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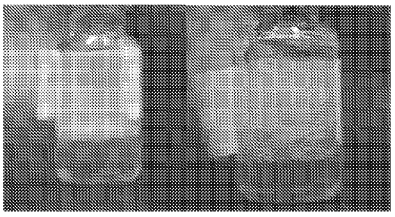
(54) **Title**: SLOW DISSOLUTION METHOD FOR RECONSTITUTION OF LYOPHILIZED MATERIAL

Figure 1



(57) **Abstract**: The present invention relates to robust, reproducible and convenient methods for reconstituting lyophilized substance. Among other things, the present invention provides methods for reconstituting lyophilized substances without involving substantial mixing, shaking or other types of agitation. In some embodiments, the present invention provides a method of reconstituting a lyophilized substance by including the steps of providing a container containing a lyophilized substance, introducing a diluent into the container so that the diluent and the lyophilized substance come into contact with each other while maintaining the container substantially still until the lyophilized substance is substantially dissolved.

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SLOW DISSOLUTION METHOD FOR RECONSTITUTION OF LYOPHILIZED MATERIAL

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to United States Provisional Patent Application serial number 61/218,390, filed on June 18, 2009; the entirety of which is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] Lyophilization or freeze-drying is a process widely used in the pharmaceutical industry for the preservation of biological and pharmaceutical materials. In many instances, a liquid formulation is desirable due to factors of clinical and patient convenience as well as ease of manufacture. However, for many compounds, a liquid formulation is either not feasible due to molecular instability or because of stresses encountered during manufacturing, packaging and shipping. When a liquid formulation is not an option, lyophilization provides a reasonable assurance of producing a stable dosage form under acceptable shipping and storage conditions.

[0003] However, lyophilized substances typically need to be reconstituted prior to administration to the patient. To ensure the intended dose as well as quality of the drug product, the reconstitution process must be reproducible and robust, especially, when a patient is responsible for reconstitution of the lyophilized material to a ready-for-injection solution.

[0004] Dissolution of lyophilized powders is proven to be not a trivial task. It has been shown in the literature that reconstitution of lyophilized protein formulations sometimes results in a cloudy solution depending on composition and manufacturing conditions (see, e.g., Hirakura Y, et al., *International Journal of Pharmaceutics* (2004), 286(1-2), pp. 53 – 67; and Hirakura, et al., *International Journal of Pharmaceutics* (2007) 340(1-2), pp. 34 – 41). In addition, most reconstitution studies that have been done so far were performed at low protein concentrations.

In modern biotech industry, it is desirable to lyophilize a protein drug at a high concentration. However, it has been reported that intensive foaming always occurred upon injection of diluent for reconstitution (see, e.g., International Patent Publication WO2007/014073), which could prevent proper reconstitution of lyophilized material by separating dry product from the diluent.

[0005] Therefore, there is a great need for more reliable and reproducible reconstitution method, especially for concentrated drug product.

SUMMARY OF THE INVENTION

[0006] The present invention provides a robust, reproducible and convenient method for reconstitution of lyophilized substance (e.g., proteins). The present invention encompasses the surprising discovery that lyophilized substances including highly concentrated proteins may be slowly and completely dissolved without involving substantial mixing, shaking, or other types of agitation.

[0007] In one aspect, the present invention provides a method of reconstituting a lyophilized substance. In some embodiments, the method includes (a) providing a container containing a lyophilized substance, and (b) introducing a diluent into the container so that the diluent and the lyophilized substance come into contact with each other while maintaining the container substantially still until the lyophilized substance is substantially dissolved.

[0008] In some embodiments, the lyophilized substance is a lyophilized protein. In some embodiments, the protein is an antibody or a fragment thereof, a growth factor, a clotting factor, a cytokine, a fusion protein, a pharmaceutical drug substance, a vaccine, an enzyme or a Small Modular ImmunoPharmaceutical (SMIP^{TM}). In some embodiments, the lyophilized substance is an amorphous mixture.

[0009] In some embodiments, the protein is present in the reconstituted formulation at a concentration of at least about 100 mg/ml (e.g., at least about 150 mg/ml, at least about 200 mg/ml, at least about 250 mg/ml, at least about 300 mg/ml, at least about 350 mg/ml, at least about 400 mg/ml). In some embodiments, the protein is present in the reconstituted formulation at a concentration ranging between about 50 mg/ml and about 400 mg/ml (e.g., about 50 mg/ml

and about 350 mg/ml; about 50 mg/ml and about 300 mg/ml; about 50 mg/ml and about 250 mg/ml; about 50 mg/ml and about 200 mg/ml; about 50 mg/ml and about 150 mg/ml; about 100 mg/ml and about 250 mg/ml; about 100 mg/ml and about 300 mg/ml, about 200 mg/ml and about 400 mg/ml, about 300 mg/ml and about 400 mg/ml).

[0010] In some embodiments, step (b) includes maintaining the container substantially still for at least about 10 minutes (e.g., at least 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours, 12 hours, 18 hours, 24 hours, 36 hours, 48 hours, 60 hours, or 72 hours). In some embodiments, step (b) includes a step of maintaining the container substantially still until the content is dissolved.

[0011] In some embodiments, the method includes maintaining the container substantially still at 2-8 ^{o}C. In some embodiments, the method includes maintaining the container substantially still at room temperature.

[0012] In some embodiments, the method further includes a step of swirling, shaking, mixing and/or agitating the container following step (b).

[0013] In some embodiments, a suitable diluent is water. In some embodiments, a suitable diluent contains an isotonicity agent (e.g., sucrose, mannitol, sodium chloride, trehalose, dextrose, glycine and combinations thereof). In some embodiments, a suitable diluent contains a buffering agent (e.g., histidine, phosphate buffers, sodium acetate, citrate, succinate, tris(hydroxymethyl)aminomethane ("Tris") and combinations thereof). In some embodiments, a suitable diluent contains sucrose and histidine. In some embodiments, a suitable diluent contains sodium chloride and histidine. In some embodiments, a suitable diluent further contains a surfactant (e.g., polysorbate 20, polysorbate 80, poloxamers, Triton and combinations thereof).

[0014] In some embodiments, the diluent is introduced by injection. In some embodiments, the injection takes at least 10 seconds (e.g., at least 12 seconds, 14 seconds, 16 seconds, 18 seconds, 20 seconds, 25 seconds, 30 seconds) to complete.

[0015] In some embodiments, the container is a vial, a tube, a syringe, a dual chamber container or any container that is suitable for freeze-drying.

[0016] In some embodiments, the container is made of glass, plastics, metal or any other material that is compatible with the product.

[0017] In some embodiments, the reconstituted formulation is suitable for intravenous or subcutaneous injection.

[0018] In another aspect, the present invention also provides reconstituted formulation according to various methods described herein. In some embodiments, a reconstituted formulation includes a protein at a concentration greater than 50 mg/ml, wherein the reconstituted formulation is prepared by slowly dissolving a lyophilized protein into a diluent without continuous agitation and wherein less than 5% (e.g., less than 4%, 3.5%, 3.0%, 2.5%, 2.0%, 1.5%, 1.0%) of the protein exists in aggregated form in the reconstituted formulation. In some embodiments, the protein concentration is greater than 100 mg/ml. In some embodiments, the protein concentration is greater than 200 mg/ml. In some embodiments, the protein concentration is greater than 300 mg/ml. In some embodiments, the protein concentration ranges between 50 mg/ml and 400 mg/ml. In some embodiments, the protein concentration ranges between 50 mg/ml and 150 mg/ml.

[0019] In some embodiments, the reconstituted formulation contains sucrose, mannitol, glycine, dextran, trehalose, and/or sorbitol. In some embodiments, the reconstituted formulation further comprises a buffering agent. In some embodiments, the buffering agent in the reconstituted formulation is selected from the group consisting of histidine, sodium acetate, citrate, phosphate, succinate, Tris and combinations thereof.

[0020] In some embodiments, the reconstituted formulation further comprises a surfactant. In some embodiments, the surfactant in the reconstituted formulation is selected from the group consisting of polysorbate 20, polysorbate 80, poloxamers, Triton and combinations thereof.

[0021] In some embodiments, the lyophilized protein is an amorphous mixture.

[0022] In some embodiments, the diluent is bacteriostatic water. In some embodiments, the diluent is distilled water. In some embodiments, the diluent is water purified by reverse osmosis and or deionization. In some embodiments, the diluent contains an isotonicity agent

(e.g., sucrose, mannitol, sodium chloride, trehalose, dextrose, glycine and combinations thereof). In some embodiments, the diluent contains a buffering agent (e.g., histidine, phosphate buffers, sodium acetate, citrate, succinate, Tris and combinations thereof). In some embodiments, the diluent further contains a surfactant (e.g., polysorbate 20, polysorbate 80, poloxamers, Triton and combinations thereof).

[0023] In some embodiments, the protein is an antibody or a fragment thereof, a growth factor, a clotting factor, a cytokine, a fusion protein, a pharmaceutical drug substance, a vaccine, an enzyme or a Small Modular ImmunoPharmaceutical (SMIP^{TM}).

[0024] In this application, the use of "or" means "and/or" unless stated otherwise. As used in this application, the term "comprise" and variations of the term, such as "comprising" and "comprises," are not intended to exclude other additives, components, integers or steps. As used in this application, the terms "about" and "approximately" are used as equivalents. Any numerals used in this application with or without about/approximately are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0025] Other features, objects, and advantages of the present invention are apparent in the detailed description, drawings and claims that follow. It should be understood, however, that the detailed description, the drawings, and the claims, while indicating embodiments of the present invention, are given by way of illustration only, not limitation. Various changes and modifications within the scope of the invention will become apparent to those skilled in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The drawings are for illustration purposes only, not for limitation.

[0027] Figure 1 illustrates exemplary effect of injection time on reconstitution of 100 mg/ml TRU-015 in 10% sucrose formulation. Left picture (a) represents reconstituted material when injection time was approximately 14 seconds. The vial on the right (b) represents the same material with the injection time only about 2 seconds. Higher injection rate resulted in separation of dry cake from the solution due to formation of effervescence.

[0028] Figure 2 illustrates exemplary effect of polysorbate-80 (Tween-80) on clarity of reconstituted solution. Both formulations contain 100 mg/ml TRU-015, 20 mM histidine and 10 % sucrose.

[0029] Figure 3 illustrates exemplary effect of formulation on reconstitution behavior of TRU-015 lyophilized cakes at a concentration of 100 mg/ml. The left vial (a) represents the formulation containing 10% sucrose. The formulation in the center vial (b) has 5% sucrose and 1% glycine, while formulation in the right vial (c) has 5% sucrose and 2.4% sorbitol. All formulations contain 20 mM histidine as a buffer and 0.01% polyosorbate-80 as a surfactant. The pictures were taken immediately after water injection. Slow injection (about 30 seconds) was applied in all cases.

[0030] Figure 4 illustrates exemplary reconstitution of 100 mg/ml TRU-015 in 10% sucrose, 20 mM histidine, and 0.01% polysorbate-80 formulation. The chunk of undissolved solids was attached to the vial wall during intensive swirling. Sticking of particles to the vial wall resulted in an increase in reconstitution time of at least 2 minutes.

[0031] Figure 5 illustrates exemplary lyophilization cycle for 200 mg/ml TRU-015 in 5% sucrose, 10 mM histidine, 0.01% polysorbate-80 formulation. Lyophilization was performed in 2/4 Schott tubing vials at fill volume of 1 ml. Product temperature at the end of primary drying was at glass transition temperature of -21°C.

[0032] Figure 6 illustrates exemplary lyophilization cycle for 200 mg/ml TRU-015 in 10% sucrose, 10 mM histidine, 0.01% polysorbate-80 formulation. Lyophilization was performed in 2/4 Schott tubing vials at fill volume of 1 ml. Product temperature before break was approximately 2 degrees higher than glass transition temperature of -26°C.

[0033] Figure 7 illustrates exemplary reconstitution of 200 mg/ml TRU-015 in 5% sucrose, 10 mM histidine, 0.01% polysorbate-80 formulation. Reconstitution was performed with 0.8 ml of RODI water at room temperature. Injection time was approximately 10 seconds. Lyophilized cake was completely dissolved after 21 min of continuous swirling. Solution cleared in less than 1 min.

[0034] Figure 8 illustrates exemplary reconstitution of 200 mg/ml TRU-015 in 10 % sucrose, 10 mM histidine, 0.01% polysorbate-80 formulation. Reconstitution was performed with 0.8 ml of RODI water at room temperature. Injection time was approximately 10 seconds. Lyophilized cake was completely dissolved after 30 min of continuous swirling (a chunk of the cake apparently stuck to the vial wall slowing reconstitution). Solution cleared in less than 1 minute.

[0035] Figure 9 illustrates exemplary reconstitution of 200 mg/ml TRU-015 in 5% sucrose, 10 mM histidine, 0.01% polysorbate-80 formulation. Reconstitution was performed with 0.8 ml of 5% sucrose and 10 mM histidine solution at room temperature. Injection time was approximately 10 seconds. Lyophilized cake was completely dissolved after 35 min of continuous swirling. Solution cleared in less than 1 min.

[0036] Figure 10 illustrates exemplary reconstitution of 200 mg/ml TRU-015 in 5% sucrose, 10 mM histidine, 0.01% polysorbate-80 formulation. Reconstitution was performed with 0.8 ml of 60 mM sodium chloride and 10 mM histidine solution at room temperature. Injection time was approximately 10 seconds. Lyophilized cake was completely dissolved after 46 min of continuous swirling. Solution cleared in less than 1 minute.

[0037] Figure 11 illustrates exemplary dissolution of 200 mg/ml TRU-015 in 10% sucrose, 10 mM histidine, 0.01% polysorbate-80 formulation. Lyophilized cake was broken into a fine powder prior to reconstitution. Reconstitution was performed with 0.8 ml of RODI water. Approximately 31 minutes of continuous swirling was required to dissolve the powder. Despite the presence of polysorbate, the solution did not clear of turbidity even within 10 min.

[0038] Figure 12 illustrates exemplary slow dissolution of 200 mg/ml TRU-015 in 10% sucrose, 10 mM histidine, 0.01% polysorbate-80 formulation. Approximately 0.8 ml of RODI water was slowly injected into the vial. The vial was not disturbed until all solids apparently were dissolved (4 hours). Then, the vial contents were gently mixed for approximately 10 seconds to ensure homogeneity of the solution.

[0039] Figure 13 illustrates exemplary slow dissolution of 200 mg/ml TRU-015 in 5% sucrose and 10 mM histidine formulation with RODI water as diluent. Approximately 0.8 ml of

RODI was slowly (at least 10 seconds) injected in all three vials (3 minutes and 12 seconds apart). Pictures were taken at different stages of the dissolution process. No agitation was applied to the vials to affect the dissolution rate.

[0040] Figure 14 illustrates exemplary slow dissolution of 200 mg/ml TRU-015 in 5% sucrose and 10 mM histidine formulation with 5% sucrose and 10 mM histidine as diluent. Approximately 0.8 ml of diluent was slowly (at least 10 seconds) injected in all three vials (2.5 minutes apart). Pictures were taken at different stages of the dissolution process. No agitation was applied to the vials to affect the dissolution rate.

[0041] Figure 15 illustrates exemplary dissolution of remaining solids (jelly-like floaters) in 200 mg/ml TRU-015 after 3 hours of slow dissolution. 5% sucrose and 10 mM histidine solution was used as a diluent.

[0042] Figure 16 illustrates exemplary slow dissolution of 200 mg/ml TRU-015 in 5% sucrose and 10 mM histidine formulation with 60 mM NaCl and 10 mM histidine as diluent. Approximately 0.8 ml of diluent was slowly (at least 10 seconds) injected in all three vials (2.5 minutes apart). Pictures were taken at different stages of the dissolution process. No agitation was applied to the vials to affect the dissolution rate.

[0043] Figure 17 illustrates exemplary dissolution of remaining particles of 200 mg/ml TRU-015 dissolved with 60 mM NaCl based diluent. The particles remained undissolved even up to 4 hours after diluent injection.

