm pores were purchased from GE Healthcare (Chicago, IL, catalog number 6780-2502). 1-mL Luer-Lok syringes were purchased from Becton, Dickinson and Company (Franklin Lakes, NJ, reference number 309628). A 20-mM histidine buffer, pH 6.0 was prepared using L-histidine, DI water, and titrated to pH 6.0 with 1 M HCl. A 15 mg/mL solution of caffeine was prepared using the histidine buffer. The caffeine-free and caffeine-containing buffers were used to reconstitute BGG to a final concentration of about 280 mg/mL. The protein concentration, c, was calculated using:

$$c = \frac{m_p}{b + v m_p} \qquad \text{(Eq. 4)}$$

where m_p is the protein mass, b is the volume of buffer added, and v is the partial specific volume of BGG, here taken to be 0.74 mL/g. The viscosity of each sample was measured using microVisc rheometer (RheoSense, San Ramon, CA) at a temperature of 23°C and shear rate of 250 s^{-1}. The energies required to pass the BGG solutions through the sterile filters were measured using a Tensile Compression Tester (TCT, Instron, Needham, MA, part number 3343) fitted with a 100 N load cell (Instron, Needham, MA, part number 2519-103). The syringe plungers were depressed at a rate of 159 mm/min for a distance of 50 mm. The energy requirements were calculated by integrating the load-versus-extension curves measured by the TCT, and results are summarized in Table 20 below.

TABLE 20

Sample	Protein concentration (mg/mL)	Caffeine concentration (mg/mL)	Viscosity (cP)	Energy requirement (mJ)
1	280	0	106	198
2	280	15.1	68.9	181

Example 28: Excipients to improve Protein-A chromatography elution

[00157] Four purified, research-grade biosimilar antibodies, ipilimumab, ustekinumab, omalizumab, and tocilizumab were purchased from Bioceros (Utrecht, The Netherlands). They were provided as frozen aliquots at protein concentrations of 20, 26, 15 and 23 mg/mL, respectively, in an aqueous 40 mM sodium acetate, 50 mM tris-HCl buffer at pH 5.5. The protein solutions were thawed at room temperature prior to measurement and afterwards, were filtered through a 0.2 μm polyethersulfone filters. The filtered protein stock solutions were mixed in 1:1 ratio of protein stock solution to a binding buffer. The binding buffer, used to promote the binding of the antibodies to the Protein-A resin, was composed of 0.1 M sodium phosphate and 0.15 sodium chloride at pH 7.2 in deionized (DI) water. The DI water was produced by purifying tap water with a Direct-Q 3 UV purification system from EMD Millipore (Billerica, MA). These solutions were employed to perform Protein-A binding and elution studies using a Pierce™ Protein-A Spin Plate for IgG Screening (ThermoFisher Scientific catalog # 45202). The plate had 96 wells, each containing 50 μL of Protein-A resin. The resin was washed with binding buffer by adding 200 μL of binding buffer to each well and centrifuging the plate at 1000 x g for 1 minute and discarding the flow-through. All subsequent centrifugation steps were performed at 1000 x g for 1 minute. This wash procedure was repeated once. Following these initial washing steps, the diluted protein samples, i.e., samples containing ipilimumab, ustekinumab, omalizumab, and tocilizumab, were added to the wells in the plate (200 μL per well). The plate was then placed on a Daigger Scientific (Vernon Hills, IL) Labgenius orbital shaker and agitated at 260 rpm for 30 minutes, following which the plate was centrifuged and the flow-through was discarded. The wells were then washed by adding 500 μL of binding buffer to each well, centrifuging the plate and discarding the flow-through. This wash step was repeated twice. After these washing steps, the proteins were eluted from the plate using elution buffers to which different excipients had been added. For each elution, 50 μL of a neutralization buffer consisting of 1

M sodium phosphate at pH 7 was added to each well of the collection plate, and then two hundred μL of elution buffer was added to each well of the plate. The plate was agitated at 260 rpm for 1 minute and then centrifuged. The flow-through was recovered for analysis. This elution step was repeated once. The control buffer, with no excipients, contained 20 mM citrate and had a pH of 2.6. Because Protein-A elution buffers often contain some amount of salt, an elution buffer of 100 mM NaCl in the citrate buffer was prepared as a secondary control.

[00158] Table 21 lists the excipient solutions used in this example, their concentrations, and final pH of the elution buffers. All excipients were purchased from Sigma Aldrich (St. Louis, MO), with the exception of aspartame, which was purchased from Herb Store USA (Los Angeles, CA), trehalose, which was purchased from Cascade Analytical Reagents and Biochemicals (Corvallis, OR), and sucrose which was purchased from Research Products International (Mt. Prospect, IL, product number S24060). All excipient-containing elution buffers were prepared by mixing the appropriate quantity of the excipient with approximately 10 mL of the salt-free citrate buffer control. The elution buffers were prepared at approximately 100 mM excipient. However, not all of the excipients are soluble at this level; Table 21 therefore lists all of the excipient concentrations that were used. The pH of each elution buffer was adjusted to about 2.6 ± 0.1 using either hydrochloride or sodium hydroxide as needed.

[00159] For each protein sample, ASD High performance size-exclusion chromatography (SEC) analysis was performed using a TSKgel SuperSW3000 column (30 cm x 4.6 mm ID, Tosoh Bioscience, King of Prussia, PA) connected to an HPLC workstation (Agilent HP 1100 system). The separation was carried out at a flow of 0.35 mL/min at room temperature. The mobile phase was an aqueous buffer of 100 mM sodium phosphate, 300 mM sodium chloride, pH 7. The protein concentration was monitored by absorbance at 280 nm using an Agilent 1100 Series G1315B diode array detector. The total amount of protein eluted from the Protein-A resin for each protein, i.e., ipilimumab, ustekinumab, omalizumab, and tocilizumab, was estimated by integrating the chromatograms. The integrated peak areas for each protein, i.e., ipilimumab, ustekinumab, omalizumab, and tocilizumab, are listed in Tables 22-25. Tables 22-25 also compare the experimental peak areas to those of the salt-free and salt-containing controls. Values greater than 100% indicate that the elution buffer recovered more protein from the Protein-A resin than the control whereas values less than 100% indicate that the elution buffer recovered less protein from the Protein-A resin than the control.

Table 21. Excipients used in Example 28

Excipient	Sigma-Aldrich product number for excipient	Excipient concentration (mM)	pH
caffeine	C7731	79	2.6
acesulfame potassium	04054	110	2.5
1-methyl-2-pyrrolidone	M6762	117	2.6
aspartame	N/A	20	2.6
taurine	T8691	114	2.5
trehalose	N/A	100	2.7
sucrose	N/A	101	2.7
niacinamide	N5535	99	2.7
sodium chloride control	S7653	117	2.6
control	N/A	N/A	2.5

Table 22. Ipilimumab recovery from Protein-A resin

Excipient	Peak area (mAU*min)	Peak area normalized to salt-free control (%)	Peak area normalized to salt control (%)
citrate	3409	77.9	83.6
Acesulfame potassium	1567	35.8	38.4
1-methyl-2-pyrrolidone	386	8.8	9.5
aspartame	4012	91.7	98.3
taurine	3958	90.4	97.0
trehalose	3667	83.8	89.9
sucrose	4585	104.8	112.4
niacinamide	4295	98.2	105.3
sodium chloride control	4080	93.2	100.0
control	4376	100.0	107.2

Table 23. Ustekinumab recovery from Protein-A resin

Excipient	Integrated peak area (mAU*min)	Peak area normalized to salt-free control (%)	Peak area normalized to salt control (%)
caffeine	2301	86.6	75.2
acesulfame potassium	307	16.2	14.0
aspartame	417	17.4	15.1
1-methyl-2-pyrrolidone	2952	108.8	94.4
taurine	3257	118.6	103.0
trehalose	1549	56.6	49.1
sucrose	1274	51.2	44.4
niacinamide	3204	116.1	100.8
sodium chloride control	3176	115.2	100.0

Table 24. Omalizumab recovery from Protein-A resin

Excipient	Integrated peak area (mAU*min)	Peak area normalized to salt-free control (%)	Peak area normalized to salt control (%)
caffeine	4040	105.5	117.5
acesulfame potassium	3620	94.5	105.3
1-methyl-2-pyrrolidone	3334	87.0	97.0
aspartame	3605	94.1	104.8
taurine	4337	113.2	126.1
trehalose	3571	93.2	103.8
sucrose	3639	95.0	105.8
niacinamide	4812	125.6	139.9
sodium chloride control	3439	89.8	100.0
control	3831	100.0	111.4

Table 25. Tocilizumab recovery from Protein-A resin

Excipient	Integrated peak area (mAU*min)	Peak area normalized to salt-free control (%)	Peak area normalized to salt control (%)
caffeine	3120	111.2	100.3
acesulfame potassium	3083	109.9	99.1
1-methyl-2-pyrrolidone	261	9.3	8.4
aspartame	556	19.8	17.9
taurine	3054	108.8	98.2
trehalose	2781	99.1	89.4
sucrose	1037	37.0	33.3
niacinamide	2550	90.9	82.0
sodium chloride control	3111	110.9	100.0
control	2806	100.0	90.2

Example 29: Excipients to improve Protein-A chromatography elution

[00160] The test proteins used in this Example are identical to those in Example 28, i.e., ipilimumab, ustekinumab, omalizumab, and tocilizumab. Protein-A binding and elution studies were performed using an identical plate to that in Example 28. The methods for loading and eluting the antibodies from the Protein-A plate were identical to those in Example 28 with the exception of the elution step. In Example 28, two elution washes were performed. However, in this Example, only one wash is performed. As in Example 28, elution buffers were prepared from a 20 mM citrate, pH 2.6 control buffer. The elution buffers are listed in Table 26 below. All of the excipients were purchased from Sigma-Aldrich (St. Louis, MO). The recovered protein was analyzed by HPLC in an identical fashion to that in Example 28, and results of protein recovery for each protein, i.e., ipilimumab, ustekinumab, omalizumab, and tocilizumab, are documented in Tables 27-30 below.

Table 26. Excipients used in Example 29

Excipient	Sigma-Aldrich product number	Excipient concentration (mM)	pH
control	N/A	N/A	2.5
sodium chloride control	S7653	117	2.6
niacinamide	N5535	99	2.7
taurine	T8691	114	2.5
imidazole	I5513	100	2.6
4-hydroxybenzesulfonic acid	171506	107	2.6
Caffeine	C7731	79	2.6

Table 27. Ipilimumab recovery from Protein-A resin

Excipient	Peak area (mAU*min)	Peak area normalized to salt-free control (%)	Peak area normalized to salt control (%)
control	4841	100.0	88.3
sodium chloride control	5485	113.3	100.0
niacinamide	6300	130.1	114.8
taurine	7557	156.1	137.8
imidazole	6071	125.4	110.7
4-hydroxybenzesulfonic acid	5836	120.6	106.4
caffeine	6051	125.0	110.3

Table 28. Ustekinumab recovery from Protein-A resin

Excipient	Integrated peak area (mAU*min)	Peak area normalized to salt-free control (%)	Peak area normalized to salt control (%)
control	4572	100.0	107.9
sodium chloride control	4238	92.7	100.0
niacinamide	5848	127.9	138.0
taurine	4744	103.8	112.0
imidazole	4617	101.0	108.9
4-hydroxybenzesulfonic acid	4132	90.4	97.5
caffeine	5084	111.2	120.0

Table 29. Omalizumab recovery from Protein-A resin

Excipient	Integrated peak area (mAU*min)	Peak area normalized to salt-free control (%)	Peak area normalized to salt control (%)
control	4194	100.0	91.7
sodium chloride control	4574	109.1	100.0
niacinamide	5748	137.0	125.7
taurine	4676	111.5	102.2
imidazole	2589	61.7	56.6
4-hydroxybenzesulfonic acid	3190	76.1	69.7
caffeine	5807	138.5	127.0

Table 30. Tocilizumab recovery from Protein-A resin

Excipient	Integrated peak area mAU*min)	Peak area normalized to salt-free control (%)	Peak area normalized to salt control (%)
control	4667	100.0	97.5
sodium chloride control	4786	102.6	100.0
niacinamide	5225	111.9	109.2
taurine	5396	115.6	112.7
imidazole	4754	101.9	99.3
4-hydroxybenzesulfonic acid	4539	97.3	94.8
caffeine	5656	121.2	118.2

Example 30: Excipients that improve omalizumab elution from Protein-A chromatography column

[00161] Research-grade omalizumab was purchased from Bioceros (Utrecht, The Netherlands) and provided frozen at 15 mg/mL in an aqueous 40 mM sodium acetate, 50 mM tris-HCl buffer, pH 5.5. The protein was thawed at room temperature prior to experiments and filtered through a 0.2 µm polyethersulfone filter. The filtered material was mixed in a 1:1 ratio with a binding buffer that consisted of 20 mM sodium phosphate, pH 7 in DI water. Tap water was purified with a Direct-Q 3 UV purification system from EMD Millipore (Billerica, MA) to produce the DI water. Protein-A purification was performed using a HiTrap Protein-A HP 1 mL column from GE Healthcare (Chicago, IL, product number 29048576). For each experiment, the column was first equilibrated with 10 mL of binding buffer. Following equilibration, 30 mg of protein were loaded onto the Protein-A column. The column was then washed with 5 mL of binding buffer. After washing the column, bound omalizumab was eluted from the column using fractions of one of the elution buffers listed in Table 31 below. The elution buffers were prepared by dissolving the indicated excipients in a 20 mM citrate buffer, pH 4.0. All elution buffers were adjusted to pH 4.0. Five 1-mL fractions were collected. Finally, Protein-A was regenerated by washing the column with 5 mL of 100 mM citrate, pH 3.0 buffer. The flowrate for each step was 1 mL/min, which was maintained by a Fusion 100 infusion pump (Chemyx, Stafford, TX). 10-mL NormJect Luer Lok syringes were used (Henke Sass Wolf, Tuttlingen, Germany, reference number 4100-000V0).