[0044] Figure 18 illustrates exemplary dissolution kinetics of 200 mg/ml TRU-015 reconstituted with less than the original volume. Vials were reconstituted with 0.48 ml (left vial) and 0.32 ml (right vial) targeting approximately 300 mg/ml and 400 m/ml concentrations respectively. Dissolution was performed at room temperature (~21°C).

[0045] Figure 19 illustrates exemplary reconstitution of TRU-015 lyophilized material (initial concentration of 200 mg/ml) to solutions in the protein concentration range of 200 to 400 mg/ml. Dissolution was performed at refrigerated temperature. The diluent volume was 0.8 ml, 0.55 ml, 0.41 ml, 0.32 ml and 0.25 ml targeting 200 mg/ml, 250 mg/ml, 300 mg/ml, 350 mg/ml and 400 mg/ml respectively. The calculations of diluent volume were made based on the initial

protein concentration and dry solids content. Estimated cake volume was approximately (0.19 ± 0.02) ml.

[0046] Figure 20 illustrates exemplary freeze-drying of 200 mg/ml TRU-015 in Lyo-Ject®. Fill volume was 1 ml.

[0047] Figure 21 illustrates exemplary cake appearance of 200 mg/ml TRU-015 lyophilized in Lyo-Ject®. After lyophilization, plastic caps were placed over the stoppers securing them in place. Syringes were turned up side down and filled with 0.8 ml of water as diluent. Cap was removed prior to water injection.

[0048] Figure 22 illustrates exemplary slow dissolution of 200 mg/ml TRU-015 in 5% sucrose and 10 mM histidine formulation. Water was carefully injected into the chamber with the dry product, completely covering the lyophilized cake. Lyophilized material slowly dissolved without agitation within two hours.

[0049] Figure 23 illustrates exemplary reconstitution of 200 mg/ml TRU-015 (pre-lyo concentration) to the target concentration of approximately 300 mg/ml. Approximately 0.49 ml of water was injected to the chamber with dry product through the syringe pass. No agitation was applied. Slow dissolution was performed at room temperature.

[0050] Figure 24 illustrates exemplary cake appearance of proteins 1-5 after lyophilization in dual chamber syringes (Lyo-Ject®).

[0051] Figure 25 illustrates filling of syringes with diluent (0.9 ml of RODI).

[0052] Figure 26 illustrates exemplary appearance of dual chamber syringes after injection of water into the dry product chamber.

[0053] Figure 27 illustrates exemplary reconstitution behavior of cakes lyophilized in Lyo-Ject® after 30 min of the slow dissolution process.

[0054] Figure 28 illustrates exemplary reconstitution behavior of cakes lyophilized in Lyo-Ject® after 2 hours of the slow dissolution process.

[0055] Figure 29 illustrates the evidence of protein/excipients stratification in protein 4 reconstituted solution. The solution clears after 5 inversions of the syringe.

[0056] Figure 30 illustrates exemplary osmolality of reconstituted solutions as a function of protein concentration. For pure buffer, testing solutions were prepared at the theoretical concentrations that were expected after partial volume reconstitution. The contribution of protein-ion and protein-protein association to osmolality was estimated by subtraction of buffer data from reconstituted solutions data.

[0057] Figure 31 illustrates measurement of glide force during injection of reconstituted protein 4 solution through a 27 gauge needle. Injection speed is 10 mm/min.

DEFINITIONS

[0058] In order for the present invention to be more readily understood, certain terms are first defined. Additional definitions for the following terms and other terms are set forth throughout the specification.

[0059] *Approximately:* As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0060] *Antibodies*: As used herein, the term "antibodies" is intended to include immunoglobulins and fragments thereof which are specifically reactive to the designated protein or peptide, or fragments thereof. Suitable antibodies include, but are not limited to, human antibodies, primatized antibodies, chimeric antibodies, bi-specific antibodies, humanized antibodies, conjugated antibodies (*i.e.*, antibodies conjugated or fused to other proteins, radiolabels, cytotoxins), and antibody fragments. As used herein, the term "antibodies" also includes intact monoclonal antibodies, polyclonal antibodies, multi-specific antibodies (*e.g.* bi-

specific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity. As used herein, an "antibody fragment" includes a portion of an intact antibody, such as, for example, the antigen-binding or variable region of an antibody. Examples of antibody fragments include the Fab, Fab', F(ab')2, and Fv fragments of an intact antibody.

[0061] *Binding protein*: As used herein, the term "binding protein" includes any naturally occurring, synthetic or genetically engineered protein that binds an antigen or a target protein or peptide. Binding proteins can be derived from naturally occurring or synthetically engineered antibodies. A binding protein can function similarly to an antibody by binding to a specific antigen to form a complex and elicit a biological response (e.g., agonize or antagonize a particular biological activity). Binding proteins can include isolated fragments, "Fv" fragments consisting of the variable regions of the heavy and light chains of an antibody, recombinant single chain polypeptide molecules in which light and heavy chain variable regions are connected by a peptide linker ("ScFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

[0062] *Bulking agent*: As used herein, the term "bulking agent" refers to a compound which adds mass to the lyophilized mixture and contributes to the physical structure of the lyophilized cake (e.g., facilitates the production of an essentially uniform lyophilized cake which maintains an open pore structure). Exemplary bulking agents include mannitol, glycine, sodium chloride, hydroxyethyl starch, lactose, sucrose, trehalose, polyethylene glycol and dextran.

[0063] *Diluent*: As used herein, the term "diluent" refers to a pharmaceutically acceptable (e.g., safe and non-toxic for administration to a human) diluting substance useful for the preparation of a reconstituted formulation. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (e.g. phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

[0064] *High molecular weight ("HMW") aggregates*: As used herein, the term "high molecular weight ("HMW") aggregates" refers to an association of at least two protein monomers. For the purposes of this invention, a monomer refers to the single unit of any biologically active form of the protein of interest. For example, a monomer of a small modular

immunopharmaceutical protein can be a monomeric polypeptide, or a homodimer, or a dissociable dimer, or a unit of multivalent complex of SMIP™ protein.

[0065] *Lyoprotectant*: As used herein, the term "lyoprotectant" refers to a molecule that prevents or reduces chemical and/or physical instability of a protein or other substance upon lyophilization and subsequent storage. Exemplary lyoprotectants include sugars such as sucrose or trehalose; an amino acid such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate: a polyol such as trihydric or higher sugar alcohols, e.g. glycerin, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; Pluronics; and combinations thereof. In some embodiments, a lyoprotectant is a non-reducing sugar, such as trehalose or sucrose.

[0066] *Preservative*: As used herein, the term "preservative" refers to a compound which can be added to the diluent to reduce bacterial action in the reconstituted formulation, thus facilitating the production of a multi-use reconstituted formulation. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechoi, resorcinol, cyclohexanol. 3-pentanol, and m-cresol. The most preferred preservative herein is benzyl alcohol.

[0067] *Protein*: As used herein, the term "protein" refers to a sequence of amino acids. Typically, the chain length of a protein is sufficient to produce the higher levels of tertiary and/or quaternary structure. Examples of proteins encompassed within the definition herein include mammalian proteins, such as, e.g., growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; α-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or tissue-type plasminogen activator (t-PA); bombazine; thrombin; tumor necrosis factor-α and -β;

enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-α); serum albumin such as human serum albumin; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; an integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as αFGF and βFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-α and TGF-β, including TGF-β1, TGF-β2, TGF-β3, TGF-β4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(l-3)-IGF-I (brain IGF-I); insulin-like growth factor binding proteins; CD proteins such as CD3, CD4, CD8, CD19 and CD20; erythropoietin (EPO); thrombopoietin (TPO); osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-α, -β, and -γ; colony stimulating factors (CSFs), e.g., M-CSF, GM-CSF, and G-CSF; interleukins (ILs), e.g., IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor (DAF); a viral antigen such as, for example, a portion of the AIDS envelope; transport proteins: homing receptors; addressins; regulatory proteins; immunoadhesins; antibodies; antibody fragments; binding proteins; Small Modular ImmunoPharmaceutical (SMIP™) proteins.

[0068] *Reconstitution*: As used herein, the term "reconstitution" refers to a process of dissolving a lyophilized substance (e.g., protein) in a diluent such that the substance (e.g., protein) is dispersed in the reconstituted formulation.

[0069] *Single-chain Fv (ScFv)*: As used herein, "single-chain Fv" or "ScFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the ScFv to form the desired structure for antigen binding. See, Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994).

[0070] *Single domain antibodies*: As used herein, "single domain antibodies" can include antibodies whose complementarity determining regions are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain antibodies, antibodies naturally devoid of light chains, single domain antibodies derived from conventional 4-chain antibodies. Single domain antibodies may be any of the art, or any future single domain antibodies. Single domain antibodies may be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, and bovine.

[0071] *Single domain binding proteins*: As used herein, "single domain binding proteins" can be any single domain binding scaffold that binds to an antigen, protein or peptide. Single domain binding proteins can include natural, synthetic or genetically engineered protein scaffold that act like an antibody by binding to specific antigen to form a complex and elicit a biological response (e.g., agonize or antagonize a particular biological activity). Single domain binding proteins may be derived from naturally occurring or synthetically engineered antibodies. Single domain binding proteins may be any of the art or any future single domain binding proteins, and may be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, and bovine. In some embodiments, of the invention, a single domain binding protein scaffold can be derived from a variable region of the immunoglobulin found in fish, such as, for example, that which is derived from the immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain binding scaffolds derived from a variable region of NAR ("IgNARs") are described in WO 03/014161 and Streltsov (2005) Protein Sci. 14:2901-2909. In other embodiments, a single domain binding protein is a naturally occurring single domain binding protein known as a heavy chain antibody devoid of light chains. Such single domain binding proteins are disclosed in WO 9404678, for example. For clarity reasons, the variable domain derived from a heavy chain antibody naturally devoid of light chain is known herein as a VHH or "nanobody" to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from antibodies raised in Camelidae species, for example in camel, llama, dromedary, alpaca and guanaco. Other species besides those in the Camelidae family may produce heavy chain antibodies naturally devoid of light chain, and such VHHs are within the scope of the invention.

[0072] *Small Modular ImmunoPharmaceuticals ("SMIP™")*: As used herein, the term "Small Modular ImmunoPharmaceuticals ("SMIP™")" typically refers to binding domain-immunoglobulin fusion proteins including a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge or hinge-acting region polypeptide, which in turn is fused or otherwise connected to a region comprising one or more native or engineered constant regions from an immunoglobulin heavy chain, other than CH1, for example, the CH2 and CH3 regions of IgG and IgA, or the CH3 and CH4 regions of IgE (*see e.g.*, U.S. 2005/0136049 by Ledbetter, J. *et al.* for a more complete description). The binding domain-immunoglobulin fusion protein can further include a region that includes a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide (or CH3 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the hinge region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide (or CH4 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the CH2 constant region polypeptide (or CH3 in the case of a construct derived in whole or in part from IgE). Typically, such binding domain-immunoglobulin fusion proteins are capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement fixation, and/or binding to a target, for example, a target antigen.

[0073] *Substantially*: As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

DETAILED DESCRIPTION

[0074] The present invention provides robust and reproducible reconstitution methods. Among other things, the present invention provides reconstitution methods based on slow dissolution without compromising patient convenience or product quality. Inventive methods in

accordance with the present invention are useful in reconstituting various lyophilized substances including proteins. In particular, the inventive methods of the present invention are useful in reconstituting lyophilized proteins to a high concentration, generally at least 100 mg/ml or higher.

[0075] The present invention is, in part, based on the unexpected discovery that lyophilized proteins may be slowly dissolved, regardless of the formulation, temperature or container design, simply by slowly injecting diluent (e.g., over 10-30 seconds) into the container and letting the container sit without mixing, swirling or agitation. As described in the Examples section, for all examined proteins, this slow dissolution method resulted in a clear solution with protein concentration as high as 400 mg/ml and without compromising protein quality.

[0076] Prior to the present invention, reconstitution typically required continuous agitation such as shaking and/or swirling. Although there was no requirement for reconstitution time from a regulatory perspective, it was traditionally thought that faster reconstitution was superior. An unofficial rule was that reconstitution of lyophilized material should not take longer that 1 minute. However, at high protein concentration, reconstitution time could be as long as 20 min (see, e.g., XOLAIR Preparation and Administration, available at http://www.xolairhcp.com/xolairhcp/how-to-administer.html) or even 2 hours (see Blue L, Yoder H, (2009) "Successful lyophilization development of protein therapeutics," American Pharmaceutical Review, Jan/Feb 2009, pp. 91 – 6) with continuous agitation. To decrease the reconstitution time, scientists had tried to modify formulation, reconstitution, or freeze-drying process. For example, for 50 mg/ml etanercept (see, e.g., International Patent Publication WO2007/014073), annealing at -12°C resulted in decrease of reconstitution time from an average of 125 sec to 63 sec. However, in this case, it was thought that crystalline mannitol served as a slowly dissolving matrix that enhanced penetration of diluent into the internal surface of the dry cake improving the gross dissolution rate. In the case of amorphous material, this method might not work especially if the concentration of proteins is increased. As described in the Examples section, the inventors of the present application found that, for substantially amorphous protein-containing materials at concentrations of ≥100 mg/ml protein, reconstitution time of lyophilized solids could take as long as 40 minutes of continuous swirling. In addition, reconstitution time of highly concentrated protein-containing lyophilized materials was not

reproducible, and varied depending on formulation, injection conditions, intensity of swirling and vial configuration. Furthermore, swirling and shaking during dissolution (process steps normally used in dissolution of lyophilized powders containing proteins at low concentration) might not work in dissolution of proteins at high concentration. The chunks (particles) of undissolved, highly concentrated protein could stick to the wall of the container leaving material without direct contact with the diluent during the dissolution process. A higher viscosity of concentrated protein solution results in a higher probability of this event.

[0077] Contrary to the traditional methods, the inventors of the present application discovered that if a diluent was slowly (typically over 10-30 seconds) injected into the container and the container was allowed to sit without agitation; the container content was slowly and completely dissolved regardless of the formulation, temperature or container design. This method is referred to as the "slow dissolution method" (SDM). As described in the Examples 2, 3, and 4, despite the differences in tested proteins or containers, lyophilized powders can be reconstituted to a clear solution with concentrations up to 400 mg/ml by a slow dissolution method. Although the slow dissolution process may sometimes take up to 17 hours or longer, the protein quality is not compromised. For example, the percentage of HMW species in the reconstituted solution by slow dissolution is unexpectedly low (e.g., only around 2% or less). Therefore, the inventors have concluded that the slow dissolution method of the invention can be applied to any dehydrated material as long as the material has the physical potential to become a solution.

[0078] Thus, the present invention provides a particularly convenient and reliable method for reconstitution of lyophilized proteins and other drug substances by patients for self-administration because reconstitution of a lyophilized drug product can be done in advance without intensive labor.

Lyophilized Substances and preparation thereof

[0079] Inventive methods according to the invention may be used to reconstitute any type of lyophilized substances including, but not limited to, proteins, peptides, nucleic acids (e.g.,

RNAs, DNAs, or RNA/DNA hybrids, aptamers), chemical compounds, small molecules, drug substances, natural products. In some embodiments, lyophilized proteins suitable for the invention include, but are not limited to, antibodies (e.g., monoclonal antibodies) or fragments thereof, growth factors, clotting factors, cytokines, fusion proteins, pharmaceutical drug substances, vaccines, enzymes, Small Modular ImmunoPharmaceutical™ (SMIP™) proteins. In some embodiments, lyophilized antibodies or antibody fragments suitable for the invention include, but are not limited to, IgG, (IgG1, IgG2, IgG3, IgG4), F(ab')2, F(ab)2, Fab', Fab, ScFv, single domain antibodies (e.g., shark single domain antibodies (e.g., IgNAR or fragments thereof)), diabodies, triabodies, tetrabodies, nanobodies.