[00162] Elution fractions, E1, E2, E3, E4, and E5, were assayed for total protein content by high performance size-exclusion chromatography (SEC) analysis. SEC analysis was performed using a TSKgel SuperSW3000 column (30 cm x 4.6 mm ID, Tosoh Bioscience, King of Prussia, PA) connected to an HPLC workstation (Agilent HP 1100 system). The separation was carried out at a flow of 0.35 mL/min at room temperature. The mobile phase was an aqueous buffer of 100 mM sodium phosphate, 300 mM sodium chloride, pH 7. The protein concentration was monitored by absorbance at 280 nm using an Agilent 1100 Series G1315B diode array detector. The total amount of protein eluted from the Protein-A resin was estimated by integrating the chromatograms.

[00163] Citrate is a common excipient used in Protein-A chromatography and was therefore used here as a control. The eluate fractions for the control sample exhibited insoluble aggregates on storage overnight at 4°C as evidenced by the formation of a precipitate phase. Therefore, the peak areas reported in Table 31 below represent the total soluble protein amounts in the eluate fractions. We note that insoluble aggregates were only observed in the control sample and none of the other samples exhibited such aggregates. Peak areas greater than that of the control (using the citrate excipient) indicate that the use of the test excipient can enable a more efficient separation of protein from the column.

Table 31. Omalizumab elution from Protein-A column

Elution excipient	Elution excipient concentration (mM)	E1 peak area (mAU*min)	E2 peak area (mAU*min)	E3 peak area (mAU*min)	E4 peak area (mAU *min)	E5 peak area (mAU* min)	Total peak area (mAU *min)
citrate (control)	103	352	9670	4098	4245	2953	21318
imidazole	100	236	10224	7373	3894	2620	24348
taurine	125	408	17018	7676	3349	2211	30662
niacinamide	102	228	14492	5307	2914	2014	24955
caffeine	81	617	21965	8069	3301	1911	35863

Example 31: Formulations of BGG with different amounts of caffeine excipient

[00164] Formulations were prepared with different molar concentrations of caffeine (at concentrations listed in Table 32 below) and a test protein, where the test protein was intended to simulate a therapeutic protein that would be used in a therapeutic formulation. The formulations for this Example were prepared in 20 mM histidine buffer for viscosity measurement in the following way. Stock solutions of 0 and 80 mM caffeine were prepared in 20 mM histidine and the resulting solution pH adjusted with small amounts of sodium hydroxide or hydrochloric acid to achieve pH 6 prior to dissolution of the model protein. Additional solutions at various caffeine concentrations were prepared by blending the two stock solutions at various volume ratios, to provide a series of caffeine-containing solutions, at concentrations listed in Table 32 below. Once these excipient solutions had been prepared, the test protein bovine gamma globulin (BGG) was dissolved into each test solution at a ratio to achieve a final protein concentration of about 280 mg/mL by adding 0.7 mL of each excipient solution to 0.25 g lyophilized BGG powder. The BGG-containing solutions were formulated in 5 mL sterile polypropylene tubes and allowed to shake at 100 rpm on an orbital shaker table overnight. These solutions were then transferred to 2 mL microcentrifuge tubes and centrifuged for about five minutes at 2400 rpm in an IEC MicroMax microcentrifuge to remove entrained air prior to viscosity measurement.

[00165] Viscosity measurements of formulations prepared as described above were made with a microVisc viscometer (RheoSense, San Ramon, CA). The viscometer was equipped with an A-10 chip having a channel depth of 100 microns, and was operated at a shear rate of 250 1/s and 25°C. To measure viscosity, the test formulation was loaded into the viscometer, taking care to remove all air bubbles from the pipet. The pipet containing the loaded sample formulation was placed in the instrument and allowed to incubate at the measurement temperature for about five minutes. The instrument was then run until the channel was fully equilibrated with the test fluid, indicated by a stable viscosity reading, and then the viscosity recorded in centipoise. Viscosity results that were obtained are presented in Table 32 below.

TABLE 32

Caffeine conc (mM)	Viscosity (cP)	Normalized Viscosity
0	83	1.00
5	67	0.81
10	70	0.84
20	77	0.92
30	63	0.76
40	65	0.78
50	65	0.78
60	57	0.69
70	50	0.60
80	50	0.60

Example 32: Preparation of solutions of co-solutes in deionized water

[00166] Compounds used as co-solutes to increase caffeine solubility in water were obtained from Sigma-Aldrich (St. Louis, MO) and included niacinamide, proline, procaine HCl, ascorbic acid, 2,5-dihydroxybenzoic acid, lidocaine, saccharin, acesulfame K, tyramine, and aminobenzoic acid. Solutions of each co-solute were prepared by dissolving dry solid in deionized water and in some cases adjusting the pH to a value between pH of about 6 and pH of about 8 with 5 M hydrochloric acid or 5 M sodium hydroxide as necessary. Solutions were then diluted to a final volume of either 25 mL or 50 mL using a Class A volumetric flask and concentration recorded based on the mass of compound dissolved and the final volume of the solution. Prepared solutions were used either neat or diluted with deionized water.

Example 33: Caffeine solubility testing

[00167] The impact of different co-solutes on the solubility of caffeine at ambient temperature (about 23°C) was assessed in the following way. Dry caffeine powder (Sigma-Aldrich, St. Louis, MO) was added to 20 mL glass scintillation vials and the mass of caffeine recorded. 10 mL of a co-solute solution prepared in accordance with Example 32 was added to the caffeine powder in certain cases; in other cases, a blend of a co-solute solution and deionized water was added to the caffeine powder, maintaining a final addition volume of 10 mL. The volume contribution of the dry caffeine powder was assumed to be negligible in any of these mixtures. A small magnetic stir bar was added to the vial, and the solution was allowed to mix vigorously on a stir plate for about 10 minutes. After about 10 minutes the vial was observed for dissolution of the dry caffeine powder, and the results are given in Table 33 below. These observations indicated that niacinamide, procaine HCl, 2,5-dihydroxybenzoic acid sodium salt, saccharin sodium salt, and tyramine chloride salt all enabled dissolution of caffeine to at least about four times the reported caffeine solubility limit (~16 mg/mL at room temperature according to Sigma-Aldrich).

TABLE 33

Test No.	**Co-solute**		**Caffeine**	**Observation**
	Name	Conc. (mg/mL)	(mg/mL)	
33.1	Proline	100	50	DND
33.2	Niacinamide	100	50	CD
33.3	Niacinamide	100	60	CD
33.4	Niacinamide	100	75	CD
33.5	Niacinamide	100	85	CD
33.6	Niacinamide	100	100	CD
33.7	Niacinamide	80	85	CD
33.8	Niacinamide	50	80	CD
33.9	Procaine HCl	100	85	CD
33.10	Procaine HCl	50	80	CD
33.11	Niacinamide	30	80	DND
33.12	Procaine HCl	30	80	DND
33.13	Niacinamide	40	80	MD
33.14	Procaine HCl	40	80	DND
33.15	Ascorbic acid, Na	50	80	DND
33.16	Ascorbic acid, Na	100	80	DND
33.17	2,5 DHBA, Na	40	80	CD
33.18	2,5 DHBA, Na	20	80	MD
33.19	Lidocaine HCl	40	80	DND
33.20	Saccharin, Na	90	80	CD
33.21	Acesulfame K	80	80	DND
33.22	Tyramine HCl	60	80	CD

Test No.	**Co-solute**		**Caffeine**	**Observation**
	Name	Conc. (mg/mL)	(mg/mL)	
33.23	Na Aminobenzoate	46	80	DND
33.24	Saccharin, Na	45	80	DND
33.25	Tyramine HCl	30	80	DND

CD=completely dissolved; MD=mostly dissolved; DND=did not dissolve

Example 34: Profile of HUMIRA®

[00168] HUMIRA® (AbbVie Inc., Chicago, IL) is a commercially available formulation of the therapeutic monoclonal antibody adalimumab, a TNF-alpha blocker typically prescribed to reduce inflammatory responses of autoimmune diseases such as rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, moderate to severe chronic psoriasis and juvenile idiopathic arthritis. HUMIRA® is sold in 0.8 mL single use doses containing 40 mg of adalimumab, 4.93 mg sodium chloride, 0.69 mg sodium phosphate monobasic dihydrate, 1.22 mg sodium phosphate dibasic dihydrate, 0.24 mg sodium citrate, 1.04 mg citric acid monohydrate, 9.6 mg mannitol and 0.8 mg polysorbate 80. A viscosity vs. concentration profile of this formulation was generated in the following way. An Amicon Ultra 15 centrifugal concentrator with a 30 kDa molecular weight cut-off (EMD-Millipore, Billerica, MA) was filled with about 15 mL of deionized water and centrifuged in a Sorvall Legend RT (ThermoFisher Scientific) at 4000 rpm for 10 minutes to rinse the membrane. Afterwards the residual water was removed and 2.4 mL of HUMIRA® liquid formulation was added to the concentrator tube and was centrifuged at 4000 rpm for 60 minutes at 25°C. Concentration of the retentate was determined by diluting 10 microliters of retentate with 1990 microliters of deionized water, measuring absorbance of the diluted sample at 280 nm, and calculating the concentration using the dilution factor and extinction coefficient of 1.39 mL/mg-cm. Viscosity of the concentrated sample was measured with a microVisc viscometer equipped with an A05 chip (RheoSense, San Ramon, CA) at a shear rate of 250 sec^{-1} at 23°C. After viscosity measurement, the sample was diluted with a small amount of filtrate and concentration and viscosity measurements were repeated. This process was used to generate viscosity values at varying adalimumab concentrations, as set forth in Table 34 below.

TABLE 34

Adalimumab concentration (mg/mL)	Viscosity (cP)
277	125
253	63
223	34
202	20
182	13

Example 35: Reformulation of HUMIRA® with viscosity-reducing excipient

[00169] The following example describes a general process by which HUMIRA® was reformulated in buffer with viscosity-reducing excipient. A solution of the viscosity-reducing excipient was prepared in 20 mM histidine by dissolving about 0.15 g histidine and 0.75 g caffeine (Sigma-Aldrich, St. Louis, MO) in deionized water. The pH of the resulting solution was adjusted to about 5 with 5 M hydrochloric acid. The solution was then diluted to a final volume of 50 mL in a volumetric flask with deionized water. The resulting buffered viscosity-reducing excipient solution was then used to reformulate HUMIRA® at high mAb concentrations. Next, about 0.8 mL of HUMIRA® was added to a rinsed Amicon Ultra 15 centrifugal concentrator tube with a 30 kDa molecular weight cutoff and centrifuged in a Sorvall Legend RT at 4000 rpm and 25°C for 8-10 minutes. Afterwards about 14 mL of the buffered viscosity-reducing excipient solution prepared as described above was added to the concentrated HUMIRA® in the centrifugal concentrator. After gentle mixing, the sample was centrifuged at 4000 rpm and 25°C for about 40-60 minutes. The retentate was a concentrated sample of HUMIRA® reformulated in a buffer with viscosity-reducing excipient. Viscosity and concentration of the sample were measured, and in some cases then diluted with a small amount of filtrate to measure viscosity at a lower concentration. Viscosity measurements were completed with a microVisc viscometer in the same way as with the concentrated HUMIRA® formulation in the previous example. Concentrations were determined with a Bradford assay using a standard curve generated from HUMIRA® stock solution diluted in deionized water. Reformulation of HUMIRA® with the viscosity-reducing excipient gave viscosity reductions of 30% to 60% compared to the viscosity values of HUMIRA® concentrated in the commercial buffer without reformulation, as set forth in Table 35 below.

TABLE 35

Adalimumab concentration (mg/mL)	**Viscosity (cP)**
290	61
273	48
244	20
205	14

Example 36: Improved stability of adalimumab solutions with caffeine as excipient

[00170] The stability of adalimumab solutions with and without caffeine excipient was evaluated after exposing samples to 2 different stress conditions: agitation and freeze-thaw. The adalimumab drug formulation HUMIRA® (AbbVie) was used, having properties described in more detail in Example 34. The HUMIRA® sample was concentrated to 200 mg/mL adalimumab concentration in the original buffer solution as described in Example 39; this concentrated sample is designated "Sample 1." A second sample was prepared with ~200 mg/mL of adalimumab and 15 mg/mL of added caffeine as described in Example 40; this concentrated sample with added caffeine is designated "Sample 2." Both samples were diluted to a final concentration of 1 mg/mL adalimumab with the diluents as follows: Sample 1 diluent is the original buffer solution, and Sample 2 diluent is a 20 mM histidine, 15 mg/mL caffeine, pH=5. Both HUMIRA® dilutions were filtered through a 0.22 µm syringe filter. For every diluted sample, 3 batches of 300 µL each were prepared in a 2 mL Eppendorf tube in a laminar flow hood. The samples were submitted to the following stress conditions: for agitation, samples were placed in an orbital shaker at 300 rpm for 91 hours; for freeze-thaw, samples were cycled 7 times from -17 to 30°C for an average of 6 hours per condition. Table 36 describes the samples prepared:

TABLE 36

Sample #	**Excipient added**	**Stress condition**
1-C	None	None
1-A	None	Agitation
1-FT	None	Freeze-Thaw
2-C	15 mg/mL caffeine	None
2-A	15 mg/mL caffeine	Agitation
2-FT	15 mg/mL caffeine	Freeze-Thaw

Example 37: Evaluation of stability by Dynamic Light Scattering (DLS)

[00171] A Brookhaven Zeta Plus dynamic light scattering instrument was used to measure the hydrodynamic radius of the adalimumab molecules in the samples from Example 36, and to look for evidence of the formation of aggregate populations. Table 37 shows the DLS results for the 6 samples prepared according to Example 36: some of them (1-A, 1-FT, 2-A, and 2-FT) had been exposed to stress conditions (“Stressed Samples”), and others (1-C and 2-C) had not been stressed. The DLS data in Table 37 show a multimodal particle size distribution of the monoclonal antibody in Stressed Samples that do not contain caffeine. In the absence of caffeine as an excipient, the Stressed Samples 1-A and 1-FT showed higher effective diameter than non-stressed Sample 1-C, and in addition they showed a second population of particles of significantly higher diameter; this new grouping of particles with a larger diameter is evidence of aggregation into subvisible particles. The Stressed Samples containing the caffeine (Samples 2-A and 2-FT) only display one population of particles, at a particle diameter similar to the unstressed Sample 2-C. These results demonstrate that adding caffeine to these samples reduced the formation of aggregates or subvisible particles.

TABLE 37

Sample #	**Effective Diameter (nm)**	**Diameter of Population #1 (nm)**	**% by Intensity of Population #1**	**Diameter of Population #2 (nm)**	**% by Intensity of Population #2**
1-C	10.9	10.8	100	-	-
1-A	11.5	10.8	87	28.9	13
1-FT	20.4	11.5	66	112.2	44
2-C	10.5	10.5	100	-	-
2-A	10.8	10.8	100	-	-
2-FT	11.4	11.4	100	-	-

[00172] Tables 38A and Table 38B display the DLS raw data of adalimumab samples from Example 36 showing the particle size distributions. In these Tables, G(d) is the intensity-weighted differential size distribution. C(d) is the cumulative intensity-weighted differential size distribution.

TABLE 38A

Sample 1-C			Sample 1-A			Sample 1-FT		
Diameter (nm)	G (d)	C(d)	Diameter (nm)	G (d)	C(d)	Diameter (nm)	G (d)	C(d)
10.6	14	4	9.3	13	3	8.2	12	2
10.6	53	20	9.8	47	15	9.2	55	13
10.7	92	46	10.3	87	37	10.3	98	32
10.8	100	76	10.8	100	63	11.5	100	52
10.9	61	93	11.4	67	80	12.9	57	63
10.9	22	100	12	27	87	14.5	14	66
			26.1	4	88	89.3	5	67
			27.5	10	91	100.1	27	72
			28.9	13	94	112.2	52	83
			30.5	13	97	125.7	52	93
			32.1	7	99	140.8	30	99
			33.8	4	100	157.8	7	100

TABLE 38B

Sample 2-C			Sample 2-A			Sample 2-FT		
Diameter (nm)	G (d)	C(d)	Diameter (nm)	G (d)	C(d)	Diameter (nm)	G (d)	C(d)
10.3	14	4	10.6	7	2	11.3	28	9
10.4	52	19	10.6	43	16	11.3	64	29
10.5	91	46	10.7	79	40	11.4	100	60
10.5	100	75	10.8	100	71	11.5	79	85
10.6	62	93	10.8	64	91	11.5	43	98
10.7	23	100	10.9	29	100	11.6	7	100

Example 38: Evaluation of stability by size-exclusion chromatography (SEC)

[00173] Size exclusion chromatography was used to detect subvisible particulates of less than about 0.1 microns in size from the stressed and unstressed adalimumab samples described in Example 36. To perform the SEC, a TSKgel SuperSW3000 column (Tosoh Biosciences, Montgomeryville, PA) with a guard column was used, and the elution was monitored at 280 nm. A total of 10 μL of each stressed and unstressed sample from Example 36 was eluted isocratically with a pH 6.2 buffer (100 mM phosphate, 325 mM NaCl), at a flow rate of 0.35 mL/min. The retention time of the adalimumab monomer was approximately 9 minutes. No detectable aggregates were identified in the samples containing the caffeine excipient, and the amount of monomer in all 3 samples remained constant.

Example 39: Viscosity reduction of HERCEPTIN® formulation

[00174] The monoclonal antibody trastuzumab (HERCEPTIN® from Genentech) was received as a lyophilized powder and reconstituted to 21 mg/mL in DI water. The resulting solution was concentrated as-is in an Amicon Ultra 4 centrifugal concentrator tube (molecular weight cut-off, 30 kDa) by centrifuging at 3500 rpm for 1.5 hrs. The concentration was measured by diluting the sample 200 times in an appropriate buffer and measuring absorbance at 280 nm using the extinction coefficient of 1.48 mL/mg. Viscosity was measured using a RheoSense microVisc viscometer.

[00175] Excipient buffers were prepared containing salicylic acid and caffeine either alone or in combination by dissolving histidine and excipients in distilled water, then adjusting pH to the appropriate level. The conditions of Buffer Systems 1 and 2 are summarized in Table 39.

TABLE 39

Buffer System #	Salicylic Acid concentration	Caffeine concentration	Osmolality (mOsm/kg)	pH
1	10 mg/mL	10 mg/mL	145	6
2	0	15 mg/mL	86	6

[00176] HERCEPTIN® solutions were diluted in the excipient buffers at a ratio of ~1:10 and concentrated in Amicon Ultra 15 (MWCO 30 kDa) concentrator tubes. Concentration was determined using a Bradford assay and compared with a standard calibration curve made from the stock HERCEPTIN® sample. Viscosity was measured using the RheoSense microVisc viscometer. The concentration and viscosity measurements of the various HERCEPTIN® solutions are shown in Table 40 below, where Buffer Systems 1 and 2 refer to those buffers described in Table 39.

TABLE 40

Control solution with no added excipients		**Buffer System 1: Solution with 10 mg/mL Caffeine + 10 mg/ml Salicylic Acid added**		**Buffer System 2: Solution with 15 mg/mL Caffeine added**	
Viscosity (cP)	Antibody Concentration (mg/mL)	Viscosity (cP)	Antibody Concentration (mg/mL)	Viscosity (cP)	Antibody Concentration (mg/mL)
37.2	215	9.7	244	23.4	236
9.3	161	7.7	167	12.2	200
3.1	108	2.9	122	5.1	134
1.6	54	2.4	77	2.1	101

[00177] Buffer System 1, containing both salicylic acid and caffeine, had a maximum viscosity reduction of 76% at 215 mg/mL compared to the control sample. Buffer System 2, containing just caffeine, had viscosity reduction up to 59% at 200 mg/mL.

Example 40: Viscosity reduction of AVASTIN® formulation

[00178] AVASTIN® (monoclonal antibody bevacizumab formulation marketed by Genentech) was received as a 25 mg/mL solution in a histidine buffer. The sample was concentrated in Amicon Ultra 4 centrifugal concentrator tubes (MWCO 30 kDa) at 3500 rpm. Viscosity was measured by RheoSense microVisc and concentration was determined by absorbance at 280 nm (extinction coefficient, 1.605 mL/mg). The excipient buffer was prepared by adding 10 mg/mL caffeine along with 25 mM histidine HCl. AVASTIN® stock solution was diluted with the excipient buffer then concentrated in Amicon Ultra 15 centrifugal concentrator tubes (MWCO 30 kDa). The concentration of the excipient samples was determined by Bradford assay and the viscosity was measured using the RheoSense microVisc. Results are shown in Table 41 below.

TABLE 41

Concentration (mg/mL)	Viscosity without added excipient (cP)	Viscosity with 10 mg/mL added caffeine excipient (cP)	% Viscosity Reduction from Excipient
266	297	113	62%
213	80	22	73%
190	21	13	36%

[00179] AVASTIN® showed a maximum viscosity reduction of 73% when concentrated with 10 mg/mL of caffeine to 213 mg/mL when compared to the control AVASTIN® sample.

Example 41: Preparation of formulations containing caffeine, a secondary excipient and test protein

[00180] Formulations were prepared using caffeine as the excipient compound or a combination of caffeine and a second excipient compound, and a test protein, where the test protein was intended to simulate a therapeutic protein that would be used in a therapeutic formulation. Such formulations were prepared in 20 mM histidine buffer with different excipient compounds for viscosity measurement in the following way. Excipient combinations (Excipients A and B, as described in Table 28 below) were dissolved in 20 mM histidine and the resulting solution pH adjusted with small amounts of sodium hydroxide or hydrochloric acid to achieve pH 6 prior to dissolution of the model protein. Once excipient solutions had been prepared, the test protein bovine gamma globulin (BGG) was dissolved at a ratio to achieve a final protein concentration of about 280 mg/mL. Solutions of BGG in the excipient solutions were formulated in 20 mL glass scintillation vials and allowed to shake at 80-100 rpm on an orbital shaker table overnight. BGG solutions were then transferred to 2 mL microcentrifuge tubes and centrifuged for about ten minutes at 2300 rpm in an IEC MicroMax microcentrifuge to remove entrained air prior to viscosity measurement.

[00181] Viscosity measurements of formulations prepared as described above were made with a DV-IIT LV cone and plate viscometer (Brookfield Engineering, Middleboro, MA). The viscometer was equipped with a CP-40 cone and was operated at 3 rpm and 25°C. The formulation was loaded into the viscometer at a volume of 0.5 mL and allowed to incubate at the given shear rate and temperature for 3 minutes, followed by a measurement collection period of twenty seconds. This was then followed by 2 additional steps consisting of 1 minute of shear incubation and subsequent twenty second measurement collection period. The three data points collected were then averaged and recorded as the viscosity for the sample in Table

42 below. Viscosities of solutions with excipient were normalized to the viscosity of the model protein solution without excipient. The normalized viscosity is the ratio of the viscosity of the model protein solution with excipient to the viscosity of the model protein solution with no excipient.

TABLE 42

Excipient A		**Excipient B**		Normalized Viscosity
Name	Conc. (mg/mL)	Name	Conc. (mg/mL)	
-	0	-	0	1.00
Caffeine	15	-	0	0.77
Caffeine	15	Sodium acetate	12	0.77
Caffeine	15	Sodium sulfate	14	0.78
Caffeine	15	Aspartic acid	20	0.73
Caffeine	15	$CaCl_2$ dihydrate	15	0.65
Caffeine	15	Dimethyl Sulfone	25	0.65
Caffeine	15	Arginine	20	0.63
Caffeine	15	Leucine	20	0.69
Caffeine	15	Phenylalanine	20	0.60
Caffeine	15	Niacinamide	15	0.63
Caffeine	15	Ethanol	22	0.65

Example 42: Preparation of formulations containing dimethyl sulfone and test protein

[00182] Formulations were prepared using dimethyl sulfone (Jarrow Formulas, Los Angeles, CA) as the excipient compound and a test protein, where the test protein was intended to simulate a therapeutic protein that would be used in a therapeutic formulation. Such formulations were prepared in 20 mM histidine buffer for viscosity measurement in the following way. Dimethyl sulfone was dissolved in 20 mM histidine and the resulting solution pH adjusted with small amounts of sodium hydroxide or hydrochloric acid to achieve pH 6 and then filtered through a 0.22 micron filter prior to dissolution of the model protein. Once excipient solutions had been prepared, the test protein bovine gamma globulin (BGG) was dissolved at a concentration of about 280 mg/mL. Solutions of BGG in the excipient solutions were formulated in 20 mL glass scintillation vials and allowed to shake at 80-100 rpm on an orbital shaker table overnight. BGG solutions were then transferred to 2 mL microcentrifuge tubes and centrifuged for about ten minutes at 2300 rpm in an IEC MicroMax microcentrifuge to remove entrained air prior to viscosity measurement.