[0080] In some embodiments, suitable SMIP proteins are derived from an immunoglobulin such as IgG1, IgA, IgE, or the like. For example, a hinge region can be a mutant IgG1 hinge region polypeptide having either zero, one or two cysteine residues. In some embodiments, SMIP proteins suitable for the invention can be mono-specific (i.e., they recognize and attach to a single antigen target to initiate biological activity) or multi-specific (such as SCORPION™ therapeutics, which incorporate a SMIP protein and also have an additional binding domain located C-terminally to the SMIP™ protein portion of the molecule). In some embodiments, the binding domains of SCORPION therapeutics each bind to a different target. In some embodiments, suitable SMIP proteins may contain a binding domain that specifically recognizes and binds to a cognate biological molecule, such as an antigen, a receptor (e.g., CD20), or complex of more than one molecule or assembly or aggregate. Exemplary small modular immunopharmaceuticals include, but are not limited to, small modular immunopharmaceuticals that target CD3, CD4, CD8, CD19, CD20 and CD34; members of the HER receptor family such as the EGF receptor, HER2, HER3 or HER4 receptor; cell adhesion molecules such as LFA-1, Mol, p150,95, VLA-4, ICAM-1, VCAM, growth factors such as VEGF; IgE; blood group antigens; flk2/flt3 receptor; obesity (OB) receptor; protein C; EGFR, RAGE, P40, Dkk1, NOTCH1, IL-13, IL-21, IL-4, and IL-22, etc.

[0081] In some embodiments, suitable SMIP proteins can be humanized and chimeric SMIP™ proteins. Humanized and chimeric SMIP™ proteins are further described in International Application Publication No. WO 2008/156713, which is incorporated by reference herein.

[0082] Additional small modular immunopharmaceuticals are further described in, e.g., US Patent Publications 20030133939, 20030118592, 20040058445, 20050136049, 20050175614, 20050180970, 20050186216, 20050202012, 20050202023, 20050202028, 20050202534, 20050238646, and 20080213273; International Patent Publications WO 2002/056910, WO 2005/037989, and WO 2005/017148, which are all incorporated by reference herein.

[0083] Proteins and other substances can be lyophilized using the methods described in the Examples section and various other methods known in the art. Typically, proteins and other substances are lyophilized in a pre-lyophilization formulation at a desirable concentration. In some embodiments, pre-lyophilization formulations may contain a protein of interest at a concentration of at least approximately 25 mg/ml, 50 mg/ml, 75 mg/ml, 100 mg/ml, 125 mg/ml, 150 mg/ml, 175 mg/ml, 200 mg/ml, 250 mg/ml, 300 mg/ml, 350 mg/ml or 400 mg/ml. In some embodiments, pre-lyophilization formulations may contain a protein of interest at a concentration ranging from about 1 mg/ml to 400 mg/ml (e.g., from about 1 mg/ml to 50 mg/ml, from 1 mg/ml to 100 mg/ml, from about 1 mg/ml to about 150 mg/ml, from about 1 mg/ml to about 200 mg/ml, from about 1 mg/ml to about 250 mg/ml, from about 1 mg/ml to about 300 mg/ml, from about 1 mg/ml to about 350 mg/ml, from about 50 mg/ml to about 400 mg/ml, from about 50 mg/ml to about 350 mg/ml, from about 50 mg/ml to about 300 mg/ml, from about 50 mg/ml to about 250 mg/ml, from about 50 mg/ml to about 200 mg/ml, from about 50 mg/ml to about 150 mg/ml, from about 50 mg/ml to about 100 mg/ml, from about 100 mg/ml to about 150 mg/ml, from about 100 mg/ml to about 200 mg/ml, from about 100 mg/ml to about 250 mg/ml).

[0084] Typically, a pre-lyophilization formulation further contains an appropriate choice of excipients or other components such as stabilizers, buffering agents, bulking agents, and surfactants to prevent compound of interest from degradation (e.g., protein aggregation, deamidation, and/or oxidation) during freeze-drying and storage. The formulation for lyophilization can include one or more additional ingredients including lyoprotectants or stabilizing agents, buffers, bulking agents, isotonicity agents and surfactants.

[0085] In some embodiments, the lyoprotectant or stabilizing agent may be a non-reducing sugar such as sucrose, raffinose, trehalose, or amino acids such as glycine, arginine and

methionine. The amount of stabilizing agent or lyoprotectant in the pre-lyophilized formulation is generally such that, upon reconstitution, the resulting formulation will be isotonic.

[0086] Exemplary lyoprotectant concentrations in the pre-lyophilized formulation may range from about 10 mM to about 400 mM (e.g., from about 30 mM to about 300 mM, and from about 50 mM to about 100 mM), or alternatively, from 0.5% to 15% (e.g., from 1% to 10%, from 5% to 15%, from 5% to 10%) by weight. In some embodiments, the ratio of the mass amount of the stabilizing agent and the compound of interest is about 1:1. In other embodiments, the ratio of the mass amount of the stabilizing agent and the compound can be about 0.1:1, 0.2:1, 0.5:1, 2:1, 5:1, 10:1, or 20:1.

[0087] In some embodiments, compounds of interest may be present in a pH-buffered solution at a pH from about 4-8 (e.g., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0) and, in some embodiments, from about 5-7. Exemplary buffers include histidine, phosphate, Tris, citrate, acetate, sodium acetate, phosphate, succinate and other organic acids. The buffer concentration can be from about 1 mM to about 30 mM, or from about 3 mM to about 20 mM, depending, for example, on the buffer and the desired isotonicity of the formulation (e.g., of the reconstituted formulation). In some embodiments, a suitable buffering agent is present at a concentration of approximately 1 mM, 5 mM, 10 mM, 15 mM, 20 mM, 25 mM, 30 mM, or 50 mM.

[0088] In some embodiments, suitable formulations for lyophilization may further include one or more bulking agents. Suitable bulking agents include, but are not limited to, sodium chloride, lactose, mannitol, glycine, sucrose, trehalose, hydroxyethyl starch. Exemplary concentrations of bulking agents are from about 1% to about 10% (e.g., 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%, 4.5%, 5.0%, 5.5%, 6.0%, 6.5%, 7.0%, 7.5%, 8.0%, 8.5%, 9.0%, 9.5%, and 10.0%).

[0089] In some embodiments, formulations for lyophilization contain an isotonicity agent to keep the pre-lyophilization formulations or the reconstituted formulations isotonic. Typically, by "isotonic" is meant that the formulation of interest has essentially the same osmotic pressure as human blood. Exemplary isotonicity agents include, but are not limited to, glycine, sorbitol, mannitol, sodium chloride, dextrose and arginine. In some embodiments, suitable isotonic

agents may be present in pre-lyophilized formulations at a concentration from about 0.01 – 5 % (e.g., 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 3.0, 4.0 or 5.0%) by weight.

[0090] In some embodiments, it is desirable to add a surfactant to formulations for lyophilization. Exemplary surfactants include nonionic surfactants such as Polysorbates (e.g., Polysorbates 20 or 80); poloxamers (e.g., poloxamer 188); Triton; sodium dodecyl sulfate (SDS); sodium laurel sulfate; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g., lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl ofeyl-taurate; and the MONAQUAT^{TM} series (Mona Industries, Inc., Paterson, N.J.), polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (e.g., Pluronics, PF68, etc). Typically, the amount of surfactant added is such that it reduces aggregation of the reconstituted protein and minimizes the formation of particulates after reconstitution. For example, a surfactant may be present in a pre-lyophilized formulation at a concentration from about 0.001 – 0.5% (e.g., about 0.005 – 0.05%, or 0.005 – 0.01%). In particular, a surfactant may be present in a pre-lyophilized formulation at a concentration of approximately 0.005%, 0.01%, 0.02%, 0.1%, 0.2%, 0.3%, 0.4%, or 0.5%, etc. Alternatively, or in addition, the surfactant may be added to the lyophilized formulation and/or the reconstituted formulation.

[0091] Other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980) may be included in the pre-lyophilized formulation (and/or the lyophilized formulation and/or the reconstituted formulation) provided that they do not adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include, but are not limited to, additional buffering agents; preservatives; co-solvents; antioxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (e.g., Zn-protein complexes); biodegradable polymers such as polyesters; and/or salt-forming counterions such as sodium.

[0092] Exemplary formulations suitable for lyophilization are described in the Examples section. Additional formulations are described in three co-pending provisional applications, each of which is entitled "Lyophilized Formulations for Small Modular Immunopharmaceuticals" and filed on even date, the contents of all of which are hereby incorporated by reference.

[0093] After the substance of interest and any additional components are mixed together, the formulation is lyophilized. Lyophilization generally includes three main stages: freezing, primary drying and secondary drying. Freezing is necessary to convert water to ice or some amorphous formulation components to the crystalline form. Primary drying is the process step when ice is removed from the frozen product by direct sublimation at low pressure and temperature. Secondary drying is the process step when bounded water is removed from the product matrix utilizing the diffusion of residual water to the evaporation surface. Product temperature during secondary drying is normally higher than during primary drying. See, Tang X. et al. (2004) "Design of freeze-drying processes for pharmaceuticals: Practical advice," *Pharm. Res.*, 21:191-200; Nail S.L. et al. (2002) "Fundamentals of freeze-drying," in Development and manufacture of protein pharmaceuticals. Nail S.L. editor New York: Kluwer Academic/Plenum Publishers, pp 281-353; Wang et al. (2000) "Lyophilization and development of solid protein pharmaceuticals," *Int. J. Pharm.*, 203:1-60; Williams N.A. et al. (1984) "The lyophilization of pharmaceuticals; A literature review." *J. Parenteral Sci. Technol.*, 38:48-59. Generally, any lyophilization process can be used in connection with the present invention. Exemplary lyophilization processes are described in the Examples section. Additional lyophilization processes are described in e.g., US Patent Publication 20080098614, International Patent Publication WO2008/042408, and U.S. Patent Application Serial Numbers 61/076129 and 61/086426, which are all incorporated by reference herein.

[0094] In some embodiments, a protein solution is de-gassed prior to freezing. Methods of de-gassing are described in Krishnan S. et al. International Patent Publication WO2007/014073. Without wishing to be bound by any theory, it is contemplated that de-gassing may reduce foaming upon reconstitution.

[0095] In some embodiments, an annealing step may be introduced during the initial freezing of the product. The annealing step may reduce the overall cycle time. Without wishing to be bound by any theories, it is contemplated that the annealing step can help promote

excipient, particularly mannitol, crystallization and formation of larger ice crystals due to re-crystallization of small crystals formed during supercooling, which, in turn, improves reconstitution. Typically, an annealing step includes an interval or oscillation in the temperature during freezing. For example, the freeze temperature may be -40 °C, and the annealing step will increase the temperature to, for example, -10 °C and maintain this temperature for a set period of time. The annealing step time may range from 0.5 hours to 8 hours (e.g., 0.5, 1.0 1.5, 2.0, 2.5, 3, 4, 6, and 8 hours). The annealing temperature may be between the freezing temperature and 0 °C.

[0096] Lyophilization may be performed in a container, such as a tube, a bag, a bottle, a tray, a vial (e.g., a glass vial), syringe or any other suitable containers. The containers may be disposable. Lyophilization may also be performed in a large scale or small scale. In some instances, it may be desirable to lyophilize the protein formulation in the container in which reconstitution of the protein is to be carried out in order to avoid a transfer step. The container in this instance may, for example, be a 3, 4, 5, 10, 20, 50 or 100 cc vial.

[0097] Many different freeze-dryers are available for this purpose such as Hull pilot scale dryer (SP Industries, USA), Genesis (SP Industries) laboratory freeze-dryers, or any freeze-dryers capable of controlling the given lyophilization process parameters. Freeze-drying is accomplished by freezing the formulation and subsequently subliming ice from the frozen content at a temperature suitable for primary drying. Initial freezing brings the formulation to a temperature below about –20 °C (e.g., -50 °C, -45 °C, -40 °C, -35 °C, -30 °C, -25 °C, etc.) in typically not more than about 4 hours (e.g., not more than about 3 hours, not more than about 2.5 hours, not more than about 2 hours). Under this condition, the product temperature is typically below the eutectic point or the collapse temperature of the formulation. Typically, the shelf temperature for the primary drying will range from about -30 to 25 °C (provided the product remains below the melting point during primary drying) at a suitable pressure, ranging typically from about 20 to 250 mTorr. The formulation, size and type of the container holding the sample (e.g., glass vial) and the volume of liquid will mainly dictate the time required for drying, which can range from a few hours to several days. A secondary drying stage is carried out at about 0-60°C, depending primarily on the type and size of container and the type of SMIP™ employed.

Again, volume of liquid will mainly dictate the time required for drying, which can range from a few hours to several days.

[0098] Lyophilized product can be assessed based on product quality analysis, reconstitution time, quality of reconstitution, high molecular weight, moisture, and glass transition temperature. Typically, protein quality and dry product analysis include product degradation rate analysis using methods including, but not limited to, size exclusion HPLC (SE-HPLC), cation exchange-HPLC (CEX-HPLC), X-ray diffraction (XRD), modulated differential scanning calorimetry (mDSC), reversed phase HPLC (RP-HPLC), multi-angle light scattering (MALS), fluorescence, ultraviolet absorption, nephelometry, capillary electrophoresis (CE), SDS-PAGE, and combinations thereof.

[0099] As a general proposition, lyophilization will result in a lyophilized formulation in which the moisture content thereof is less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%, and less than about 0.5%.

[0100] In general, the goal of lyophilization is to produce stable lyophilized formulations containing the compound of interest in addition to the combinations of stabilizers, buffering agents, bulking agents, and/or other excipients. As used herein, a "stable" formulation is one in which the protein therein essentially retains its physical and chemical stability and integrity during lyophilization and upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery*, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10: 29-90 (1993). Stability can be measured after storage at a selected temperature (e.g., 0 °C, 5°C, 25 °C (room temperature), 30 °C, 40 °C) for a selected time period (e.g., 2 weeks, 1 month, 1.5 months, 2 months, 3, months, 4 months, 5 months, 6 months, 12 months, 18 months, 24 months, etc.). For rapid screening, the formulation may be kept at 40 °C for 2 weeks to 1 month, at which time stability is measured. Where the formulation is to be stored at 2-8 °C, generally the formulation should be stable at 25 °C (i.e., room temperature) or 40 °C for at least 1 month and/or stable at 2-8 °C for at least 3 months, 6 months, 1 year or 2 years. Where the formulation is to be stored at 30 °C, generally the formulation should be stable for at least 3 months, 6 months, 1 year or 2 years at 30 °C and/or stable at 40 °C for at least 2 weeks, 1 month,

3 months or 6 months. In some embodiments, the extent of aggregation following lyophilization and storage can be used as an indicator of protein stability (see Examples herein). As used herein, the term "high molecular weight ("HMW") aggregates" refers to an association of at least two protein monomers. For the purposes of this invention, a monomer refers to the single unit of any biologically active form of the protein of interest. For example, a monomer of a small modular immunopharmaceutical protein can be a monomeric polypeptide, or a homodimer, or a dissociable dimer, or a unit of multivalent complex of SMIP™ protein. The association may be covalent, non-covalent, disulfide, non-reducible crosslinking, or by other mechanism.

Reconstitution by slow dissolution method

[0101] At the desired stage, typically at an appropriate time prior to administration to the patient, the lyophilized formulation may be reconstituted with a diluent such that the protein concentration in the reconstituted formulation is desirable.

[0102] Typically, reconstitution typically begins by introducing a diluent into the container containing the lyophilized substance so that the diluent and the lyophilized substance come into contact with each other. According to the present invention, it is desirable to introduce a diluent slowly. It is contemplated that slow introduction of a diluent allows better contact between the diluent and the lyophilized solids. In some embodiments, a diluent is introduced slowly such that the diluent covers the surface of the lyophilized cake. In some embodiments, a diluent is introduced slowly such that the diluent does not disturb the lyophilized cake. In some embodiments, a diluent is introduced slowly such that the diluent does not cause the lyophilized solids to stick to a wall of the container. For example, a diluent may be introduced by slow injection over a period of at least 5 (e.g., at least 10 seconds, at least 12 seconds, at least 14 seconds, at least 16 seconds, at least 18 seconds, at least 20 seconds, or at least 30 seconds). In some embodiments, a diluent may be introduced by slow injection over a period of 10-30 seconds, (e.g., 10-25 seconds, 10-20 seconds, 10-15 seconds, 12-30 seconds, 14-30 seconds, 16-30 seconds, 18-30 seconds, or 20-30 seconds). In addition to injection, various other methods can be used to introduce a diluent slowly. For example, diluent and dry powder could be separated by impermeable barrier during storage. When it is needed, the barrier could

be broken to bring diluent and lyophilized material (or any dried material) in direct contact followed by slow dissolution process.