[00183] Viscosity measurements of formulations prepared as described above were made with a DV-IIT LV cone and plate viscometer (Brookfield Engineering, Middleboro, MA). The viscometer was equipped with a CP-40 cone and was operated at 3 rpm and 25°C. The formulation was loaded into the viscometer at a volume of 0.5 mL and allowed to incubate at the given shear rate and temperature for 3 minutes, followed by a measurement collection period of twenty seconds. This was then followed by 2 additional steps consisting of 1 minute of shear incubation and subsequent twenty second measurement collection period. The three data points collected were then averaged and recorded as the viscosity for the sample. Viscosities of solutions with excipient were normalized to the viscosity of the model protein solution without excipient. The normalized viscosity recorded in Table 43 is the ratio of the viscosity of the model protein solution with excipient to the viscosity of the model protein solution with no excipient.

TABLE 43

Dimethyl sulfone concentration (mg/mL)	Normalized viscosity
0	1.00
15	0.92
30	0.71
50	0.71
30	0.72

Example 43: Process benefit observed during concentrating HGG by centrifugation

[00184] Centrifugation was used for quick assessment of the effect of caffeine in the concentrating process of human-serum-derived gamma globulin (HGG). HGG stock solution at 100 mg/mL (10% Octagam) was first exchanged into PBS buffer with or without 50 mM caffeine using Amicon-15 centrifugal units with 30 kDa-MWCO membrane; 7 mL of HGG stock was pipetted into an Amicon-15 centrifugal unit, followed by addition of 7 mL of formulation vehicle into the centrifugal unit. After mixing the solution by pipetting, the centrifugal units were centrifuged at 2,844 x g for about 40 min until about 7 mL of filtrate was collected. The filtrate was discarded. About 7 mL of corresponding formulation vehicle was then added to the centrifugal unit and mixed. This process of centrifugation and dilution with vehicle was repeated two times, then the buffer-exchanged HGG solution was collected from the centrifugal units. The corresponding formulation vehicle was added to the HGG solution to a final mass of about 14 g. The HGG concentration in the starting formulations was about 50 mg/mL, which was subsequently verified by BCA assay.

[00185] Next, about 13 mL of HGG formulation was added to the outer tubes of CentriPrep centrifugal units with a 30 kDa NMWL (nominal molecular weight limit) membrane. The formulations were concentrated by centrifugation at 1,300 x g and the mass of filtrate for each formulation was recorded every 10 min. HGG concentration in retentate was estimated using the mass of filtrate collect using 13 g and 50 mg/mL as the initial sample weight and HGG concentration, respectively. This entire process was repeated two times, to generate the following data sets: Run #1 was conducted in phosphate buffered saline (PBS) and this sample is designated as “PBS-1”; Run #1 was conducted in PBS containing caffeine and this sample is designated as “PBS-caffeine-1”; Run #2 was conducted in PBS and this sample is designated as “PBS-2”; Run #2 was conducted in PBS containing caffeine and this sample is designated as “PBS-caffeine-2”. The centrifugation experiments Run #1 and Run #2 were conducted separately, so the control data sets (PBS-1 and PBS-2) should be compared with their respective caffeine-containing examples (PBS-caffine-1 and PBS-caffeine-2). The results of these tests are documented in the Tables 44 (Run #1) and 45 (Run #2) below, including mass of filtrate in grams and the concentration of HGG in mg/mL units. In both Run #1 and Run #2, the calculated concentration of HGG in the retentate was higher when the excipient was added, compared with the control formulation. These results are also shown in the graphs of FIGs. 3A and 3B, where an increasing amount of HGG in the retentate.

TABLE 44

Centrifuge Time (min)	Mass of filtrate (g)		Estimated HGG concentration in retentate (mg/mL)	
	PBS-1	PBS-caffeine-1	PBS-1	PBS-caffeine-1
0	0	0	50	50
10	1.3428	1.686	56	57
20	2.8042	3.4284	64	68
30	4.2015	5.0912	74	82
40	5.4708	6.5663	86	101
50	6.5555	7.9021	101	128
60	7.5734	9.0425	120	164
70	8.5464	9.9982	146	217
80	9.3322	10.6264	177	274
90	9.9302	11.0618	212	335
100	10.3502	11.3234	245	388
110	10.7023	11.5735	283	456
120	10.9384	11.7092	315	504
130	NA	NA	NA	NA
140	NA	NA	NA	NA

TABLE 45

Centrifuge Time (min)	Mass of filtrate (g)		Estimated HGG concentration in retentate (mg/mL)	
	PBS-2	PBS-caffeine-2	PBS-2	PBS-caffeine-2
0	0	0	50	50
10	1.3754	1.5748	56	57
20	2.8867	3.1872	64	66
30	4.241	4.623	74	78
40	5.4953	5.9191	87	92
50	6.6583	6.9913	102	108
60	7.3571	7.9329	115	128
70	7.973	8.7046	129	151

Centrifuge Time (min)	**Mass of filtrate (g)**		**Estimated HGG concentration in retentate (mg/mL)**	
	PBS-2	PBS-caffeine-2	PBS-2	PBS-caffeine-2
80	8.5071	9.3864	145	180
90	8.9538	9.9148	161	211
100	9.3573	10.3398	178	244
110	9.6367	10.6072	193	272
120	9.8756	10.8152	208	298
130	10.0687	11.0051	222	326
140	10.1515	11.011	228	327

Example 44: DLS viscosity measurements of concentrated human immune globulin

[00186] 10X phosphate buffered saline (PBS) from Fisher Scientific (Hampton, NH) was diluted with Milli-Q Type 1 ultra-pure water to 1X concentration prior to use. Nicotinamide, acesulfame K, 1,3-dimethyluracil, arginine monohydrochloride, saccharin, caffeine, tyramine, and imidazole were purchased from Sigma-Aldrich (St. Louis, MO), sodium benzoate from Spectrum Chemical (New Brunswick, NJ) and hordenine HCl from Bulk Supplements (Henderson, NV) and all were used as excipients in the following experiment.

[00187] A purified human immune globulin (Octagam 10%) was purchased from NOVA Biologics, Inc (Oceanside, CA), buffer exchanged into 1X PBS, using a benchtop EMD Millipore (Billerica, MA) tangential flow-filtration unit, and concentrated using an Amicon Ultra 15 centrifugal concentrator tube with a 30 kDa molecular weight cut-off (EMD Millipore, Billerica, MA). Stock excipient solutions were prepared in 1X PBS at a concentration of 1 M or the solubility limit of the compound, and pH adjusted to about 7.4 as necessary with either concentrated hydrochloric acid or sodium hydroxide. In a PCR tube, the concentrated human IgG and excipient solutions were mixed together (9 parts IgG concentrate, 1 part excipient solution or buffer). To the mixture was added a solution of PEG surface modified gold nanoparticles (nanoComposix, San Diego, CA) in deionized water. The resulting mixture of IgG, excipient and particles was loaded in duplicate into a 384-well Aurora (Whitefish, MT) microplate. The microplate was then centrifuged at 400 x g in a Sorvall Legend RT centrifuge, and then placed in a DynaPro II DLS plate reader (Wyatt Technology Corp., Goleta, CA) to measure the apparent particle size of the gold nanoparticles at 25 degrees Centigrade. The ratio of the apparent particle size of the gold nanoparticle in a protein formulation to the apparent particle size of the gold nanoparticle in buffer (no protein) was used to determine the viscosity of the protein formulation according to the stokes-Einstein equation. In this example, the ratio of apparent radius to the actual radius of the gold nanoparticle was multiplied by the viscosity of water at 25 degrees Centigrade to calculate the viscosity of the protein formulation in centipoise (cP). Viscosity results in the presence of excipient were compared with results in the presence of no excipient to determine magnitude of viscosity reduction achieved.

TABLE 46

Excipient added	**DLS viscosity (cP)**	
	replicate 1	**replicate 2**
1,3-dimethyluracil	42.36	40.44
Hordenine HCl	42.8	43.72
Acesulfame K	45.59	42.28
Nicotinamide	46.4	49.1
Arginine HCl	49.09	52.95
Aspartame	50.64	53.74
Saccharin	51.46	56.22
Caffeine	57.43	47.25
Tyramine	65.76	59.98
Imidazole	61.75	57.41
Sodium benzoate	71.02	87.49
None (control)	67.07	63.39

Example 45: DLS viscosity measurements of concentrated human immune globulin

[00188] To examine the effects of dimethyluracil and hordenine as excipients, HGG formulations were prepared by mixing concentrated HGG (215 mg/mL) with appropriate amount of PBS and 10x excipient stock to achieve a 50 mg/mL HGG formulations in PBS with or without 100 mM excipients. Next, 13 mL of each formulation was added to a CentriPrep centrifugal unit and conduct the centrifugal study as described in Example 43 above. The filtrate volume and retentate concentration were recorded as a function of centrifugation time, and the results of these tests with 100 mM concentration of excipients dimethyluracil and hordenine vs. control (PBS buffer) are summarized in Table 47 below. Addition of the excipients hordenine and dimethyluracil resulted in improved filtration rate compared with the control. The results in Table 47 are also shown in the graph of FIG. 4, where an increasing amount of HGG in the retentate correlates with improved processing performance.

TABLE 47

Time (min)	**Mass of filtrate (g)**			**Estimated HGG concentration in retentate (mg/mL)**		
	PBS	100 mM dimethyluracil	100 mM hordenine	PBS	100 mM dimethyluracil	100 mM hordenine
0	0	0	0	50	50	50
10	0.6804	1.0751	2.2692	53	54	60
20	1.6566	2.2902	3.2286	57	61	66
30	2.5385	3.4562	4.1148	62	68	73
40	3.3715	4.4359	4.9049	66	76	80
50	4.2098	5.4398	5.6059	71	86	87
60	4.856	6.2301	6.1031	76	95	93
70	5.5011	7.0011	6.6934	81	107	102
80	6.0436	7.6977	7.0824	87	121	108
90	6.5227	8.2152	7.5251	93	134	117
100	7.0035	8.7504	7.8367	99	151	124
110	7.4411	9.137	8.119	105	165	131
120	7.8312	9.4786	8.3418	111	181	137
130	8.1512	9.7263	8.5544	116	194	143

Example 46: Improving Tangential Flow filtration using caffeine

[00189] 400 mL of human gamma globulin (Octagam, Octapharma, USA) at a concentration of 35 mg/mL was prepared by diluting the stock at 100 mg/mL into phosphate buffered saline (PBS). The buffer was prepared by dissolving 1.8 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl in 1 L of Milli-Q water. A caffeine PBS solution was prepared by dissolving 50 mM caffeine, 1.8 mM KH_2PO_4, 10 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl in 1 L of Milli-Q water. Tap water was purified with a Direct-Q 3 UV purification system from EMD Millipore (Billerica, MA) to produce the DI water. The human gamma globulin (HGG) solution was transferred to a reservoir of a Labscale tangential flow filtration (TFF) system (Millipore, Billerica, MA) equipped with 30 KDa MWCO Pellicon XL TFF cassette (Millipore, Billerica, MA). Prior to use, the cassette was flushed with Milli-Q water followed by PBS and a water permeability test was carried out to ensure membrane integrity and efficiency. The HGG solution was pumped using a Quattroflow pump (Cole-Parmer, IL) through the cassette with the retentate line going back to the sample reservoir and the permeate collected in a graduated measuring cylinder. A stirrer bar ensured proper mixing of the feed with the retentate. The feed pump was set to deliver 120 mL/min feed to the cassette. The retentate restrictor was used to get the transmembrane pressure (TMP) roughly in the 20 to 30 psi range, and it was ensured that the TMP remained constant throughout the run by adjusting the feed pump and retentate restrictor. Data logging of the pressure and flow rates was carried out and samples taken every 30 minutes. To calculate the feed concentration, samples were analyzed by SE-HPLC where 50 mg was loaded onto an Agilent 1100 HPLC system fitted with TSKgel SuperSW3000 column (30 cm × 4.6 mm ID, Tosoh Bioscience, King of Prussia, PA) and Agilent G1351B Diode array detector. PBS was used as mobile phase at a flow rate of 0.35 mL/min. The protein concentration was calculated by integrating the area under the peaks. The feed concentration plotted as a function of time was used to compare TFF efficiency in presence of caffeine with the TFF using the control system. Higher percent concentration change from the initial feed concentration was observed in a shorter time with the caffeine as compared to the control, as shown in Table 48 below, demonstrating increased TFF efficiency.

TABLE 48

Time (min)	Control system, protein conc (mg/mL)	Control system, % change	Caffeine system, protein conc (mg/mL)	Caffeine system, % change
0	38.62	0	37.07	0
30	67.42	74.6	82.11	121.5
60	78.14	102.3	120.63	225.4
90	101.94	163.9	141.88	282.7
120	140.81	264.6	198.96	436.7
150	162.39	320.4	222.50	500.2
180	226.06	485.3	286.68	673.3
210	254.76	559.6	305.39	723.8

240	281.58	629.0		
270	291.99	656.0		

Example 47: Using caffeine to improve purification yield from Protein A resin

[00190] Research-grade omalizumab, purchased from Bioceros (Utrecht, The Netherlands) at 15 mg/mL in 20 mM sodium phosphate, pH 7 buffer was used as test sample. This protein solution was filtered through a 0.2 µm polyethersulfone (PES) filter. The filtered material was mixed in a 1:1 ratio with a binding buffer that consisted of 20 mM sodium phosphate at pH 7 in DI water. Tap water was purified with a Direct-Q 3 UV purification system from EMD Millipore (Billerica, MA) to produce the DI water. Protein-A purification was performed using a HiTrap Protein-A HP 1 mL column from GE Healthcare (Chicago, IL). 10 mL of binding buffer was used for column equilibration, followed by loading 30 mg of protein. The column was then washed with 5 mL of binding buffer to remove unbound protein. Bound omalizumab was eluted from the column in 1 mL fractions using either 0.1 M glycine buffer at pH 3.5 as the control buffer or by using 0.1 M glycine, 50 mM caffeine buffer at pH 3.5. The control buffer was prepared by dissolving 7.5 g of glycine into DI water, adjusting the pH to 3.5 using 6M HCl and adjusting the volume to 1 L. Caffeine buffer was prepared by dissolving 7.5 g of glycine and 10 g of caffeine into DI water, adjusting the pH to 3.5 using 6M HCl and adjusting the volume to 1 L. Five 1 mL fractions were collected; these eluted fractions were labeled E1, E2, E3, E4, and E5. Finally, Protein-A was regenerated by washing the column with 5 mL of 0.1 M glycine pH 3.0 buffer. The flowrate for each step was 1 mL/min, which was maintained by a Fusion 100 infusion pump (Chemyx, Stafford, TX). 10 mL NormJect Luer Lok syringes were used (Henke Sass Wolf, Tuttlingen, Germany, reference number 4100-000V0).

[00191] The 5 eluted fractions, E1, E2, E3, E4, and E5, were assayed for total protein content by high performance size-exclusion chromatography (SEC) analysis. SEC analysis was performed using a TSKgel SuperSW3000 column (30 cm × 4.6 mm ID, Tosoh Bioscience, King of Prussia, PA) connected to an Agilent HP 1100 HPLC system. PBS was used as mobile phase at a flow rate of 0.35 mL/min at room temperature. The protein concentration was monitored by absorbance at 280 nm using a G1315B diode array detector. The total amount of protein eluted from the Protein-A resin was determined by integrating the chromatograms, and about 8% increase in yield was observed in the presence of caffeine, as shown in Table 49 below.

TABLE 49

Sample Fraction	**Protein concentration in fractions eluted without caffeine containing buffer (mg/mL)**	**Protein concentration in fractions eluted with caffeine containing buffer (mg/mL)**
E1	0.277	1.13
E2	2.14	6.72
E3	6.58	4.67
E4	2.24	1.40
E5	1.06	0.708
Total yield	12.29	14.63
% recovery	**44.38**	**52.83**

Example 48: Excipients for stabilization during low pH hold

[00192] Research-grade ipilimumab, purchased from Bioceros (Utrecht, The Netherlands) at 15 mg/mL in 20 mM sodium phosphate, pH 7 buffer was used as test sample. The protein solution was filtered through a 0.2 μm polyethersulfone (PES) filter. Raffinose pentahydrate was obtained from Sigma (St Louis, MO). The excipient stock was prepared by dissolving the raffinose pentahydrate at a concentration of 1 M in 0.15 M glycine buffer, pH 2.75. The buffer was prepared by dissolving 7.5 g of glycine in 0.9 L Milli-Q water, adjusting the pH to 2.75 using 1 M HCl, and making the volume to 0.1 L. One control formulation and three excipient-containing formulations were prepared by adding the excipient to the ipilimumab solution, with a final excipient concentration of 0 mM, 100 mM, 200 mM and 400 mM and a final ipilimumab concentration of 2 mg/mL. The samples were incubated overnight at the acidic pH (2.75) for 24 h and the samples were analyzed by SE-HPLC where 50 mg was loaded onto an Agilent 1100 HPLC system fitted with TSKgel SuperSW3000 column (30 cm × 4.6 mm ID, Tosoh Bioscience, King of Prussia, PA) and Agilent G1351B diode array detector. PBS was used as mobile phase at a flow rate of 0.35 mL/min. The monomer protein (ipilimumab) concentration was calculated by integrating the areas under the monomer peak. The monomer fraction from an untreated sample not exposed to the low pH was normalized to 100 % and the monomeric fractions of the treated samples expressed as percentage change of this untreated sample. The results in Table 50 below, show that the presence of raffinose in the samples resulted in a higher percentage monomeric form of the ipilimumab after the low pH hold.

TABLE 50

Sample	% of ipilimumab in the monomeric form
0 mM raffinose	38.69
100 mM raffinose	52.68
200 mM raffinose	63.07
400 mM raffinose	78.88
Untreated	100

Example 49: Buffer and excipient preparation

[00193] A stock 20 mM histidine hydrochloride (His HCl) buffer was prepared for use in formulating excipients and protein buffer exchange. Two liters of His HCl was prepared by dissolving 6.206 grams of histidine (Sigma-Aldrich, St. Louis, MO) in Type 1 ultrapure water. The solution of dissolved histidine was titrated to pH 6.0 using concentrated hydrochloric acid. The His HCl solution was then brought up to 2 liters using a volumetric flask and filtered through a 0.2 µm membrane bottle-top filter device (Sigma Aldrich, St. Louis, MO). Excipients to be tested in Example 51 (listed in Table 51) were prepared as excipient solutions for subsequent testing as follows. Each excipient was were prepared at 10X (1 M) by dissolving it in this His HCl buffer described above, and adjusting the pH with concentrated sodium hydroxide or concentrated hydrochloric acid. Each excipient solution was then filtered using 0.2 µm membrane filter.

Example 50: Protein solution preparation

[00194] Two test proteins, purified omalizumab purchased from Bioceros (The Netherlands) and human serum derived polyclonal IgG (Octagam 10%), were buffer exchanged into His HCl (as prepared in Example 49) using 20 kDa molecular weight cut-off dialysis cassettes (Fisher Scientific). Each protein solution was transferred into the dialysis cassette attached to a buoy and placed in a flask for buffer exchanges. A total of 3 buffer exchanges were performed into > 50x the starting protein volume. Upon the final buffer exchange step, the protein solution was removed from the dialysis cassette and filtered through 0.2 µm membrane filter and protein concentration was measured via A280 by diluting 100-fold into His HCl buffer. 100 µL was then transferred to a UV clear 96 half-well microplate (Greiner Bio-One, Austria), and absorbance measured at a wavelength of 280 nm with a Synergy HT plate reader (BioTek, Winooski, VT). The blanked, pathlength corrected A280 measurement was then divided by the respective extinction coefficient and multiplied by the dilution factor to determine the protein concentration. A subsequent concentration step was needed to concentrate the protein in preparation for dynamic light scattering (DLS) viscosity measurements in Example 51. Concentration was performed using Amicon-15 centrifugal devices with a 30 kDa molecular weight cut-off (EMD Millipore, Billerica, MA) and concentrated to 175 mg/mL based on retentate mass in the centrifugal device by centrifuging at 4000 x g on a benchtop centrifuge (Sorvall Legend RT).

Example 51: DLS measurement of the diffusion interaction parameter

[00195] In this Example, the diffusion interaction parameter (kD) of a dilute protein solution was measured by DLS in the presence of 0.1 M excipient solution. The excipients being tested are listed in Table 51 For each excipient, a 0.2M solution of the excipient was prepared separately from the previously prepared 1 M excipient stock. The kD was measured by DLS using 5 different concentrations of omalizumab (prepared as described in Example 50) ranging from 10 mg/mL to 0.6 mg/mL in the presence of 0.1M excipient. An identical set of control samples was prepared, containing the same concentrations of omalizumab in the absence of any excipient. For each test sample, 20 µL of protein solution was combined with 20 µL of 0.2 M excipient solution (1:1 mixture) onto a 384-well plate (Aurora Microplates, Whitefish, MT). After loading the samples, the well plate was shaken on a plate shaker to mix the contents for 5 minutes. Upon mixing, the well plate was centrifuged at 400 x g in a Sorvall Legend RT for 1 minute to force out any air pockets. The well plate was then loaded into a DynaPro II DLS plate reader (Wyatt Technologies Corp., Goleta, CA) and the diffusion coefficient of each sample was measured at 25°C. For each excipient, the measured diffusion coefficient was plotted as a function of protein concentration, and the slope of the linear fit of the data was recorded as the kD. In this example, each measurement was normalized to a control average and reported as a percent to the control. These results are shown in Table 51 below.

TABLE 51

Excipients	Protein	% change in kD value from control
3-(1-Pyridinio)-1-propanesulfonate	Omalizumab	22%
Aspartic Acid	Omalizumab	72%
Ornithine	Omalizumab	49%
beta-alanine	Omalizumab	25%
Lysine	Omalizumab	47%

Excipients	Protein	% change in kD value from control
Trigonelline	Omalizumab	28%
(3-carboxypropyl) trimethylammonium chloride	Omalizumab	59%
Aminohippuric acid	Omalizumab	20%
Arginine	Omalizumab	81%
1-Hexyl-3-methylimidazolium chloride	Omalizumab	25%
NaCl (200 mM)	Omalizumab	70%
ethanolamine HCL	Omalizumab	25%
spermidine	Omalizumab	54%
4-aminopyridine	Omalizumab	25%
lysine	Omalizumab	66%
cysteamine HCl	Omalizumab	38%
x-xylylenediamine	Omalizumab	56%
nicotinic acid	Omalizumab	18%
quinic acid	Omalizumab	19%
1,3-diaminopropane	Omalizumab	62%
lactobionic acid	Omalizumab	47%
Glutamic acid	Omalizumab	35%
Sodium Ascorbate	Omalizumab	35%
sodium propionate	Omalizumab	33%
Quinic acid	Omalizumab	27%
sodium benzoate	Omalizumab	33%
Glucuronic acid	Omalizumab	32%
Hydroxybenzoic acid	Omalizumab	50%
sodium bisulfite	Omalizumab	57%
Salicylic acid	Omalizumab	43%
Etidronate	Omalizumab	56%
Acesulfame K+ salt	Omalizumab	49%
Calcium propionate	Omalizumab	83%
Citric acid	Omalizumab	47%

Excipients	Protein	% change in kD value from control
hydroquinone sulfonic acid	Omalizumab	36%
Menadione sodium bisulfite	Omalizumab	25%
2-dimethylaminoethanol	Omalizumab	31%
2-methyl-2-imidazoline	Omalizumab	17%
cycloserine	Omalizumab	9%
3-aminopyridine	Omalizumab	6%
4-aminopyridine	Omalizumab	23%
agmatine sulfate	Omalizumab	69%
cytidine	Omalizumab	29%
ethanolamine	Omalizumab	29%
meglumine	Omalizumab	171%
morpholine	Omalizumab	17%
triethanolamine	Omalizumab	40%

Example 52: Viscosity measurement by DLS

[00196] Purified omalizumab purchased from Bioceros (The Netherlands) and human serum derived polyclonal IgG (Octagam 10%, Pfizer) were used as model protein systems to explore viscosity effects of excipients. Concentrated stock solutions of excipients (listed in Tables 52 and 53) were prepared at 10X (1M) in His HCl buffer, following the protocol described in Example 49. Omalizumab was buffer exchanged using Amicon-15 centrifugal (30 kDa MWCO) devices into His HCl buffer and concentrated to 175 mg/mL based on retentate mass in the centrifugal device. Excipient and concentrated protein were combined in a 200 µL PCR tube, adding 1-part 10X excipient and 9 parts protein. An additional 2 µL of a 5-fold diluted solution of polyethylene glycol surface modified gold nanoparticles (nanoComposix, San Diego, CA) was added to each PCR tube and mixed thoroughly by inversion. A control sample was prepared identically, except without adding any excipient. Each sample (test samples and the control) was transferred to a 384-well microplate (Aurora Microplates, Whitefish, MT) in duplicate (25 µL per well) and centrifuged at 400 x g for 1 minute before analysis. A DynaPro II DLS plate reader (Wyatt Technology Corp., Goleta, CA) was used to measure apparent particle size of gold nanoparticles at 25°C. The ratio of the apparent particle size of the gold nanoparticle to the known particle size of the gold nanoparticle in water was used to determine the viscosity of the protein formulation according to the Stokes-Einstein equation. In this Example, each measurement was normalized to a control average and reported as a percent reduction compared with the control, and standard deviation is shown, and the results are shown in Tables 52 and 53 below.