[0103] Various diluents may be used in accordance with the present invention. In some embodiments, a suitable diluent for reconstitution is water. The water used as the diluent can be treated in a variety of ways including reverse osmosis, distillation, deionization, filtrations (e.g., activated carbon, microfiltration, nanofiltration) and combinations of these treatment methods. In general, the water should be suitable for injection including, but not limited to, sterile water or bacteriostatic water for injection.

[0104] Additional exemplary diluents include a pH buffered solution (e.g., phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution. Suitable diluents may optionally contain a preservative. Exemplary preservatives include aromatic alcohols such as benzyl or phenol alcohol. The amount of preservative employed is determined by assessing different preservative concentrations for compatibility with the protein and preservative efficacy testing. For example, if the preservative is an aromatic alcohol (such as benzyl alcohol), it can be present in an amount from about 0.1-2.0%, from about 0.5-1.5%, or about 1.0-1.2%.

[0105] Diluents suitable for the invention may include a variety of additives, including, but not limited to, pH buffering agents, (e.g. Tris, histidine,) salts (e.g., sodium chloride) and other additives (e.g., sucrose).

[0106] In some embodiments, the container is maintained substantially still during and after the injection of the diluent. By "substantially still," it meant that the container remains substantially at the same position. In some embodiments, the container is substantially still if the change of the position does not cause any undissolved lyophilized solids to stick to a wall of the container. In some embodiments, the container is maintained substantially still for at least 5 minutes, 10 minutes, 30 minutes, 1 hour, 2 hours, 4 hours, 12 hours, 24 hours, 48 hours, 72 hours, or longer. In some embodiments, the container is maintained substantially still until the lyophilized substance is substantially dissolved. As used herein, a lyophilized substance is "substantially dissolved" if more than 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% of the original amount of the lyophilized substance is dissolved or, alternatively, if less than 20%, 15%, 10%, 5%, 4%, 3%, 2%, or 1% of the original amount of the lyophilized substance is present as

solids or semi-solids. This process is also referred to as the slow dissolution process in the present application.

[0107] In some embodiments, the slow dissolution process may take place at room temperature or a temperature higher or lower than the room temperature. For example, the container may be kept substantially still at room temperature (e.g., 20 °C – 30 °C). In some embodiments, the container with the lyophilized substance may be placed in a refrigerator (e.g., 2 °C - 8 °C) after injection of the diluent. In some embodiments, the container may be kept substantially still, for example, at 40°C, 37°C , 35°C, 30°C, 25°C, 20°C, 15°C, 10°C, 8°C, 4°C, or 2°C. In some embodiments, the container may be shifted from one temperature to another during slow dissolution (e.g., from room temperature to 2-8 °C, or from room temperature to 37°C, or from 2-8 °C to room temperature, or from 2-8 °C to 37°C).

[0108] In some embodiments, a mixing (e.g., agitation, shaking, or swirling) step is followed after the slow dissolution process to ensure that the protein or other substances are completely dissolved. For example, the lyophilized substance may not dissolve entirely during slow dissolution, with only a small percentage of the substance remaining undissolved. In these cases, the remaining undissolved solids can be quickly dissolved with agitation, swirling or other types of mixing. In some embodiments, a mixing step is followed after the slow dissolution period to ensure homogeneity of the reconstituted solution. For example, when the reconstituted formulation appears stratified, the layers can be mixed with agitation, swirling or other types of mixing prior to administration.

[0109] Typically, after the slow dissolution period, the solution may be mixed for about at least 5 seconds, 10 seconds, 20 seconds, 30 seconds, 1 minute, 5 minutes, or 10 minutes.

[0110] According to the present invention, a lyophilized substance (e.g., protein) can be reconstituted to a concentration of at least 25 mg/ml (e.g., at least 50 mg/ml, at least 75 mg/ml, at least 100 mg/ml, at least 150 mg/ml, at least 200 mg/ml, at least 250 mg/ml, at least 300 mg/ml, at least 350 mg/ml, or at least 400 mg/ml). In some embodiments, a lyophilized substance (e.g., protein) may be reconstituted to a concentration ranging from about 1 mg/ml to 400 mg/ml (e.g., from about 1 mg/ml to 50 mg/ml, from 1 mg/ml to 100 mg/ml, from about 1 mg/ml to about 150 mg/ml, from about 1 mg/ml to about 200 mg/ml, from about 1 mg/ml to about 250 mg/ml, from

about 1 mg/ml to about 300 mg/ml, from about 1 mg/ml to about 350 mg/ml, from about 50 mg/ml to about 400 mg/ml, from about 50 mg/ml to about 350 mg/ml, from about 50 mg/ml to about 300 mg/ml, from about 50 mg/ml to about 250 mg/ml, from about 50 mg/ml to about 200 mg/ml, from about 50 mg/ml to about 150 mg/ml, from about 100 mg/ml to about 150 mg/ml, from about 100 mg/ml to about 200 mg/ml, from about 100 mg/ml to about 250 mg/ml, from about 100 mg/ml to about 350 mg/ml, from about 100 mg/ml to about 400 mg/ml, from about 150 mg/ml to about 250 mg/ml, from about 150 mg/ml to about 300 mg/ml, from about 150 mg/ml to about 350 mg/ml, from about 150 mg/ml to about 400 mg/ml, from about 200 mg/ml to about 250 mg/ml, from about 200 mg/ml to about 300 mg/ml, from about 200 mg/ml to about 350 mg/ml, or from about 200 mg/ml to about 400 mg/ml). In some embodiments, the concentration of protein in the reconstituted formulation may be higher than the concentration in the pre-lyophilization formulation. High protein concentrations in the reconstituted formulation are considered to be particularly useful where subcutaneous or intramuscular delivery of the reconstituted formulation is intended. In some embodiments, the protein concentration in the reconstituted formulation may be about 2-50 times (e.g., about 2-20, about 2-10 times, or about 2-5 times) of the pre-lyophilized formulation. In some embodiments, the protein concentration in the reconstituted formulation may be at least about 2 times (e.g., at least about 3, 4, 5, 10, 20, 40 times) of the pre-lyophilized formulation.

[0111] Reconstitution according to the present invention may be performed in any container. Exemplary containers suitable for the invention include, but are not limited to, such as tubes, vials, syringes (e.g., single-chamber or dual-chamber), bags, bottles, and trays. Suitable containers may be made of any materials such as glass, plastics, metal. The containers may be disposable or reusable. Reconstitution may also be performed in a large scale or small scale.

[0112] In some instances, it may be desirable to lyophilize the protein formulation in the container in which reconstitution of the protein is to be carried out in order to avoid a transfer step. The container in this instance may, for example, be a 3, 4, 5, 10, 20, 50 or 100 cc vial. In some embodiments, a suitable container for lyophilization and reconstitution is a dual chamber syringe (e.g., Lyo-Ject,® (Vetter) syringes). For example, a dual chamber syringe may contain both the lyophilized substance and the diluent, each in a separate chamber, separated by a stopper (see Example 5). To reconstitute, a plunger can be attached to the stopper at the diluent

side and pressed to move diluent into the product chamber so that the diluent can contact the lyophilized substance and reconstitution may take place as described herein (see Example 5).

[0113] In some embodiments, reconstituted formulations can be assessed based on product quality analysis. Typically, protein quality analysis include product degradation rate analysis using methods including, but not limited to, size exclusion HPLC (SE-HPLC), cation exchange-HPLC (CEX-HPLC), reversed phase HPLC (RP-HPLC), multi-angle light scattering (MALS), fluorescence, ultraviolet absorption, nephelometry, capillary electrophoresis (CE), SDS-PAGE, and combinations thereof. In some embodiments, the extent of aggregation following reconstitution can be used as an indicator of protein quality (see Examples below). In some embodiments, a reconstituted protein formulation according to the invention contains less than 5% (e.g., less than 4%, 3.5%, 3.0%, 2.5%, 2.0%, 1.5%, 1.0%) aggregated form of the protein following reconstitution.

Administration

[0114] The reconstituted formulation is administered to a subject in need of treatment with the lyophilized substance (e.g., a protein), for example, a human, in accordance with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

[0115] In some embodiments, the reconstituted formulation is administered to the subject by subcutaneous (i.e., beneath the skin) administration. For such purposes, the formulation may be injected using a syringe (e.g. Lyo-Ject). However, other devices for administration of the formulation are available such as injection devices (e.g., the Inject-ease™ and Genject™ devices); injector pens (such as the GenPen™); needleless devices (e.g., MediJector™ and BioJector™); and subcutaneous patch delivery systems.

[0116] The appropriate dosage ("therapeutically effective amount") of the reconstituted substance (e.g., protein) will depend, for example, on the condition to be treated, the severity and course of the condition, whether the protein is administered for preventive or therapeutic

purposes, previous therapy, the patient's clinical history and response to the protein, the type of protein used, and the discretion of the attending physician. The reconstituted substance (e.g., protein) is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards. The protein may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question.

Kits

[0117] The present invention provides kits or other articles of manufacture which contains the lyophilized substance and provides instructions for its reconstitution and/or use. Kits or other articles of manufacture may include a container as described above. Suitable containers include, for example, bottles, vials, tubes, and syringes. The container may be formed from a variety of materials such as glass, metal or plastic. The container holds the lyophilized substance. Instructions such as the label on, or associated with, the container, or a user's manual may indicate directions for reconstitution by slow dissolution as described herein. The label may further indicate that the formulation is useful or intended for, for example, subcutaneous administration. The container holding the formulation may be a multi-use vial, which allows for repeat administrations (e.g., from 2-6 administrations) of the reconstituted formulation. Kits or other articles of manufacture may further include a second container comprising a suitable diluent (e.g., BWFI). Upon mixing of the diluent and the lyophilized formulation, the final protein concentration in the reconstituted formulation will generally be at least 25 mg/ml (e.g., at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml, at least 100 mg/ml, at least 150 mg/ml, at least 200 mg/ml, at least 250 mg/ml at least 300 mg/ml, or at least 400 mg/ml). In some embodiments, the container is a dual chamber syringe, as described above, containing both the lyophilized substance and the diluent. Kits or other articles of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0118] In some embodiments, a kit according to the invention includes a vial or other suitable container containing lyophilized substance and a pre-filled diluent syringe or a prefilled dual chamber syringe with both the lyophilized substance and the diluent. The pre-filled diluent

may be any solution suitable for reconstitution (e.g., BWFI, RO/DI water, or 0.9% Sodium Chloride solution, etc.). A suitable syringe may be plastic or glass and may be disposable or re-usable. A suitable syringe may also be of various sizes (e.g., 1 ml, 2 ml, 4 ml, 6 ml, 8 ml, 10 ml). In some embodiments, a syringe may have a plunger rod attached to the syringe tube. In some embodiments, a syringe may have a detached plunger rod that needs to be assembled by the user. Typically, a suitable syringe may have a tamper-resistant plastic tip cap that can be taken or broken off before administration. The cap may also be replaced to prevent possible contamination if the reconstituted substance is not immediately used. Suitable vials or other containers containing lyophilized product may be plastic or glass and may be disposable or re-usable. A suitable vial or other container such as an ampoule may be sealed with, e.g., rubber stopper, glass and/or plastic cap. In some embodiments, a kit may include an adapter that can be used to penetrate the vial stopper. In some embodiments, an adapter includes a needle that can be used to penetrate the vial stopper and is adapted to be attached to the syringe for reconstitution of the lyophilized product and injection. In some embodiments, a kit may include multiple prefilled vials, multiple pre-filled syringes, and/or a larger syringe for administering the contents of multiple vials. Typically, components of a kit can be separately packaged and sterilized. In some embodiments, a kit may include an instruction for use including specific reconstitution and/or administration procedures.

[0119] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All literature citations are incorporated by reference.

EXAMPLES

Example 1: Effect of formulation and reconstitution conditions on dissolution of 100 mg/ml TRU-015

[0120] Experiments described in this example demonstrate, among other things, that slow injection (e.g., more than 10s) facilitates reconstitution of lyophilized substances, in particular, highly concentrated proteins (e.g., ≥ 100 mg/ml).

[0121] TRU-015 bulk material was dialyzed into three isotonic formulations. Formulation 1 contained 10% sucrose as stabilizer, formulation 2 contained 5% sucrose as stabilizer and 1% glycine for isotonicity adjustment, and formulation 3 contained 5% sucrose and 2.4% sorbitol as stabilizer and isotonicity adjustment component, respectively. All formulations also contained 20 mM histidine as a buffer. Some vials also contained 0.01% polysorbate-80. The lyophilization cycle was performed below the lowest glass transition temperature (e.g., -25°C). Material was filled in 2/4 ml Schott vials at a fill volume of 1.2 ml. Drug product had acceptable cake appearance with the final residual moisture below 0.5%. Lyophilized material was reconstituted to the original volume with RODI water.

[0122] The injection procedure is important for the reconstitution process. The rate of injection was optimized with formulation 1 containing 10% sucrose. If water was injected into the vial very slowly (e.g., 14-30s), the lyophilized cake slowly collapsed and finally dissolved within 3 minutes when gentle and constant swirling was applied (left vial, Figure 1). However, when water was injected very quickly, the cake floated on the surface of the solution separated by the effervescence (foam) (right vial, Figure 1). Typically, more vigorous swirling for approximately 10.5 min was required to dissolve the vial content. We also noted that the reconstituted solution was hazy and cleared when stored overnight at 2-8°C. However, reconstituted solution cleared very quickly (in less than 1 minute) regardless of fast or slow injection if polysorbate-80 (as a surfactant) was added to the pre-lyophilization formulation. Figure 2 shows the effect of polysorbate-80 on clarity of reconstituted solution. Reconstituted vials with no polysorbate-80 also have notable foam on the surface of the solution.

[0123] The effect of formulation on reconstitution behavior of 100 mg/ml TRU-015 was further investigated with the three isotonic formulations used as an example (Figure 3). The injection time for all three formulations was approximately 30 seconds. With formulation 1 containing 10% sucrose, the lyophilized cake disintegrated immediately after injection (Figure 3a). Gentle swirling within 3 minutes was enough to dissolve the vial content. With formulations 2 and 3, the lyophilized cake remained as a tablet at the end of injection. At least 10 minutes of intensive swirling was required to dissolve the tablets. Despite the difference in composition and solids content, both formulations (b and c, Figure 3) showed similar dissolution behavior. It should be mentioned that all three formulations were amorphous (confirmed with X-Ray Powder diffraction, data not shown). Therefore, the difference in dissolution is likely attributed to the additional sucrose content: 10% sucrose in the left vial compared to center and right vials that contain only 5% sucrose (Figure 3). Alternatively, the presence of amorphous glycine or sorbitol may also decrease the apparent wetability of lyophilized cakes.

[0124] Swirling process is also important to reconstitution of highly concentrated proteins (≥100 mg/ml). Typically, if the vial was swirled slightly more aggressively, chunks of undissolved solids could stick to the wall of the vial (Figure 4), significantly increasing reconstitution time.

Example 2: Effect of formulation and reconstitution conditions on dissolution of 200 mg/ml TRU-015

[0125] Demand for an increase in delivered dose of pharmaceuticals dictates a need for an increase in protein concentration in ready to use solutions. The experiments described in this example were designed in part to address this demand. Briefly, TRU-015 protein concentration was increased to 200 mg/ml. The basic formulation contained 5% sucrose, 10 mM histidine, 0.01% polysorbate-80. This formulation was found to be desirable for drug substance manufacturing. To make the solution isotonic, the concentration of sucrose was increased to 10% by spiking in a concentrated sucrose solution. Due to the difference in formulations, glass transition temperatures of these two TRU-015 solutions were also different: Tg' for 5% sucrose based solution was approximately -21°C, whereas Tg' for 10% sucrose based formulation was 5°C lower. Therefore, each formulation required a separate lyophilization cycle (Figures 5 and

6). Product temperatures during primary drying were at glass transition temperature (Figure 5) or slightly higher than Tg' (Figure 6). However, the cake appearance for both formulations was similar and acceptable.