TABLE 52

Excipient (100 mM)	Protein	% Reduction	Std. dev.
Dimethyluracil	hIgG	36.5%	1.5%
hordenine	hIgG	33.7%	0.7%
acesulfame K	hIgG	32.6%	2.5%
nicotinamide	hIgG	26.8%	2.1%
arginine	hIgG	21.8%	3.0%
aspartame	hIgG	20.0%	2.4%
saccharin	hIgG	17.5%	3.7%
3-(1-Pyridinio)-1-propanesulfonate	hIgG	15.0%	1.9%
caffeine	hIgG	19.8%	7.8%
imidazole	hIgG	8.7%	3.3%
tyramine	hIgG	3.6%	4.4%
Control	hIgG	0.0%	2.8%
Dimethylglycine	hIgG	2.5%	10.0%
4-aminopyridine	hIgG	29.7%	2.9%
nicotinamide/caffeine	hIgG	30.0%	1.0%
nicotinamide/caffeine	hIgG	33.4%	3.5%
hordenine HCl	hIgG	32.9%	5.5%
Dimethyluracil/arginine	hIgG	22.2%	0.6%
Jeffamine M600	hIgG	16.5%	2.1%
Dimethyluracil	hIgG	18.1%	9.4%
diethylnicotinamide	hIgG	11.1%	2.8%
arginine HCl	hIgG	14.6%	6.9%
arginine/glutamic acid	hIgG	17.5%	11.2%
nicotinamide	hIgG	12.1%	5.8%
serine/theonine	hIgG	5.8%	5.1%
isonicotinamide	hIgG	8.8%	8.5%

Excipient (100 mM)	Protein	% Reduction	Std. dev.
(3-carboxylpropyl) trimethyl ammonium chloride	hIgG	5.2%	12.1%

TABLE 53

Excipient (100 mM)	Protein	% Reduction	Std. dev.
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid	omalizumab	71%	1.3%
O-(octylphosphoryl)choline	omalizumab	38%	0.1%
Nicotinamide mononucleotide	omalizumab	61%	8%
Itaconic acid	omalizumab	54%	1%
3-(1-Pyridinio)-1-propanesulfonate	omalizumab	4%	0.2%
N-methyl aspartic acid	omalizumab	73%	0.6%
L-Ornithine	omalizumab	82%	0.0%
beta-alanine	omalizumab	25%	5.4%
ethylenediaminetetraacetic acid (EDTA)	omalizumab	64%	3.1%
Trigonelline	omalizumab	63%	1.1%
(3-carboxypropyl)trimethylammonium chloride	omalizumab	64%	3.6%
Iminodiacetic acid	omalizumab	51%	0.6%
aminohippuric acid	omalizumab	66%	2.4%
caffeic acid	omalizumab	35%	8.0%
Aspartame (50 mM)	omalizumab	32%	2.0%
1-(1-Adamantyl)ethylamine hydrochloride (100 mg/mL)	omalizumab	32%	4.0%
naphthalenedisulfonic acid (100 mg/mL)	omalizumab	9%	8.0%
x-xylylenediamine	omalizumab	78%	2.4%
1,3-diaminopropane	omalizumab	78%	1.5%
isonicotinic acid	omalizumab	45%	17.2%
Lysine	omalizumab	54%	3.4%
4-aminopyridine	omalizumab	41%	2.8%
Imidazole	omalizumab	62%	1.8%
Adenosine monophosphate	omalizumab	73%	2.7%

Excipient (100 mM)	**Protein**	**% Reduction**	**Std. dev.**
Dicyclomine (100 mg/mL)	omalizumab	43%	2.4%
2-Imidazolidone	omalizumab	10%	3.0%
pyridoxine HCl	omalizumab	63%	11.0%
2-ethylimidazole	omalizumab	51%	1.7%
triethanolamine	omalizumab	69%	2.9%
Ethanolamine	omalizumab	49%	5.1%
Benzylamine	omalizumab	81%	0.6%
1-Butylimidazole	omalizumab	60%	4.6%
diphenhydramine HCl	omalizumab	77%	0.2%
procaine HCl	omalizumab	71%	8.1%
2-dimethylaminoethanol	omalizumab	72%	6.7%
Acesulfame K	omalizumab	74%	3.3%
sodium ascorbate	omalizumab	55%	0.6%
glutamic acid	omalizumab	49%	5.0%
Etidronate	omalizumab	72%	0.0%
salicylic acid	omalizumab	72%	1.1%
quinic acid	omalizumab	58%	7.8%
hydroxybenzoic acid	omalizumab	68%	5.1%
glucuronic acid	omalizumab	60%	6.0%
Lactobionic acid	omalizumab	46%	0.1%
sodium hexametaphosphate	omalizumab	75%	0.5%
sodium bisulfite	omalizumab	71%	6.5%
sodium benzoate	omalizumab	60%	12.8%
Calcium propionate	omalizumab	75%	3.5%
Sodium propionate	omalizumab	56%	-
2-dimethylaminoethanol	omalizumab	65%	10.6%
2-methyl-2-imidazoline	omalizumab	49%	12.6%
cycloserine	omalizumab	33%	1.2%
3-aminopyridine	omalizumab	59%	4.0%
4-aminopyridine	omalizumab	73%	2.3%
agmatine sulfate	omalizumab	85%	1.8%

Excipient (100 mM)	Protein	% Reduction	Std. dev.
cytidine	omalizumab	68%	1.6%
diphenhydramine	omalizumab	81%	0.1%
ethanolamine	omalizumab	93%	1.6%
meglumine	omalizumab	91%	0.2%
morpholine	omalizumab	78%	2.7%

Example 53: Viscosity measurements by viscometer

[00197] Excipient solutions for those excipients listed in Tables 54 and 55 were prepared at 0.1 M or 0.075 M in His HCl buffer and pH adjusted using concentrated sodium hydroxide or concentrated hydrochloric acid. Omalizumab and human IgG were buffer exchanged into each excipient formulation using Amicon-15 centrifugal devices (30 kDa MWCO). After buffer exchange, the protein solution was concentrated up to 150 mg/mL for omalizumab and 250 mg/mL for human IgG based on retentate mass in the centrifugal device. Control formulations were prepared in identical manner except in the absence of the excipient. Viscosity measurements were performed on a RheoSense micro-viscometer using an A05 chip enclosed in a temperature-controlled enclosure set to 25°C. The shear rate was set to 250 s^{-1}. The viscosity of each formulation was measured 3 times and then diluted by adding 20 µL of the respective buffer and viscosity was measured again. This was repeated 5-6 times each to generate viscosity data for 5-6 different protein concentrations. Protein concentration was measured by absorbance at 280 nm using an Agilent 1100 series high pressure liquid chromatography instrument paired with a size exclusion column (TOSOH TSKgel SuperSW3000). A scatter plot was generated by plotting viscosity as a function of concentration for each excipient formulation. An exponential trendline was fitted to each formulation and the viscosity at a concentration was calculated based on the exponential fit with the equation $y = a*e^{(b*x)}$, where y is viscosity in cP units, x is concentration of protein in mg/mL, a and b are fitting parameters for the equation, and R^2 is the statistical coefficient of determination. For this example, the viscosity is reported as a function of a fixed concentration and results are given in Tables 54 and 55 below.

TABLE 54

Excipient	Protein	Buffer	Exponential Equation Calculations			
			a	b	R^2	Calc. Viscosity @ 250 mg/mL
Caffeine	human IgG	His HCl pH 5.5	0.469	0.0187	0.9194	50.3
nicotinamide	human IgG	His HCl pH 5.5	0.6136	0.0198	0.9329	86.6
hordenine HCl	human IgG	His HCl pH 5.5	0.4116	0.0197	0.9535	56.7
1,3-dimethyluracil	human IgG	His HCl pH 5.5	0.0586	0.0282	0.9929	67.6
control	human IgG	His HCl pH 5.5	0.2059	0.0248	0.9995	101.5

TABLE 55

Excipient	Protein	Buffer	Exponential Equation			
			a	b	R^2	Calc. Viscosity @120 mg/mL
Sulfanilic Acid	omalizumab	His HCl pH 6.0	1.2194	0.0185	0.8218	11.2
Nicotinic acid	omalizumab	His HCl pH 6.0	1.0171	0.0244	0.8041	19.0
Ornithine	omalizumab	His HCl pH 6.0	0.1068	0.0419	0.6874	16.3
control	omalizumab	His HCl pH 6.0	0.8156	0.0319	0.9763	37.5
1,3 diaminopropane	omalizumab	His HCl pH 6.0	0.1498	0.0274	0.9835	4.0

Example 54: BLI measurement of self-interaction

[00198] In this example, biolayer interferometry (BLI) tests were done with a ForteBio Octet Red-96 instrument. Amine reactive second-generation (AR2G) biosensors (Molecular Devices, CA) were conjugated with omalizumab to detect protein self-interaction in the presence of excipients. Excipient solutions for the excipients listed in Table 56 were prepared at 0.1 M in His HCl buffer. 20 mM sodium phosphate, pH 6.4 buffer was prepared by dissolving 1.679 g of dibasic sodium phosphate, heptahydrate (Sigma, St. Louis) and 1.895 g of monobasic sodium phosphate, monohydrate (Sigma, St. Louis) in DI water and adjusting the volume to 1 L. Research-grade omalizumab, purchased from Bioceros (Utrecht, The Netherlands) was buffer exchanged using Amicon-15 centrifugal (30 kDa MWCO) devices into phosphate buffer at pH 6.4. This omalizumab stock solution at 15 mg/mL in 20 mM sodium phosphate, pH 6.4 buffer was further buffer exchanged using Sephadex G-25 PD-10 desalting columns (GE Healthcare Life Sciences) and eluted with the 20 mM sodium phosphate, pH 6.4 buffers containing the prepared excipient at 0.1 M. The control was similarly prepared by using Sephadex G-25 PD-10 desalting columns (GE Healthcare Life Sciences) and eluted with the 20 mM sodium phosphate, pH 6.4 buffer. Protein concentration was measured using UV clear 96 half-well microplate (Greiner Bio-One, Austria), and absorbance measured at a wavelength of 280 nm with a Synergy HT plate reader (BioTek, Winooski, VT). Protein concentration was adjusted to 5 mg/mL by diluting in the prepared excipient buffers. In a black bottom 96-well microplate (Greiner Bio-One, Austria), 250 µL of each excipient solution at 0.1 M in 20 mM sodium phosphate, pH 6.4 buffer was transferred to column B and 250 µL of the 5 mg/ml omalizumab solution containing 0.1 M excipients was transferred to column C. The 96-well plate was set up so the columns represented individual formulations and rows distinguished protein-containing formulations. The tray was then transferred into a ForteBio Octet Red-96 for analysis. The omalizumab conjugated biosensors were dipped into the formulations containing no protein for 120 seconds to generate a baseline. Biosensors were then removed and dipped into formulations containing protein for 300 seconds. In this example, we reported the delta in a percent of binding signal at 300 seconds compared to the binding signal of the control, and the results are summarized in Table 56 below.

TABLE 56

Excipient	**Binding (nm) at 300 s**	**% change**
control	7	0%
ornithine	2.5	64%
iminodiacetic acid	1.25	82%
nicotinic acid	0.5	93%
sulfanilic acid	0.3	96%

EQUIVALENTS

[00199] While specific embodiments of the subject invention have been disclosed herein, the above specification is illustrative and not restrictive. While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims. Many variations of the invention will become apparent to those of skilled art upon review of this specification. Unless otherwise indicated, all numbers expressing reaction conditions, quantities of ingredients, and so forth, as used in this specification and the claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth herein are approximations that can vary depending upon the desired properties sought to be obtained by the present invention.

CLAIMS

What is claimed is:

1. A method of improving a parameter of a protein-related process, comprising:

providing a viscosity-reducing excipient additive comprising at least one excipient compound selected from the group consisting of hindered amines, aromatics and anionic aromatics, functionalized amino acids, oligopeptides, short-chain organic acids, low molecular weight aliphatic polyacids, diones and sulfones, zwitterionic excipients, and crowding agents with hydrogen bonding elements, and

adding a viscosity-reducing amount of the at least one excipient compound to a carrier solution for the protein-related process containing a protein of interest, thereby improving the parameter.

2. The method of claim 1, wherein the parameter is selected from the group consisting of cost of protein production, amount of protein production, rate of protein production, purity of protein produced, and efficiency of protein production.

3. The method of claim 1, wherein the parameter is selected from the group consisting of cost of protein purification, amount of protein purification, rate of protein purification, purity of protein purified, and efficiency of protein purification.

4. The method of claim 1, wherein the parameter is a proxy parameter.

5. The method of claim 4, wherein the proxy parameter is a reduced protein-protein interaction.

6. The method of claim 5, wherein the reduced protein-protein interaction is determined by a technique selected from the group consisting of biolayer interferometry, surface plasmon resonance, intrinsic fluorescence measurement, extrinsic fluorescence measurement, dynamic light scattering, kD value, static light scattering, B22 value, isothermal titration calorimetry, and *in silico* simulation.

7. The method of claim 1, wherein the protein-related process is an upstream processing process.

8. The method of claim 7, wherein the upstream processing process uses a cell culture medium for the carrier solution.

9. The method of claim 1, wherein the protein-related process is a downstream processing process.

10. The method of claim 9, wherein the downstream processing process is a chromatography process.

11. The method of claim 10, wherein the chromatography process is a Protein-A chromatography process.

12. The method of claim 10, wherein the chromatography process recovers the protein of interest, and wherein the protein of interest is characterized by an improved protein-related parameter selected from the group consisting of improved purity, improved yield, fewer particles, less misfolding, improved biological activity, increased percentage recovered in a monomeric form, and less aggregation, as compared to a control solution.

13. The method of claim 12, wherein the improved protein-related parameter is improved yield from the chromatography process.

14. The method of claim 1, wherein the protein-related process is a process selected from the group consisting of filtration, tangential flow filtration, sterile filtration, microfiltration, ultrafiltration, diafiltration, centrifugal concentration, in-line filtration, injection, syringing, pumping, mixing, centrifugation, membrane separation, and lyophilization.

15. The method of claim 14, wherein the process requires less force than a process-specific control process.

16. The method of claim 1, wherein the protein-related process is selected from the group consisting of a cell culture process, a cell culture harvesting process, a chromatography process, a viral inactivation process, and a filtration process.

17. The method of claim 16, wherein the protein-related process is the viral inactivation process.

18. The method of claim 17, wherein the viral inactivation process is conducted at a pH level of about 2.5 to about 5.0.

19. The method of claim 17, wherein the viral inactivation process is conducted at a higher pH than a viral-inactivation-specific control process.

20. The method of claim 16, wherein the protein-related process is the filtration process.

21. The method of claim 20, wherein the filtration process is a virus removal filtration process, a sterile filtration process, or an ultrafiltration/diafiltration process.

22. The method of claim 20, wherein the filtration process is characterized by an improved filtration-related parameter.

23. The method of claim 22, wherein the improved filtration-related parameter is a faster filtration rate than the filtration rate of the control solution.

24. The method of claim 22, wherein the improved filtration-related parameter is production of a smaller amount of aggregated protein than the amount of aggregated protein produced by a control filtration process.

25. The method of claim 22, wherein the improved filtration-related parameter is a higher mass transfer efficiency than the mass transfer efficiency of the control filtration process.

26. The method of claim 22, wherein the improved filtration-related parameter is a higher concentration or a higher yield of the target protein than a concentration or yield of the target protein produced by the control filtration process.

27. The method of claim 1, wherein the viscosity-reducing excipient additive comprises two or more excipient compounds.

28. The method of claim 1, wherein the at least one excipient compound is a hindered amine.

29. The method of claim 28, wherein the hindered amine is selected from the group consisting of pyrimidines, methyl-substituted pyrimidines, and phenethylamines.

30. The method of claim 29, wherein the hindered amine is a pyrimidine compound.

31. The method of claim 29, wherein the hindered amine is a phenethylamine compound.

32. The method of claim 31, wherein the phenethylamine compound is a non-psychoactive phenethylamine.

33. The method of claim 1, wherein the at least one excipient compound is a crowding agent with hydrogen bonding elements.

34. The method of claim 33, wherein the crowding agent with hydrogen bonding elements is selected from the group consisting of raffinose, inulin, pullulan, and sinistrins.

35. The method of claim 34, wherein the crowding agent with hydrogen bonding elements is raffinose.

36. The method of claim 1, wherein the at least one excipient compound is selected from the group consisting of caffeine, nicotinamide, nicotinamide mononucleotide, diethylnicotinamide, taurine, imidazole, ornithine, iminodiacetic acid, nicotinic acid, and sulfanilic acid.

37. The method of claim 36, wherein the at least one excipient compound is selected from the group consisting of caffeine, nicotinamide, taurine, and imidazole.

38. The method of claim 37, wherein the at least one excipient compound is caffeine.

39. The method of claim 1, wherein the at least one excipient compound is selected from the group consisting of calcium propionate and potassium sorbate.

40. The method of claim 1, wherein the viscosity-reducing amount is between about 1 mM and about 1000 mM of the at least one excipient compound.

41. The method of claim 40, wherein the viscosity-reducing amount is between about 1 mM and about 400 mM of the at least one excipient compound.

42. The method of claim 41, wherein the viscosity-reducing amount is between about 1 mg/mL and about 100 mg/mL of the at least one excipient compound.

43. The method of claim 41, wherein the viscosity-reducing amount is an amount from about 2 mM and about 150 mM.

44. The method of claim 1, wherein the carrier solution comprises an additional agent selected from the group consisting of preservatives, sugars, polyols, polysaccharides, arginine, proline, surfactants, stabilizers, and buffers.

45. The method of claim 1, wherein the protein of interest is a therapeutic protein.

46. The method of claim 45, wherein the therapeutic protein is selected from the group consisting of a monoclonal antibody, a polyclonal antibody, an antibody fragment, a fusion protein, a PEGylated protein, an antibody-drug conjugate, a synthetic polypeptide, a protein fragment, a lipoprotein, an enzyme, and a structural peptide.

47. The method of claim 45, wherein the therapeutic protein is a recombinant protein.

48. The method of claim 1, further comprising a step of adding a second viscosity-reducing excipient to the carrier solution, wherein the step of adding the second viscosity-reducing compound adds an additional improvement to the parameter.

49. A carrier solution comprising:
a liquid medium in which is dissolved a protein of interest, and a viscosity-reducing additive, wherein the carrier solution has a lower viscosity that that of a control solution.

50. The carrier solution of claim 49, wherein the carrier solution further comprises an additional agent selected from the group consisting of preservatives, sugars, polyols, polysaccharides, arginine, proline, surfactants, stabilizers, and buffers.

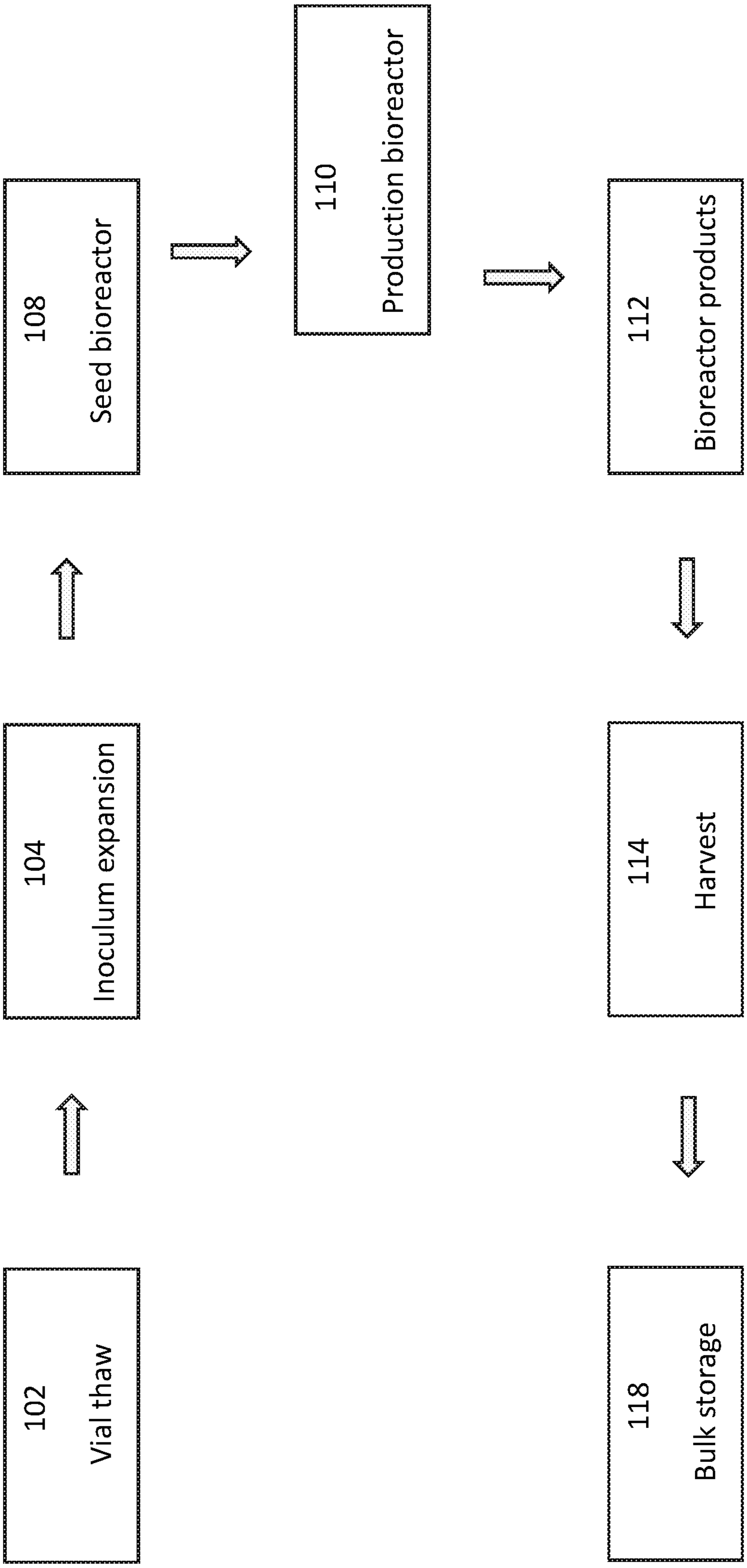
102
Vial thaw
104
Inoculum expansion
108
Seed bioreactor
110
Production bioreactor
112
Bioreactor products
114
Harvest
118
Bulk storage

FIG. 1

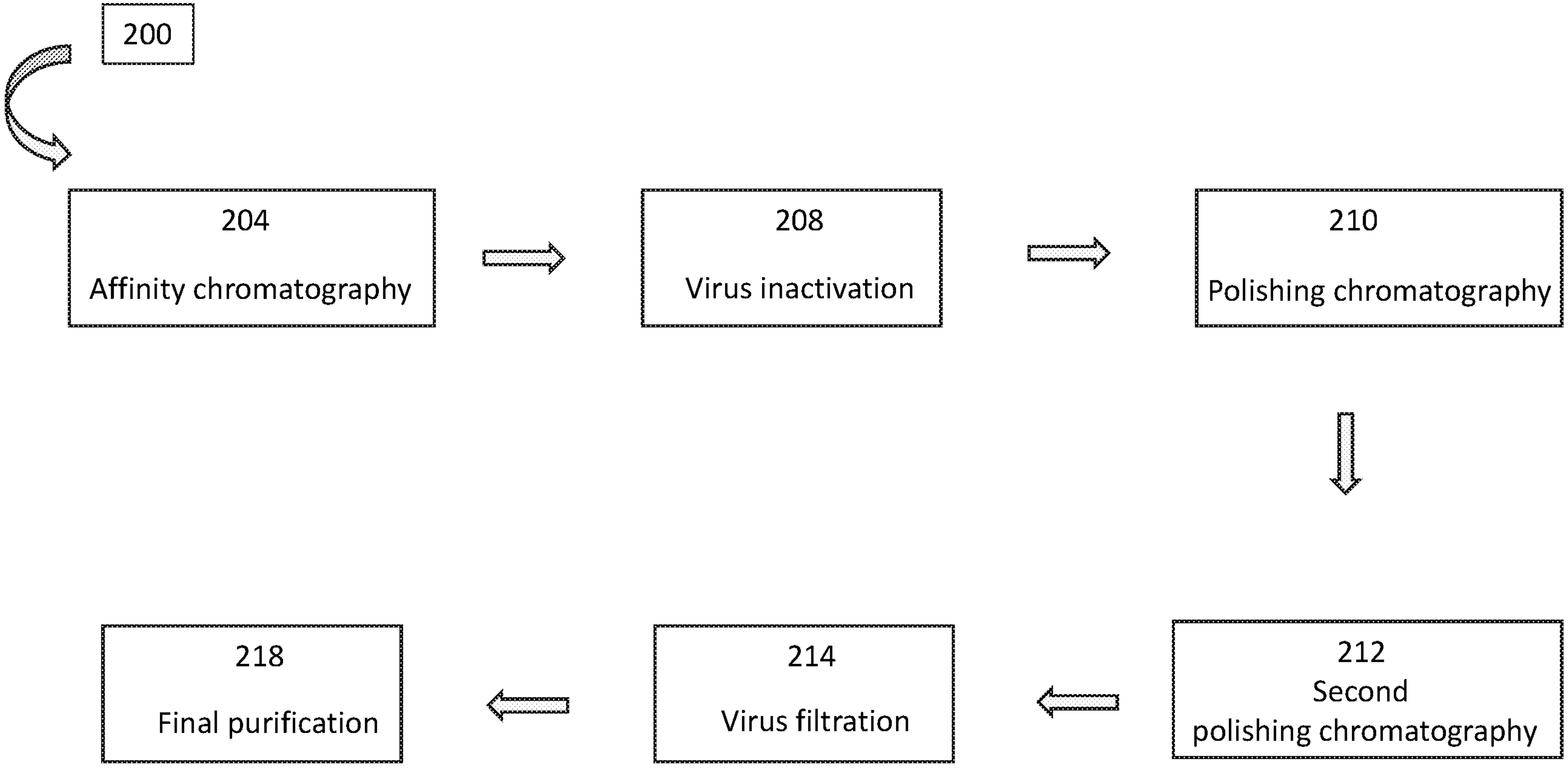
200
204
Affinity chromatography
208
Virus inactivation
210
Polishing chromatography
212
Second
polishing chromatography
214
Virus filtration
218
Final purification