[0126] Reconstitution of 200 mg/ml TRU-015 formulations is shown in Figures 7 and 8. Both lyophilized cakes were finally dissolved by prolonged and continuous swirling. The solutions cleared very quickly after the solids dissolution procedure was completed. Addition of additional sucrose to pre-lyophilization formulation (from 5% (Fig. 7) to 10% (Fig. 8)) does not appear to significantly improve the reconstitution time. Reconstitution conditions of 200 mg/ml TRU-015 (for example, the chunks of cake that stuck to the walls during continuous swirling) appeared to have more impact on reconstitution time. The viscosities of both solutions were comparable (Table 1). The increase in high molecular weight species, as a total percentage of weight, after freeze-drying and reconstitution did not exceed 1.1% (Table 1).

[0127] To reach theoretical isotonic conditions in reconstituted solutions, two different diluents were tested: 5% sucrose and 60 mM sodium chloride. Both diluents also contained 10 mM histidine. Figure 9 shows that reconstitution time was increased when 5% sucrose-based diluent was used instead of water. Reconstitution time with 60 mM NaCl-based diluent was even longer than with 5% sucrose based diluent (Figure 10).

[0128] Before the present invention, it was speculated that reconstitution of highly concentrated protein solutions could be difficult because of restricted access of water to the surface of the lyophilized cake.

[0129] The inventors of the present application tested whether the dissolution rate can be increased by increasing the surface contact between water and dry cake. Briefly, lyophilized cake was broken into a fine powder which was then dissolved using a similar procedure as was utilized for the intact cake in the same formulation (200 mg/ml TRU-015 in 10% sucrose, 10 mM histidine, 0.01% polysorbate-80). Water was slowly (e.g., at least 10 seconds) injected into the vial and remained on top of the powder during injection. During swirling the powder formed a cone that easily settled when swirling was stopped for a short time. Eventually, the powder was slowly dissolved layer by layer (Figure 11). Total dissolution time was almost the same as

for the intact lyophilized cake (see Figure 8). However, the reconstituted solution was turbid (Figure 11 and Table 1).

[0130] Therefore, no significant reduction of dissolution time was observed with increased surface area for 200 mg/ml TRU-015. Depending on formulation, diluent, or reconstitution conditions, total reconstitution times were within the range of 21 to 46 min of continuous swirling. Continuous swirling for this amount of time may not be convenient for patients, especially if the drug is a self-administered pharmaceutical. The inventors of the present application asked the question: how long would it take for lyophilized solids to dissolve if a diluent was simply added to the vial without an intensive swirling procedure? One experiment showed that only 4 hours were required to completely dissolve 200 mg/ml TRU-015 in 10 % sucrose, 10 mM histidine, 0.01% polysorbate-80 formulation (Figure 12). The diluent (RODI) was slowly injected into the vial and the vial content (i.e., the lyophilized TRU-015 mixture) was successfully dissolved without any mixing. When solids were apparently dissolved (after 4 hours) 10 seconds of swirling was applied to insure homogeneity. This procedure, though longer than traditional reconstitution, does not require any patient attention to the reconstitution process and can be done prior to the administration of drug.

[0131] Various exemplary reconstitution conditions and reconstituted product characteristics are summarized in Table 1.

Table 1. Exemplary reconstitution conditions of 200 mg/ml TRU-015.

*Basic formulation contains 5% sucrose, 10 mM histidine and 0.01% of polysorbate-80 at pH 6.0.

Pre-lyophilization Formulation	Diluent	Turbidity, NTU	Viscosity, cPs	Recon-stitution time	HMW by SEC,%		Note
					Pre-lyo	Post-lyo	
Basic*	Water	35	17.6	21 min	1.1	2.0	
Basic & 5% sucrose	Water	41	17.1	30 min	1.1	1.9	
Basic	5% sucrose & 10 mM histidine	44	14.8	35 min	1.1	2.0	
Basic	60 mM NaCl & 10 mM histidine	61	18.5	46 min	1.1	2.0	
Basic & 5% sucrose	Water	41	17.1	4 hours	1.1	2.1	No agitation
Basic	Water	489	15.5	31 min	1.1	2.2	Cake was broken into powder. 90% of recovery.

[0132] Data in Table 1 show that freeze-drying and reconstitution of 200 mg/ml TRU-15 resulted in a very small (approximately 1%) increase in total HMW percentage. The viscosities of reconstituted solutions were relatively high but comparable. When drug product was reconstituted with a diluent containing 60 mM NaCl, the turbidity of the resulting solution was higher as compared to either pure water or 5% sucrose based diluent. The turbidity of reconstituted powder (from the broken cake) was an order of magnitude higher than turbidity of the other tested solutions. Unexpectedly, the percentage of HMW species in the reconstituted solution by slow dissolution without agitation (4 hours of slow dissolution) is comparable to that in the reconstituted solution by continuous swirling (see, Table 1). These data showed that slow dissolution is a promising method for convenient reconstitution of lyophilized substances including highly concentrated proteins.

Example 3. Use of slow dissolution method ("SDM") to reconstitute 200 mg/ml TRU-015 with different diluents

[0133] The experiments described in this example demonstrated that various diluents may be used for slow dissolution.

[0134] Slow dissolution method was used to reconstitute TRU-015 lyophilized in 5% sucrose and 10 mM histidine formulation at concentration of 200 mg/ml. Polysorbate-80 was not added. Lyophilization was performed using the cycle shown in Figure 5. Lyophilized material was reconstituted with three different diluents: (1) RODI water, (2) 5% sucrose plus 10 mM histidine, and (3) 60 mM sodium chloride plus 10 mM histidine. Three vials of lyophilized material were used with each diluent. 0.8 ml diluent was used for each vial.

[0135] Reconstitution of lyophilized cakes with RODI water using SDM is shown in Figure 13. Pictures in Figure 13 show that cakes were gradually dissolved within 2.5 hours. The apparent undissolved solids seen in the last picture (Figure 13) were quickly dissolved in less than 1 min of continuous swirling. All vials showed similar reconstitution time demonstrating good reproducibility of the slow dissolution method.

[0136] The dissolution of 200 mg/ml TRU-015 with 5% sucrose based diluent is shown in Figure 14. After 2 hours and 20 minutes, the formation of floating jelly-like substances was observed in all vials. The content of the right vial shown in Figure 14 was swirled in an attempt to accelerate the dissolution process. It took, however, 10 minutes of intensive swirling and 5 minutes of additional slow dissolution to completely dissolve the content of the right vial. Jelly-like floaters were seen in the center and left vials even after 3 hours after injection. When those vials were agitated, the floaters were dissolved in less than 1 minute (Figure 15).

[0137] Undissolved solids were seen up to 4 hours of slow dissolution when 60 mM NaCl based diluent was used (Figure 16). The dissolution process varied significantly from vial to vial (Figure 17).

[0138] The contents of the right vial were dissolved with swirling within 3 minutes after 2 hours and 40 minutes of the slow dissolution process. However, 8 minutes of intensive swirling was required to dissolve the content of the left vial in addition to 4 hours of slow

dissolution. Exemplary effects of diluent and properties of reconstituted solutions are summarized in Table 2.

Table 2. Exemplary effects of diluent and properties of reconstituted solutions

Diluent	Vial	Reconstitution time		Turbidity, NTU	Viscosity, cPs	HMW (%)	
		Slow dissolution	Additional swirling			Pre-lyo	Post-lyo and recon.
Water	Left	2.5 hours	45s	38	17.6	1.1	2.0
	Center	2.5 hours	1 min				
	Right	2.5 hours	<1 min				
5% sucrose +10 mM histidine	Left	3 hours	20s	48	14.8	1.1	2.0
	Center	3 hours	1 min				
	Right	2hrs 20 min	10 min +5 min SDM*				
60 mM NaCL +10 mM histidine	Left	4 hrs	8 min	74	18.5	1.1	2.0
	Center	3hrs	7 min				
	Right	2hrs 40min	3 min				

[0139] These data showed that regardless of diluent, the slow dissolution method proved to be efficient for reconstitution of 200 mg/ml TRU-015. An increase in HMW species after slow dissolution reconstitution was consistently less than 1% (see Table 2).

Example 4. Reconstitution by SDM of TRU-015 at a concentration as high as 400 mg/ml

[0140] 1 ml of TRU-015 was lyophilized in 2/4 Schott tubing vials at an initial concentration of 200 mg/ml in 5% sucrose and 10 mM histidine formulation. Lyophilization was performed using the cycle shown in Figure 5. Lyophilized cakes were reconstituted with 0.48 ml and 0.32 ml of water (RODI) to obtain the final concentration of TRU-015 up to 300 mg/ml and 400 mg/ml, respectively. Figure 18 demonstrates the kinetics of cake dissolution during the slow dissolution process. No agitation was used during the dissolution process. The diluent was slowly (~10s) injected into the vial to make sure that lyophilized cake was covered with the diluent. The diluent remained on top of the cake for almost 7 hours until the cake collapsed. After 9 hours of slow dissolution large chunks of undissolved material remained in the vials. After 24 hours at room temperature, particles were completely dissolved. Reconstituted solutions appeared to be very viscous but contained no undissolved particles. The formation of

large bubbles was observed during the slow dissolution process (no polysorbate-80 was used in the formulation).

[0141] The experiment confirmed that lyophilized protein formulations can be reconstituted by SDM up to a concentration of approximately 400 mg/ml (the concentration was estimated based on material balance: the mass of water removed during lyophilization as well as the mass of dry powder in the vial were measured. Based on these data cake volume was calculated. The final concentration was estimated using known diluent volume). Additional experiments were performed to determine the effect of slow dissolution on the quality of reconstituted protein at a concentration within the range of 200 mg/ml to 400 mg/ml. Polysorbate-80 was added in pre-lyo formulation to a final concentration of 0.01% to prevent bubble formation during slow dissolution. To minimize aggregation during slow dissolution, vials were placed in a refrigerator at 2-8°C after diluent was added to the vials. The reconstitution process is shown in Figure 19. After 17 hours of slow dissolution at 2-8°C, solids were completely dissolved up to a concentration of 300 mg/ml (the third vial from the left in Figure 19). In the rest of the vials (i.e., protein concentration ranging between 300 mg/ml and 400 mg/ml), solids were completely dissolved after additional 2 days. Exemplary protein concentrations and characteristics after reconstitution are shown in Table 3.

Table 3. Exemplary protein concentrations and characteristics after reconstitution by SDM*
*SDM was performed at 2-8°C for 17 hours (for the range 200-300 mg/ml) or almost three days for the 350 mg/ml and 400 mg/ml materials.

Vial # from the left	Diluent volume, ml	Concentration, mg/ml		HMW (%)		Viscosity cPs
		Targeted	Actual	Pre-lyo	Post-lyo and recon	
1	0.8	200	195.9	1.1	2.0	19
2	0.55	250	251.1	1.1	2.0	78
3	0.41	300	292.4	1.1	2.1	472
4	0.32	350	362.5	1.1	1.9	2800
5	0.25	400	402.2	1.1	2.1	7400

[0142] Data in Table 3 show that despite a long reconstitution time (almost three days (e.g., about 71 hours) for the highest protein concentration) the quality of protein did not change. The increase in protein concentration from 200 mg/ml to 400 mg/ml did not result in an increase in HMW species. However, the viscosity of the solution increased almost exponentially.

[0143] Examples 1-4 demonstrate that (1) proteins can be successfully freeze-dried at a high concentration (e.g., up to 200 mg/ml) in tubing vials and reconstituted to an even higher concentration (e.g., up to 400 mg/ml) using a slow dissolution method; (2) unexpectedly, agitation does not appear to be required for reconstitution of lyophilized substance; (3) it may be desirable to inject diluent slowly to cover the entire cake; and (4) the percentage of aggregated form (i.e., HMW species) of the protein in the reconstituted solution prepared by SDM is unexpectedly low indicating that the slow dissolution process appears to have no negative impact on protein quality.

Example 5: Reconstitution of 200 mg/ml TRU-015 lyophilized in dual-chamber syringe using slow dissolution method.

[0144] The experiments described in Examples 5 and 6 demonstrated that slow dissolution methods according to the present invention may be used to reconstitute different proteins in different types of containers including dual chamber syringe (e.g., Vetter Lyo-Ject®).

[0145] TRU-015 at 200 mg/ml in 5% sucrose, 10 mM histidine formulation was lyophilized in dual chamber syringes (Lyo-Ject®(Vetter)). Briefly, 1 ml of the pre-lyophilization solution was carefully placed on top of the stopper that separates the two chambers. Syringes were then semi-stoppered with a second stopper that has vents for water vapor, allowing water to escape during lyophilization. Syringes were positioned on a special rack that supports the syringes during lyophilization. The protein was lyophilized using the cycle shown in Figure 20. After lyophilization, syringes were stoppered using a lyophilizer stoppering mechanism. Plastic caps were then placed on top of the stoppers, securing stopper in place. Cake appearance is shown in Figure 21. Syringes were then turned up side down and 0.82 ml of RODI was added to the second chamber. An additional stopper was inserted to seal the second chamber. Therefore, one chamber contained the freeze-dried cake, and the second chamber contained the diluent, separated by a water impermeable stopper.

[0146] During reconstitution, the plastic cap of the second chamber containing the diluent was removed, and a plastic plunger was attached to the stopper from the diluent side. Holding the syringe vertically, the stopper from the diluent side was moved up toward the stopper that had a lyophilized cake on the other side. Both stoppers and the water between them

were gradually moved up to the position where water was able to penetrate to the top chamber through the pass. When the lower stopper reached the upper stopper, the water from the diluent chamber completely (with the exception of the pass dead volume) moved from the diluent chamber to the product chamber (Figure 22, left picture, top row).

[0147] In this experiment, the injection of water was performed in such a way that water finally resided on top of the lyophilized cake (similarly to the slow dissolution process in the vials used in Examples 3 and 4). Two hours of slow dissolution (no agitation) was enough to dissolve all solids. In an attempt to increase protein concentration in solution, 200 mg/ml TRU-015 was reconstituted with 0.49 ml of water targeting a concentration of 300 mg/ml (Figure 23). Within 17 hours lyophilized cake was completely dissolved without agitation.

[0148] Data from Example 5 showed feasibility of the slow dissolution method to be used with different types of container (e.g., a dual chamber syringe) for reconstitution of lyophilized proteins up to a high concentration of, e.g., 300 mg/ml.

Example 6: Reconstitution of 200 mg/ml proteins lyophilized in Lyo-Ject using slow dissolution method

[0149] This example was carried out to prove that the slow dissolution method works for many types of proteins. Briefly, TRU-015 and five additional proteins with different molecular weights were tested. For convenience, TRU-015 was named as Protein 6 and the rest of proteins were Proteins 1-5. Certain molecular characteristics of Proteins 1-5 are summarized in Table 4.

[0150] Five different proteins shown in Table 4 were dialyzed into 5% sucrose, 10 mM histidine and 0.01% polysorbate-80 formulation and concentrated to approximately 200 mg/ml. Five syringes of each formulation were filled with 1.1 ml of solution. Lyophilization cycle shown in Figure 20 was used to manufacture the syringes.

Table 4. Description of proteins used in the slow dissolution study.

Protein #	MW	Short description	Concentration, mg/ml
1	105 kD	Small modular immunopharmaceutical	207
2	150 kD	Fusion protein	160*
3	150 kD	Monoclonal IgG1 antibody	196
4	36 kD	Nanobody	200
5	150 kD	Monoclonal IgG2 antibody	199

*Represents maximum concentration of protein #2 in this particular formulation.

[0151] After lyophilization, cake appearance was similar between all proteins (Figure 24). Some cracks were observed at the bottom of the cakes formed likely during secondary drying, which resulted in cakes being loose within the chamber. When the syringe was inverted for diluent filling, not all of the cake returned to the same position (see, Figure 25, Protein 4).

[0152] After filling with the diluent (0.9 ml of RODI) in the second chamber, plastic caps were removed and water was injected slowly into the dry product chamber (holding syringes in vertical position) as described in Example 5. In some syringes (Protein 3 and Protein 4 in Figure 26), the dry cake was not completely overlaid with the diluent. However, all cakes were wetted (or at least in contact with the diluent) only after 30 minutes of slow dissolution (Figure 27). No agitation was used. Syringes were in vertical position during the entire dissolution process. The results demonstrated that slow dissolution worked for all tested proteins; however, significantly different dissolution rates were observed for different proteins. Lyophilized cakes of Protein 4 were almost dissolved after only 1 hour of slow dissolution with small particles seen on top of the solution. Particles of Protein 4 were completely dissolved within 2 hours of slow dissolution process (Figure 28). Lyophilized cakes of Proteins 1-3 completely collapsed after 2 hours of slow dissolution (Figure 28) with Protein 3 having larger particles than Proteins 1 and 2. After 6 hours of slow dissolution process, all proteins, except protein 5, were dissolved. Large particles remained in solution 5 even after 6 hours of the slow dissolution process. Solutions 1-4 were clear and free of particles. When the syringe of Protein 4 was inverted, a strong diffraction of light was observed (Figure 29) indicating significant protein/excipients stratification within the reconstituted solution. Five turns were used to homogenize the Protein 4 solution.

[0153] Longer time was used to mix Proteins 1-3 by gently inverting the syringe several times. Alternatively, less than 20 seconds of intensive shaking allowed complete mixing of Protein 1-4 solutions. Protein 5 solution was kept in a refrigerator for additional 16 hours to dissolve the particles. The solution was then quickly mixed by intensive shaking (no inversion).

[0154] Data from these experiments demonstrated that there can be significant reconstitution kinetics with different proteins. However, slow dissolution methods successfully reconstituted all tested proteins.

Example 7: Osmolality of Reconstituted Protein Solutions

[0155] This experiment was performed to demonstrate that reconstituted protein solutions according to the present invention have suitable osmolality for *in vivo* administration. The osmolality of the reconstituted solution is important in the viability of injectable solutions, as the osmolality of the reconstituted solution should approximate the osmolality in the body. The osmolality of each of the reconstituted solutions of TRU-015 from Example 4 was analyzed. In addition to the reconstituted solution's osmolality, the osmolality of the buffer solution alone was measured. For the buffer, the osmolality was measured for the theoretical concentrations that were expected after partial volume reconstitution. From these two measurements the osmolality contribution of protein-protein and protein-ion associations was calculated. The results of the osmolality measurements and association contribution are shown in Figure 30. Results for the five different concentrations of TRU-015 protein are shown in Table 5.

Table 5: Properties of reconstituted solutions (TRU-015): Reconstitution to smaller volumes at refrigerated temperature with no agitation applied.

Concentration mg/ml	Reconstitution Volume	% HMW (post-lyo)	Viscosity cPs	Osmolality mOsm/kg
196	0.8	2.0	19	245 ± 2
251	0.55	2.0	78	366 ± 2
292	0.41	2.1	472	548 ± 1
363	0.32	1.9	2800	754 ± 2
402	0.25	2.1	7400	984 ± 5

Example 8 "Syringe-ability" of Reconstituted Solution

[0156] This example demonstrates that reconstituted solutions according to the present invention are suitable for syringe injection, also referred to as the "syringe-ability." Typically, the syringe-ability depends on the viscosity and other physical properties of a solution. Typically, the syringe-ability of a solution can be determined by the glide force used to inject the solution through a needle. In this example, each of reconstituted protein 1-6 solutions was injected through a 27 gauge needle and the glide force was measured. Typically, the injection speed is 10 mm/minute. Exemplary results are summarized in Table 6 and Figure 31.

Table 6: Exemplary results of the syringe-ability test

Protein	Concentration mg/ml	Reconstitution Time (hrs)	Viscosity cPs	Glide Force, N
1	207	5.5	31	43.5
2	160	5.5	169	> 95
3	196	6.0	41	46.9
4	200	2.0	13	15.9
5	199	6 (Room Temp.) + 16 (@ 5 °C)	107	>95
6	200	2.0	19	–

Note: 1. Solutions were reconstituted (with no agitation) in dual chamber syringes.

2. Protein 2 has many glycosylation sites.

Equivalents

[0157] The foregoing has been a description of certain non-limiting embodiments of the invention. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Those of ordinary skill in the art will appreciate that various changes and modifications to this description may be made without departing from the spirit or scope of the present invention, as defined in the following claims.

[0158] In the claims articles such as "a,", "an" and "the" may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, *etc.*, from one or more of the claims or from relevant portions of the description is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Furthermore, where the claims recite a composition, it is to be understood that methods of using the composition for any of the purposes disclosed herein are included, and methods of making the composition according to any of the methods of making disclosed herein or other methods known in the art are included, unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. In addition, the invention encompasses compositions made according to any of the methods for preparing compositions disclosed herein.

[0159] Where elements are presented as lists, *e.g.*, in Markush group format, it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be

removed from the group. It is also noted that the term "comprising" is intended to be open and permits the inclusion of additional elements or steps. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, steps, *etc.*, certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, steps, *etc.* For purposes of simplicity those embodiments have not been specifically set forth *in haec verba* herein. Thus for each embodiment of the invention that comprises one or more elements, features, steps, *etc.*, the invention also provides embodiments that consist or consist essentially of those elements, features, steps, *etc.*

[0160] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise. It is also to be understood that unless otherwise indicated or otherwise evident from the context and/or the understanding of one of ordinary skill in the art, values expressed as ranges can assume any subrange within the given range, wherein the endpoints of the subrange are expressed to the same degree of accuracy as the tenth of the unit of the lower limit of the range.

[0161] In addition, it is to be understood that any particular embodiment of the present invention may be explicitly excluded from any one or more of the claims. Any embodiment, element, feature, application, or aspect of the compositions and/or methods of the invention can be excluded from any one or more claims. For purposes of brevity, all of the embodiments in which one or more elements, features, purposes, or aspects is excluded are not set forth explicitly herein.

Incorporation by Reference

[0162] All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if the contents of each individual publication or patent document were incorporated herein.

[0163] What is claimed is:

CLAIMS

1. A method of reconstituting a lyophilized substance, the method comprising:

   (a) providing a container containing a lyophilized substance,

   (b) introducing a diluent into the container so that the diluent and the lyophilized substance come into contact with each other while maintaining the container substantially still until the lyophilized substance is substantially dissolved.

2. The method of claim 1, wherein the lyophilized substance is a lyophilized protein.

3. The method of claim 2, wherein the protein is an antibody or a fragment thereof, a growth factor, a clotting factor, a cytokine, a fusion protein, a pharmaceutical drug substance, a vaccine, an enzyme or a Small Modular ImmunoPharmaceutical (SMIP).

4. The method of any one of the preceding claims wherein the lyophilized substance is an amorphous mixture.

5. The method of any one of the preceding claims, wherein the protein is present in the reconstituted formulation at a concentration greater than about 100 mg/ml.

6. The method of claim 5, wherein the concentration is greater than about 200 mg/ml.

7. The method of any one of the preceding claims, wherein the protein is present in the reconstituted formulation at a concentration ranging between about 50 mg/ml and about 400 mg/ml.

8. The method of claim 7, wherein the protein concentration ranges between about 50 mg/ml and about 150 mg/ml.

9. The method of any one of the preceding claims, wherein step (b) comprises maintaining the container substantially still for at least about 10 minutes.

10. The method of any one of the preceding claims, wherein step (b) comprises maintaining the container substantially still for at least about 2 hours.

11. The method of any one of the preceding claims, wherein step (b) comprises maintaining the container substantially still for at least 4 hours.

12. The method of any one the preceding claims, wherein step (b) comprises maintaining the container substantially still for at least 24 hours.

13. The method of any one the preceding claims, wherein step (b) comprises maintaining the container substantially still for at least 48 hours.

14. The method of any one the preceding claims, wherein step (b) comprises maintaining the container substantially still for at least 72 hours.

15. The method of any one of the preceding claims, wherein the container is maintained substantially still at 2-8 ^{o}C.

16. The method of any one of the preceding claims, wherein the container is maintained substantially still at room temperature.

17. The method of any one of the preceding claims, wherein the method further comprises a step of swirling or shaking the container following step (b).

18. The method of any one of the preceding claims, wherein the diluent is water.

19. The method of any one of the preceding claims, wherein the diluent compromises an isotonicity agent.

20. The method of claim 19, wherein the isotonicity agent is selected from the group consisting of sucrose, mannitol, sodium chloride, trehalose, dextrose, glycine, and combination thereof.

21. The method of any one of the preceding claims, wherein the diluent comprises a buffering agent.

22. The method of claim 21, wherein the buffering agent is selected from the group consisting of histidine, Tris, phosphate buffers, sodium acetate, citrate, succinate, and combinations thereof.

23. The method of any one of the preceding claims, wherein the diluent comprises water, sucrose and histidine.

24. The method of any one of claims 1-22, wherein the diluent comprises water, sodium chloride and histidine.

25. The method of any one of the preceding claims, wherein the diluent further comprises a surfactant.

26. The method of claim 25, wherein the surfactant is selected from the group consisting of polysorbate 20, polysorbate 80, poloxamers, Triton and combinations thereof.

27. The method of any one of the preceding claims, wherein the diluent is introduced by injection.

28. The method of claim 25 wherein the injection takes at least 10 seconds to complete.

29. The method of claim 25 wherein the injection takes at least 30 seconds to complete.

30. The method of any one of the preceding claims, wherein the container is a vial, a tube, a syringe, or a dual chamber container.

31. The method of any one of the preceding claims, wherein the container is made of glass, plastics, or metal.

32. The method of any one of the preceding claims, wherein the reconstituted formulation is suitable for intravenous or subcutaneous injection.

33. A reconstituted formulation according to the method of any one of the preceding claims.

34. A reconstituted formulation comprising a protein at a concentration greater than 50 mg/ml, wherein the reconstituted formulation is prepared by dissolving a lyophilized protein with a diluent without continuous agitation, wherein less than 5% of the protein exists in aggregated form in the reconstituted formulation.

35. The reconstituted formulation of claim 34, wherein the protein concentration is greater than 100 mg/ml.

36. The reconstituted formulation of claim 34, wherein the protein concentration is greater than 200 mg/ml.

37. The reconstituted formulation of claim 34, wherein the protein concentration is greater than 300 mg/ml.

38. The reconstituted formulation of claim 34, wherein the protein concentration ranges between 50 mg/ml and 400 mg/ml.

39. The reconstituted formulation of claim 34, wherein the protein concentration ranges between 50 mg/ml and 150 mg/ml.

40. The reconstituted formulation of any one of claims 34-39, wherein less than 2.5% of the protein exists in aggregated form in the reconstituted formulation.

41. The reconstituted formulation of any one of claims 34-40, wherein less than 2.0% of the protein exists in aggregated form in the reconstituted formulation.

42. The reconstituted formulation of any one of claims 34-41, wherein the reconstituted formulation comprises sucrose, mannitol, glycine, dextran, trehalose, and/or sorbitol.

43. The reconstituted formulation of any one of claims 34-41, wherein the reconstituted formulation further comprises a buffering agent.

44. The reconstituted formulation of claim 43, wherein the buffering agent is selected from the group consisting of histidine, sodium acetate, citrate, phosphate, succinate, Tris and combinations thereof.

45. The reconstituted formulation of any one of claims 34-43, wherein the reconstituted formulation further comprises a surfactant.

46. The reconstituted formulation of claim 45, wherein the surfactant is selected from the group consisting of polysorbate 20, polysorbate 80, poloxamers, Triton and combinations thereof.

47. The reconstituted formulation of any one of claims 34-46, wherein the lyophilized mixture is amorphous.

48. The method of any one of the preceding claims, wherein the diluent is bacteriostatic water.

49. The reconstituted formulation of any one of claims 34-48, wherein the protein is an antibody or a fragment thereof, a growth factor, a clotting factor, a cytokine, a fusion protein, a pharmaceutical drug substance, a vaccine, an enzyme or a Small Modular ImmunoPharmaceutical (SMIP).



Figure 1

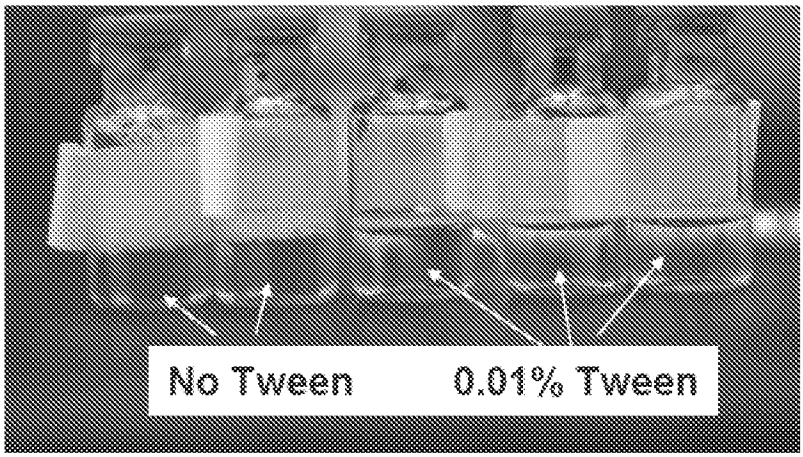
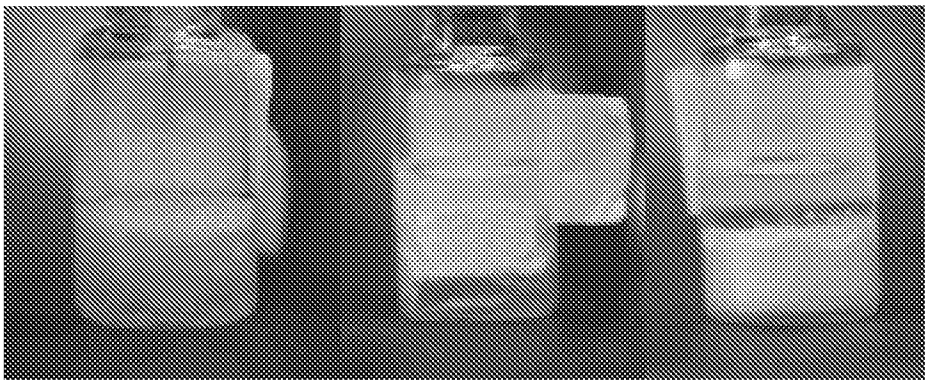


Figure 2



a b c

Figure 3

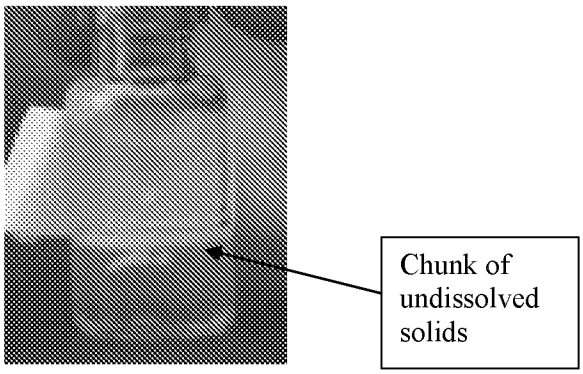


Figure 4

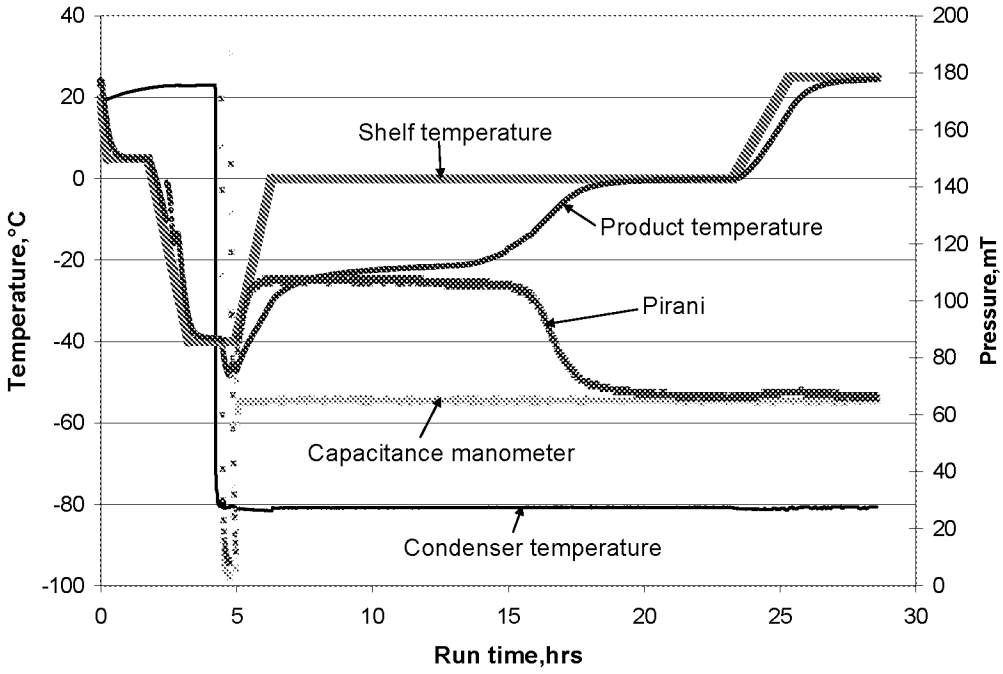

Temperature, °C
Pressure,mT
Run time,hrs
Shelf temperature
Product temperature
Pirani
Capacitance manometer
Condenser temperature
40
20
0
-20
-40
-60
-80
-100
200
180
160
140
120
100
80
60
40
20
0
0
5
10
15
20
25
30