FIG. 2

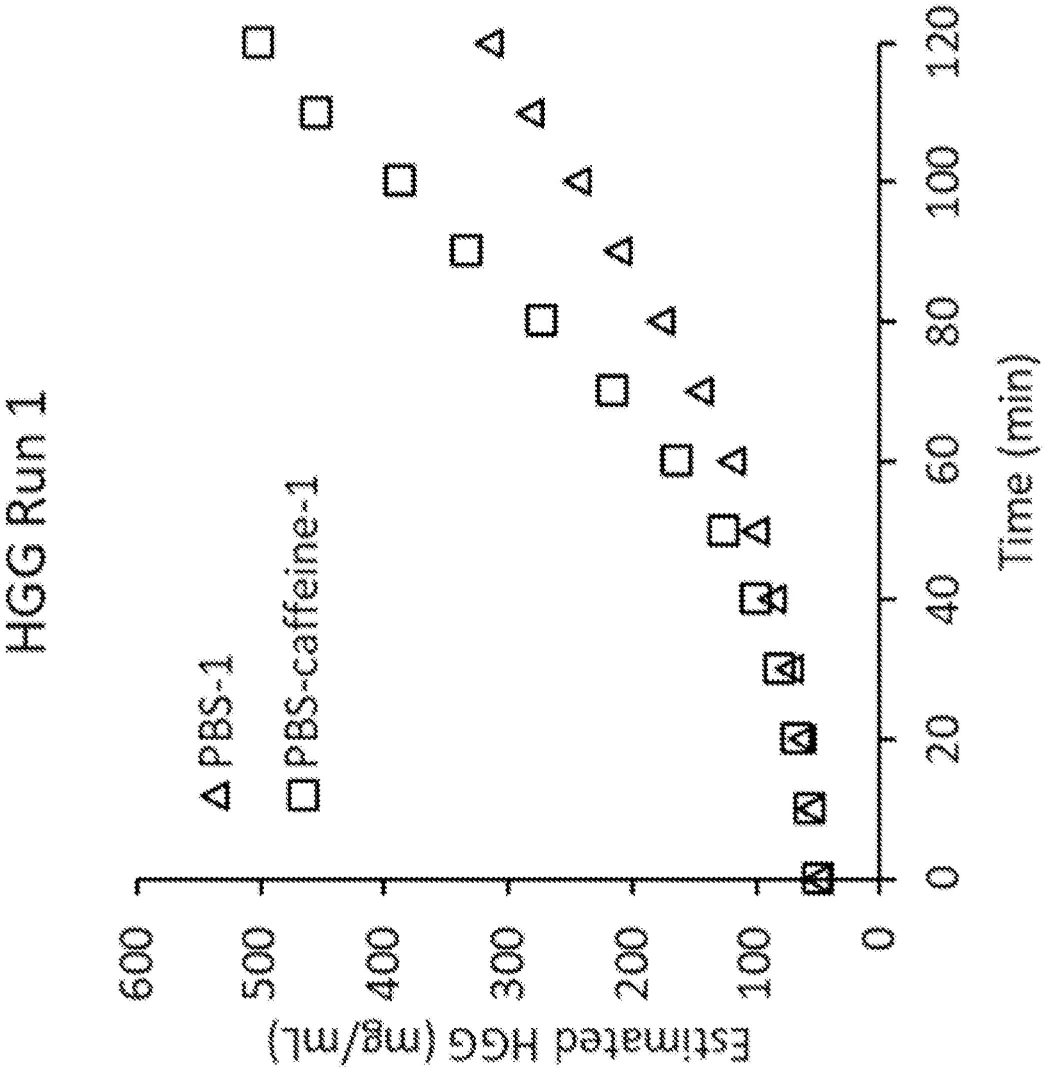
HGG Run 1
△ PBS-1
□ PBS-caffeine-1
Estimated HGG (mg/mL)
600
500
400
300
200
100
0
0
20
40
60
80
100
120
Time (min)

FIG. 3A

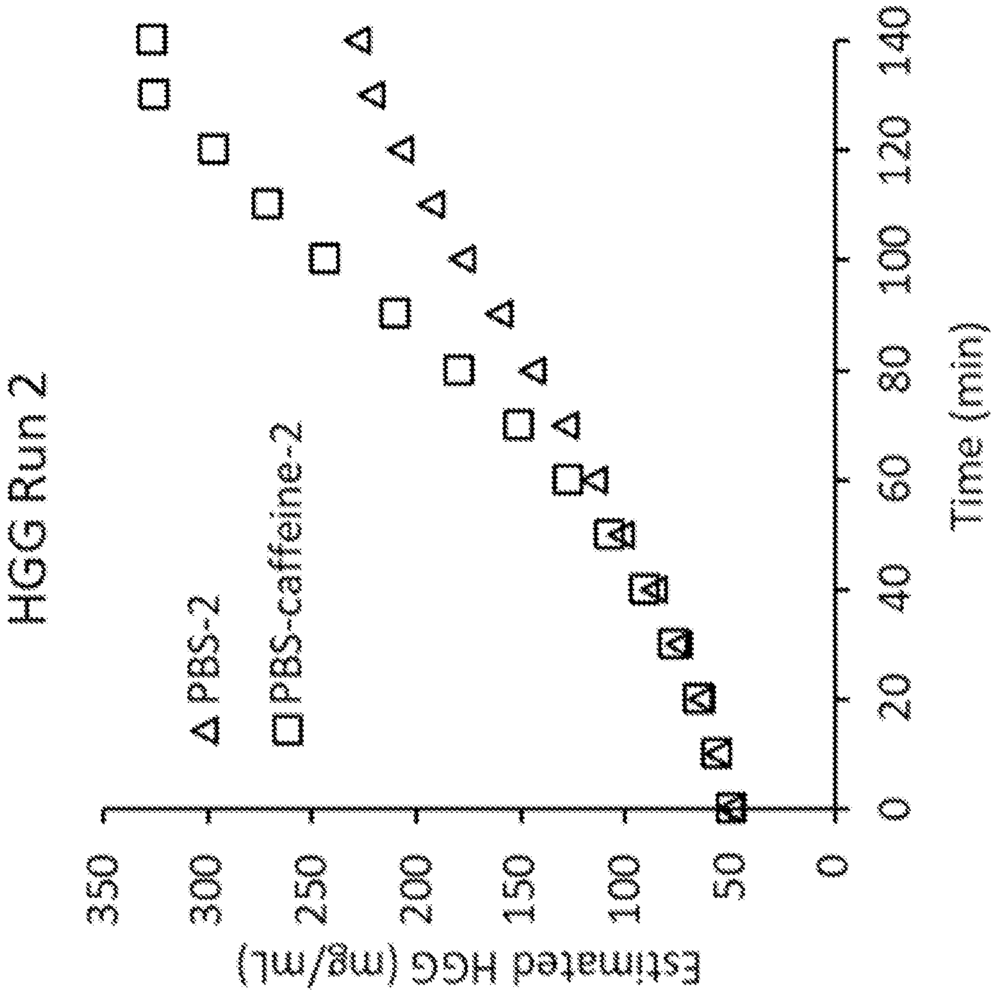
HGG Run 2
△ PBS-2
□ PBS-caffeine-2
Estimated HGG (mg/mL)
350
300
250
200
150
100
50
0
0
20
40
60
80
100
120
140
Time (min)

FIG. 3B

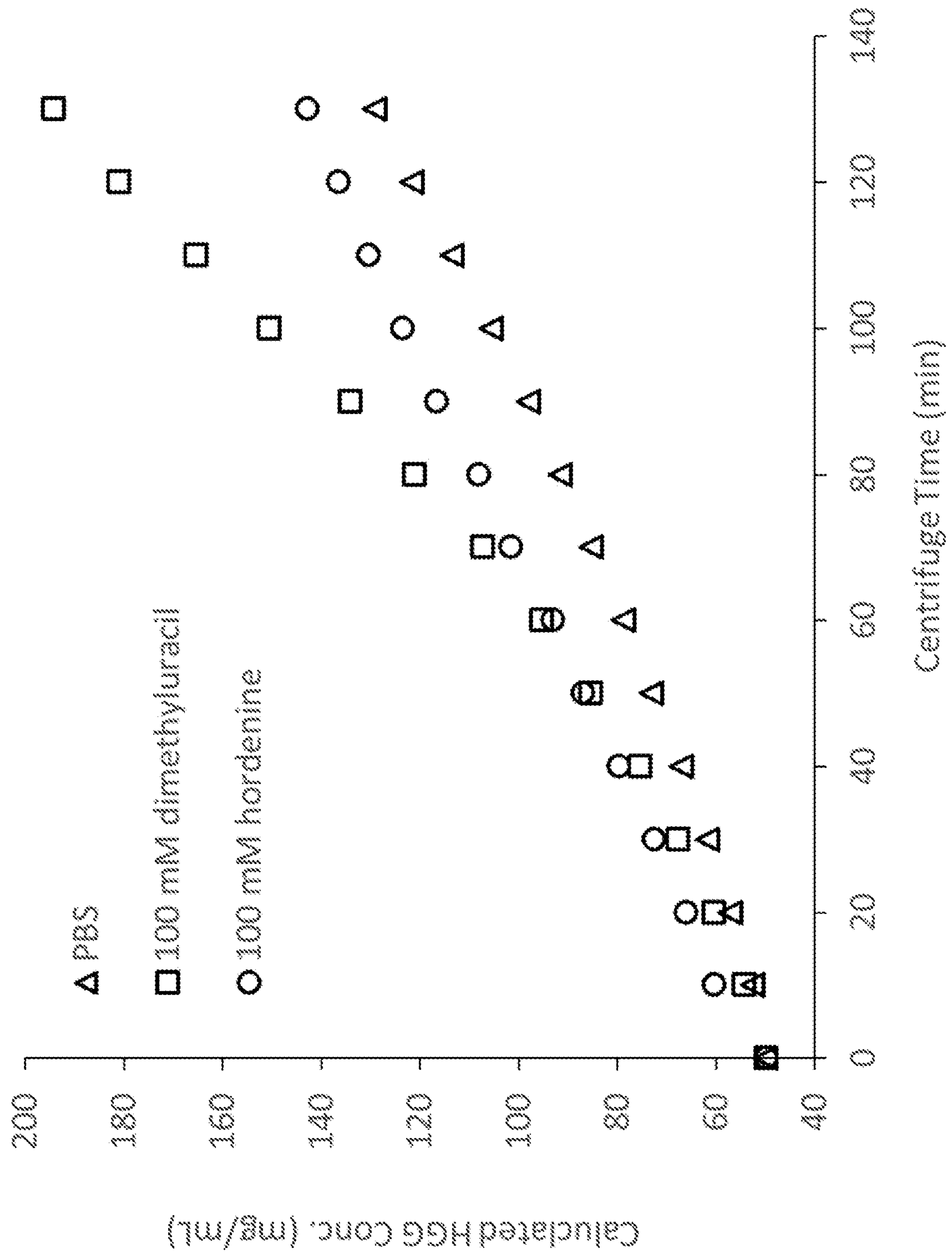
△ PBS
□ 100 mM dimethyluracil
○ 100 mM hordenine
Caluclated HGG Conc. (mg/mL)
200
180
160
140
120
100
80
60
40
Centrifuge Time (min)
0
20
40
60
80
100
120
140

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/63374

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 47/22, 39/395, 47/12, 47/18, 47/20, 47/24, 47/42; C07K 16/00 (2020.01)

CPC - A61K 39/39591, 38/385, 38/47, 47/22, 47/183, 47/24, 9/0019, 47/20, 47/42, 39/395, 47/60, 47/12, 47/18, 9/08; C12N 9/96, 9/2462; C07K 16/00; C12Y 302/01017

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 ----- Y ----- A	US 2017/0157256 A1 (REFORM BIOLOGICS LLC) 08 June 2017; abstract; paragraphs [0011], [0015], [0016], [0021], [0024], [0030], [0033], [0039], [0041], [0054], [0060], [0070], [0074], [0075], [0081], [0115], [0116]; claims 2, 9	1-8, 14-16, 20-27, 36-50 --- 9-13, 17-19, 31, 33-35 --- 32
X	US 2017/0232103 A1 (REFORM BIOLOGICS LLC) 17 August 2017; abstract; paragraphs [0012], [0014], [0094], [0156]; claim 1	1, 28-30
Y	US 2014/0255423 A1 (ABBVIE INC.) 11 September 2014; paragraphs [0004], [0153], [0229]	9-13
Y	US 2016/0010063 A1 (BIOGEN MA INC.) 14 January 2016; abstract; figure 14; paragraph [0030]	17-19
Y ----- A	US 2013/0071411 A1 (PHARMASCIENCE INC.) 21 March 2013; paragraphs [0787]-[0788]	31 --- 32
Y	DAVIDSON et al. 'Effect of Sucrose/Raffinose Mass Ratios on the Stability of Co-Lyophilized Protein During Storage Above the Tg' Pharm. Res. (2001) Vol. 18, pp. 474–479; abstract	33-35
A	US 5,063,246 A (IMAI et al.) 05 November 1991; abstract	32

☐ 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
"D" document cited by the applicant in the international application
"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 21 February 2020 (21.02.2020)	Date of mailing of the international search report 02 MAR 2020
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/63374

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:
      - ☐ in the form of an Annex C/ST.25 text file.
      - ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      - ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      - ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ *In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required* statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

An invitation to Furnish Nucleotide and/or Amino Acid Sequence Listing and to Pay, Where Applicable, Late Furnishing Fee ("ISA/225") was mailed on 10 December 2019 (10.12.2019). No approved electronic sequence listing was received in response to the ISA/225. Therefore, the international search was carried out only to the extent possible.