Figure 5

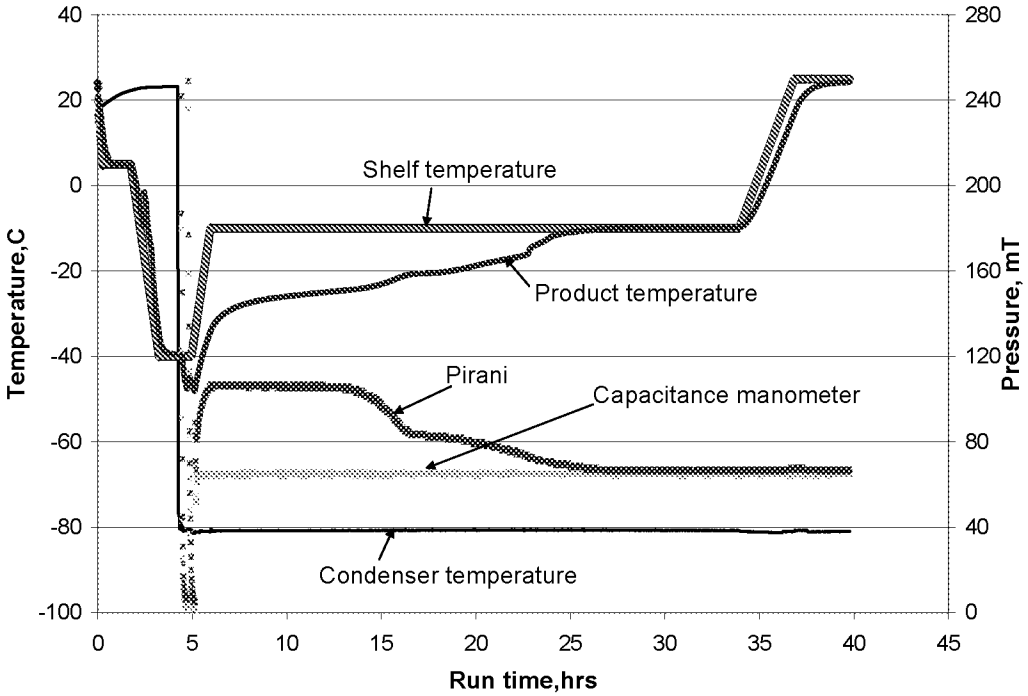

40
20
0
-20
-40
-60
-80
-100
Temperature,C
280
240
200
160
120
80
40
0
Pressure, mT
0
5
10
15
20
25
30
35
40
45
Run time,hrs
Shelf temperature
Product temperature
Pirani
Capacitance manometer
Condenser temperature

Figure 6

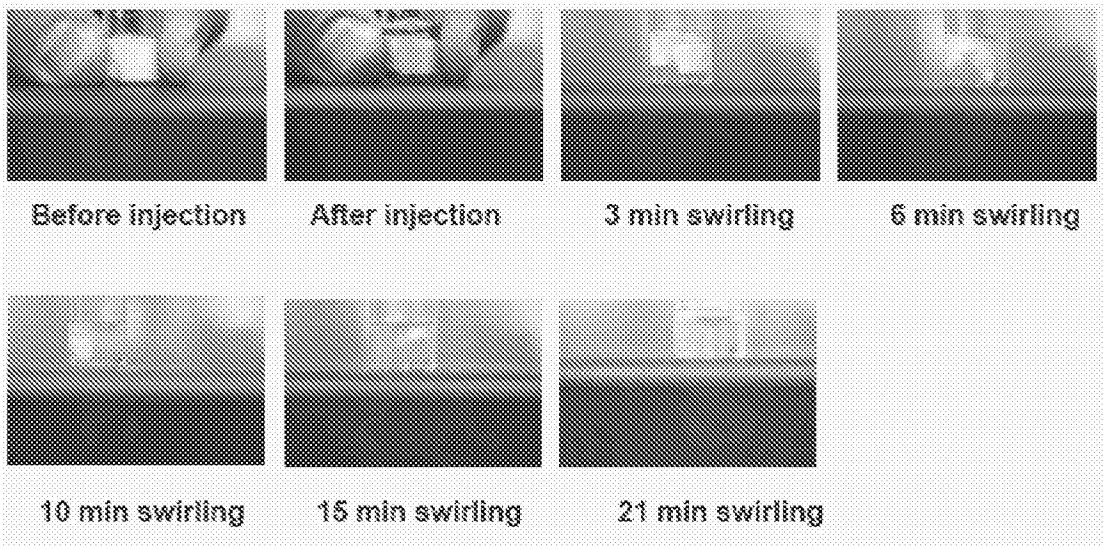

Before injection
After injection
3 min swirling
6 min swirling
10 min swirling
15 min swirling
21 min swirling

Figure 7

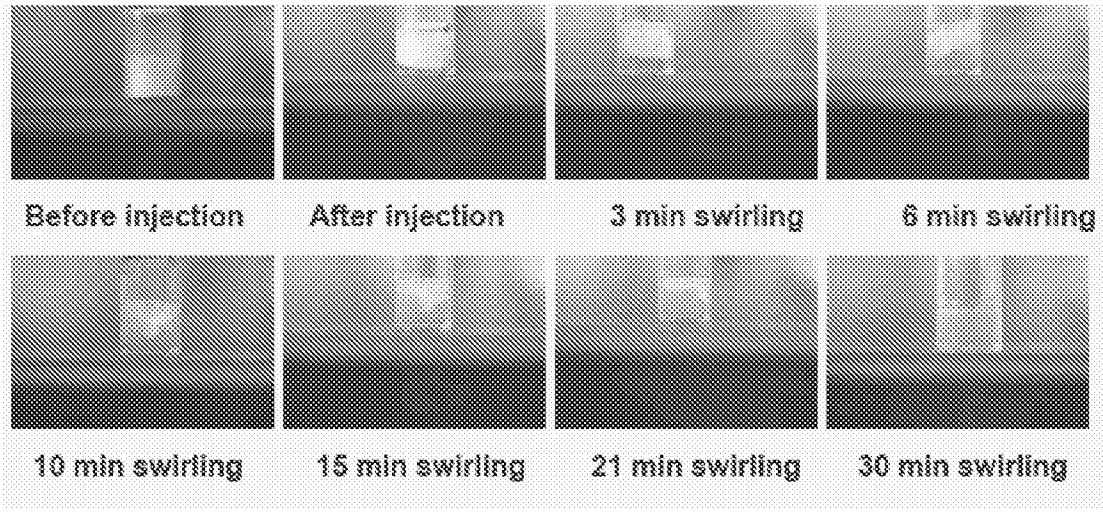

Before injection
After injection
3 min swirling
6 min swirling
10 min swirling
15 min swirling
21 min swirling
30 min swirling

Figure 8

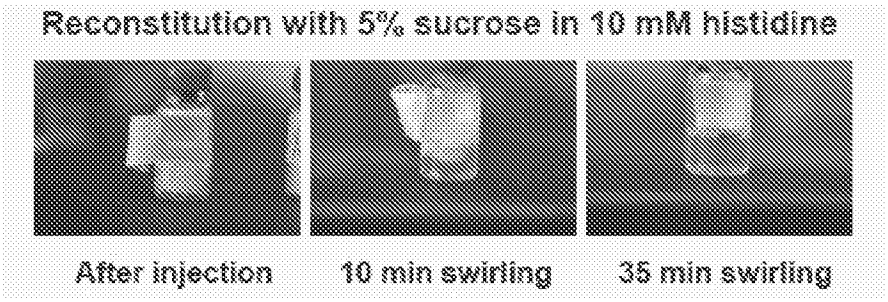

Reconstitution with 5% sucrose in 10 mM histidine
After injection
10 min swirling
35 min swirling

Figure 9

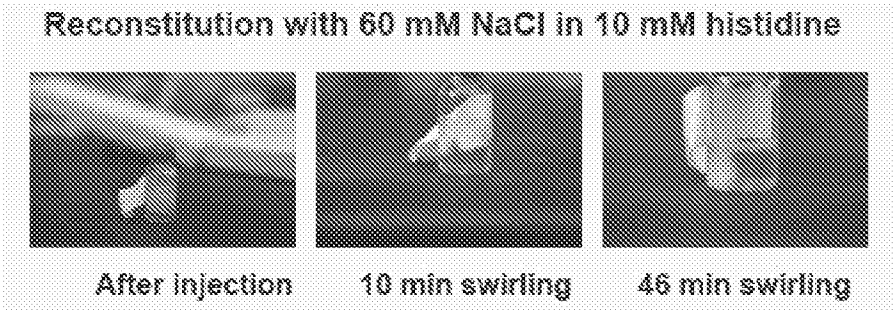

Reconstitution with 60 mM NaCl in 10 mM histidine
After injection
10 min swirling
46 min swirling

Figure 10

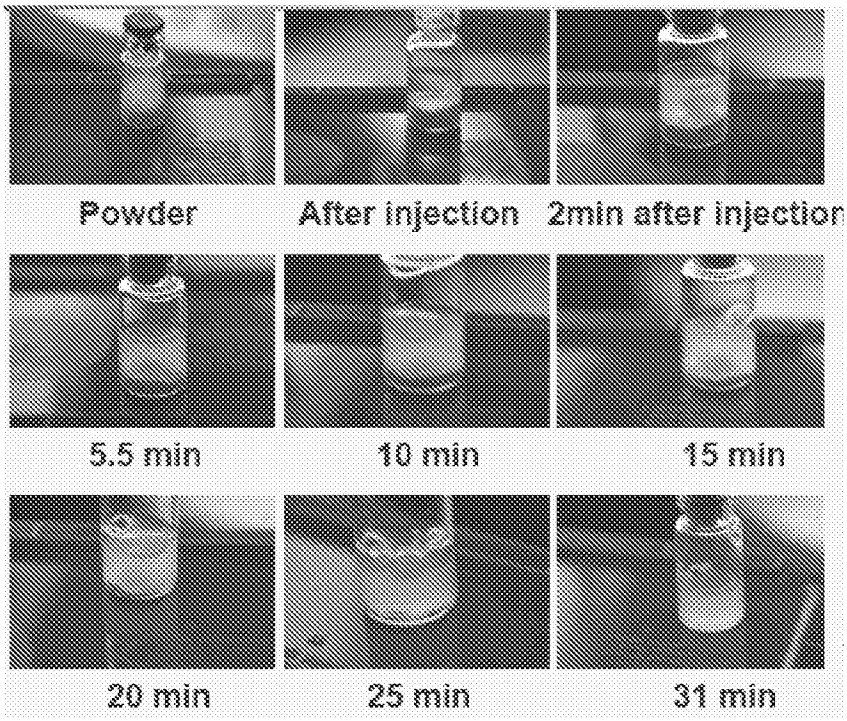

Powder
After injection
2min after injection
5.5 min
10 min
15 min
20 min
25 min
31 min

Figure 11

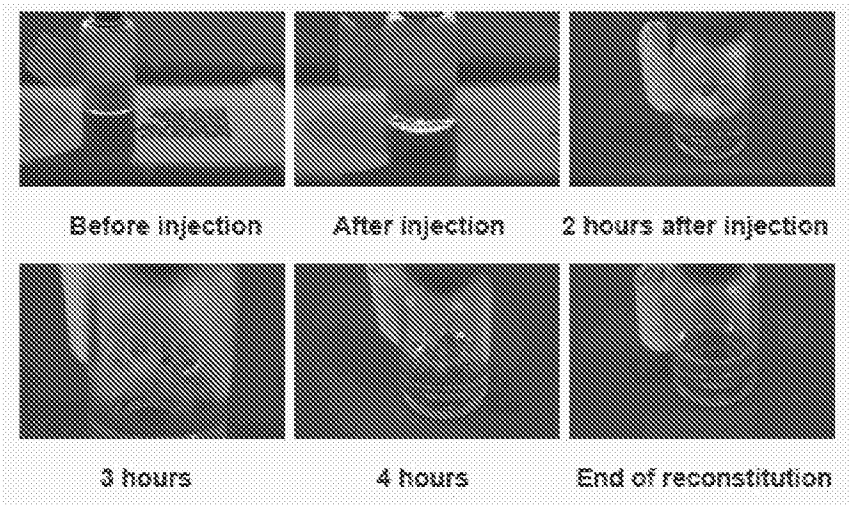

Before injection
After injection
2 hours after injection
3 hours
4 hours
End of reconstitution

Figure 12

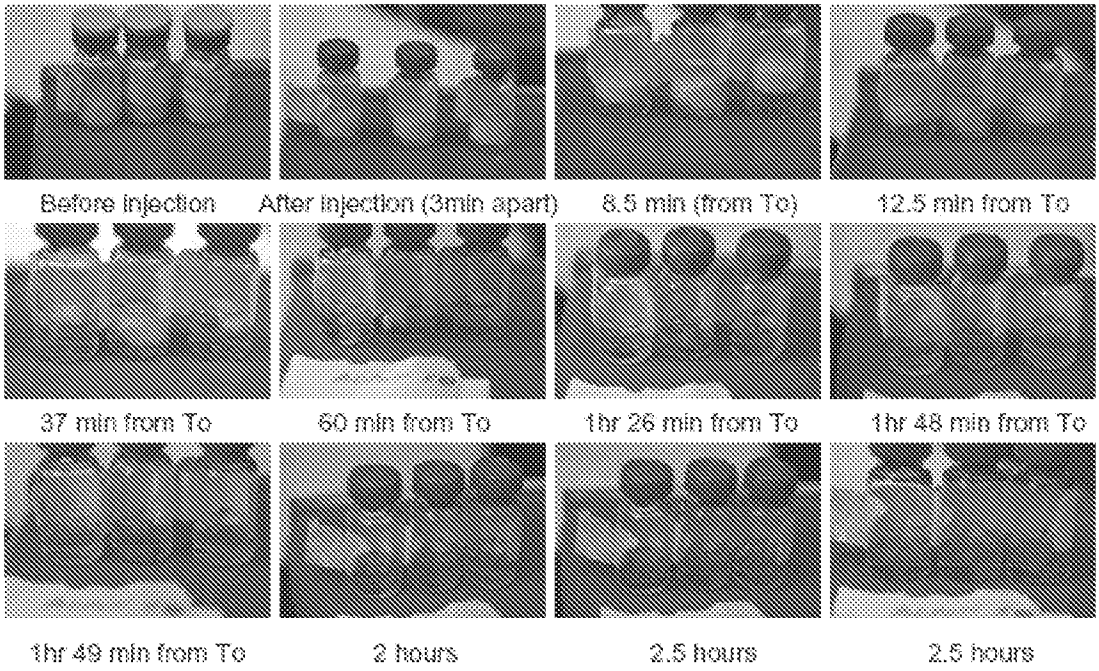

Before injection
After injection (3min apart)
8.5 min (from To)
12.5 min from To
37 min from To
60 min from To
1hr 26 min from To
1hr 48 min from To
1hr 49 min from To
2 hours
2.5 hours
2.5 hours

Figure 13

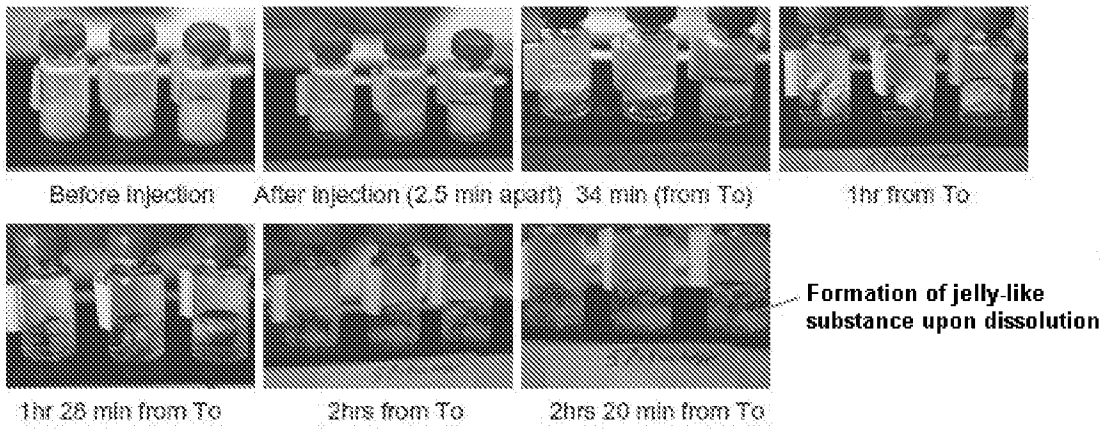

Before injection
After injection (2.5 min apart)
34 min (from To)
1hr from To
1hr 28 min from To
2hrs from To
2hrs 20 min from To
Formation of jelly-like substance upon dissolution

Figure 14

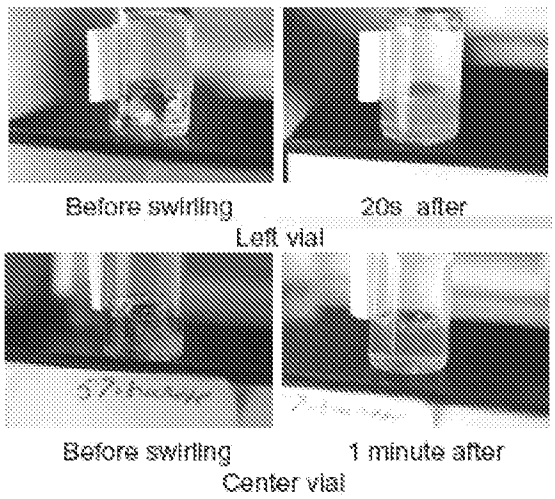

Before swirling
20s after
Left vial
Before swirling
1 minute after
Center vial

Figure 15

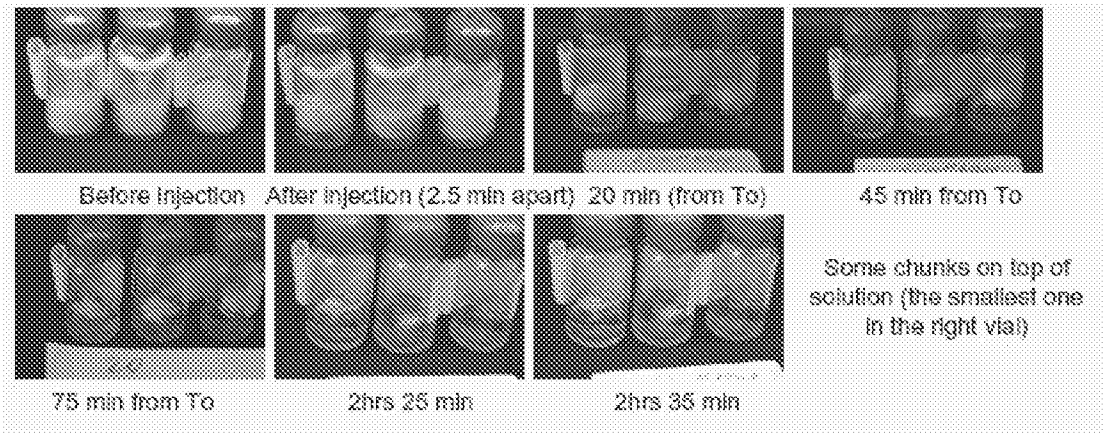

Before injection
After injection (2.5 min apart)
20 min (from To)
45 min from To
Some chunks on top of solution (the smallest one in the right vial)
75 min from To
2hrs 25 min
2hrs 35 min

Figure 16

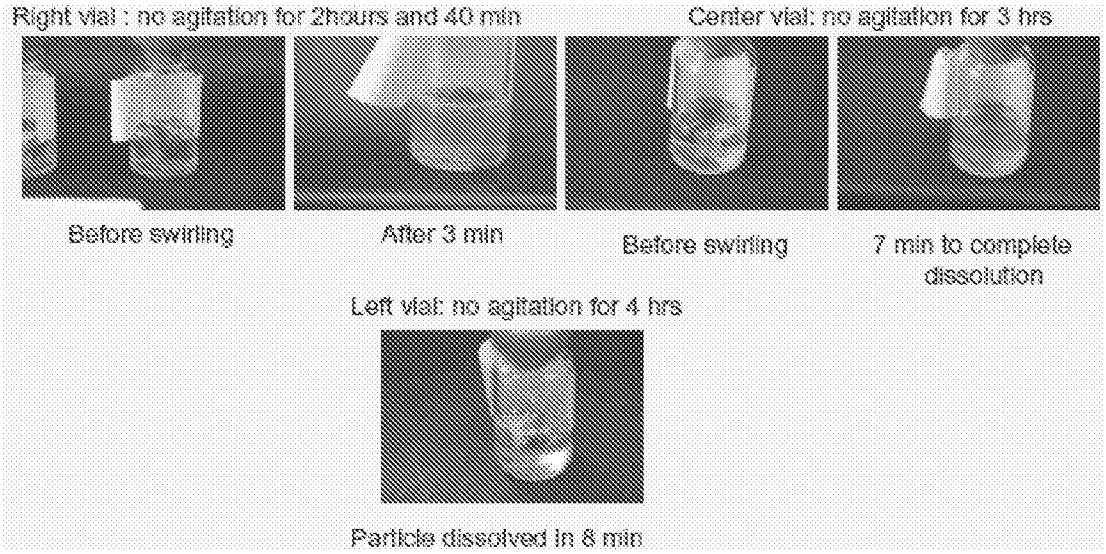

Right vial : no agitation for 2hours and 40 min
Center vial: no agitation for 3 hrs
Before swirling
After 3 min
Before swirling
7 min to complete dissolution
Left vial: no agitation for 4 hrs
Particle dissolved in 8 min

Figure 17

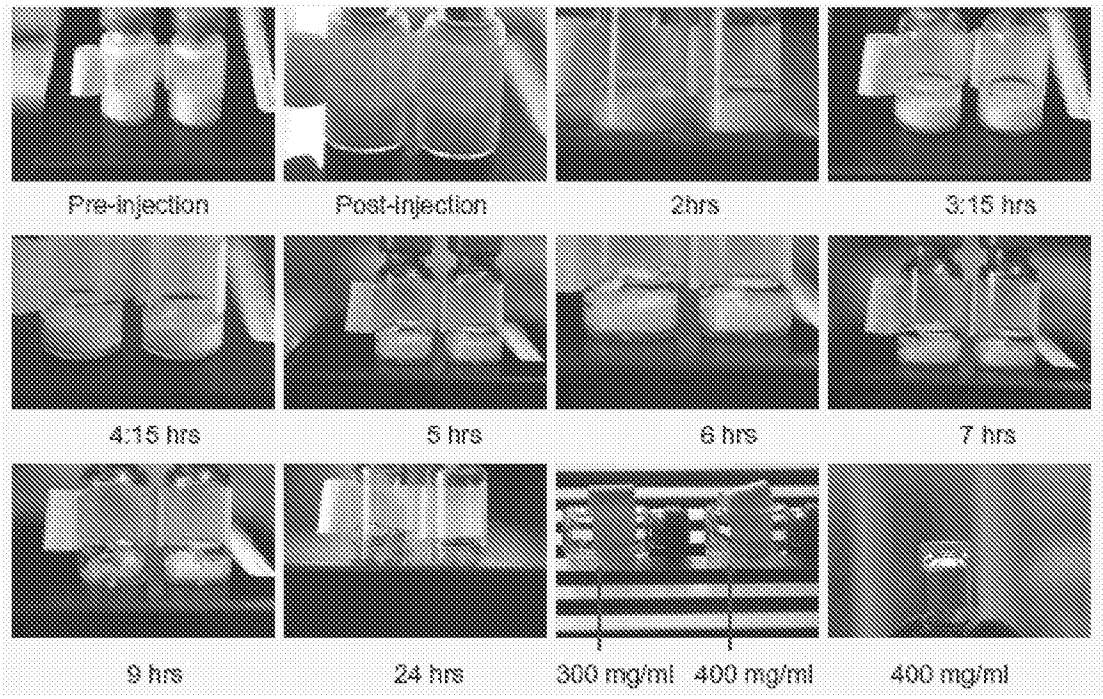

Pre-injection
Post-injection
2hrs
3:15 hrs
4:15 hrs
5 hrs
6 hrs
7 hrs
9 hrs
24 hrs
300 mg/ml
400 mg/ml
400 mg/ml

Figure 18

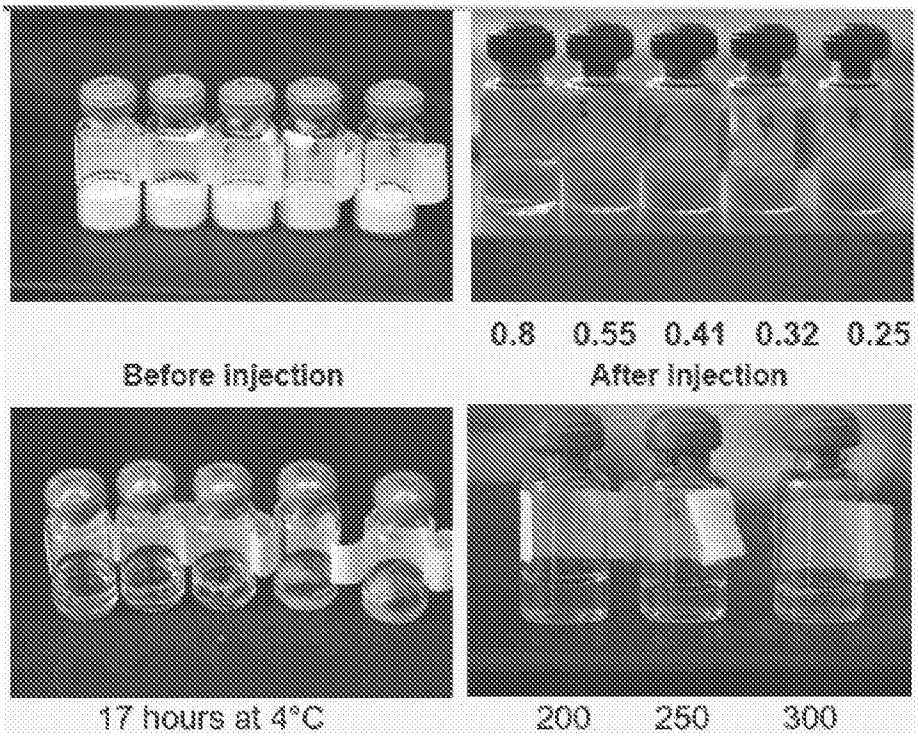

0.8 0.55 0.41 0.32 0.25
Before injection
After injection
17 hours at 4°C
200 250 300

Figure 19

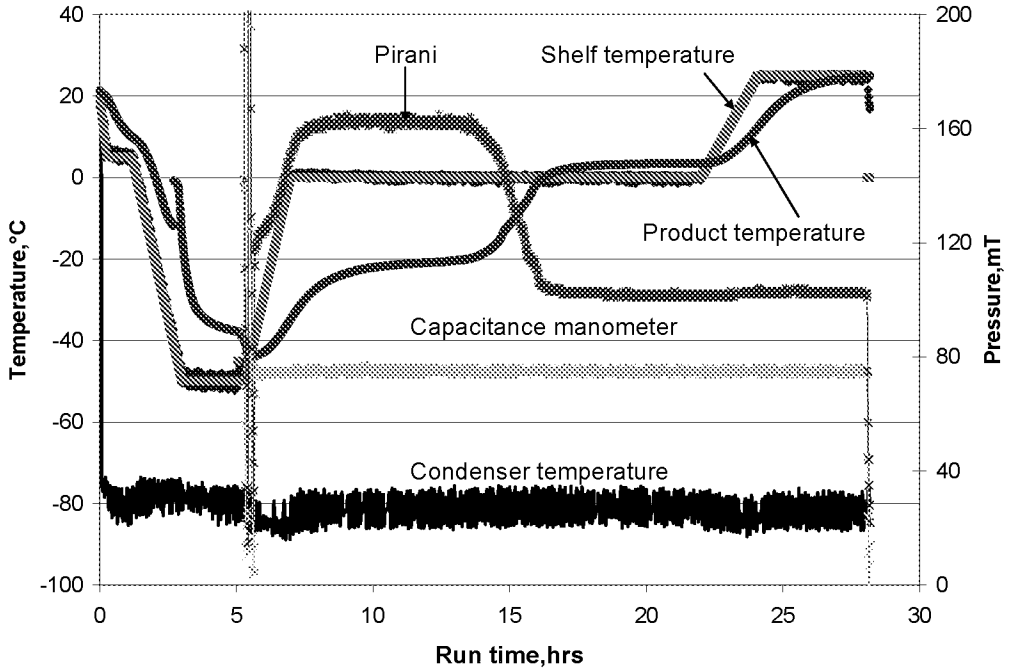

Pirani
Shelf temperature
Product temperature
Capacitance manometer
Condenser temperature
Temperature,°C
Pressure,mT
Run time,hrs
40
20
0
-20
-40
-60
-80
-100
200
160
120
80
40
0
0
5
10
15
20
25
30

Figure 20

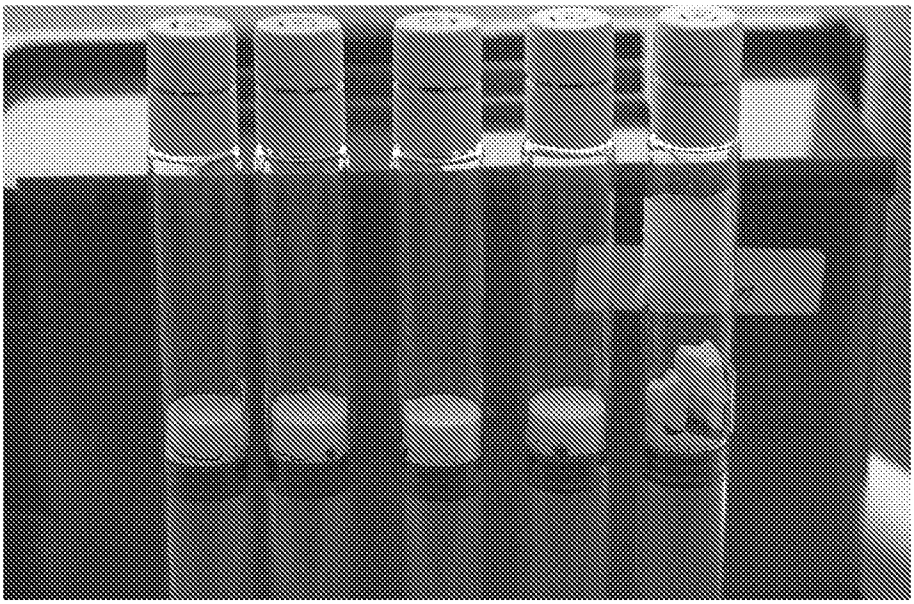


Figure 21

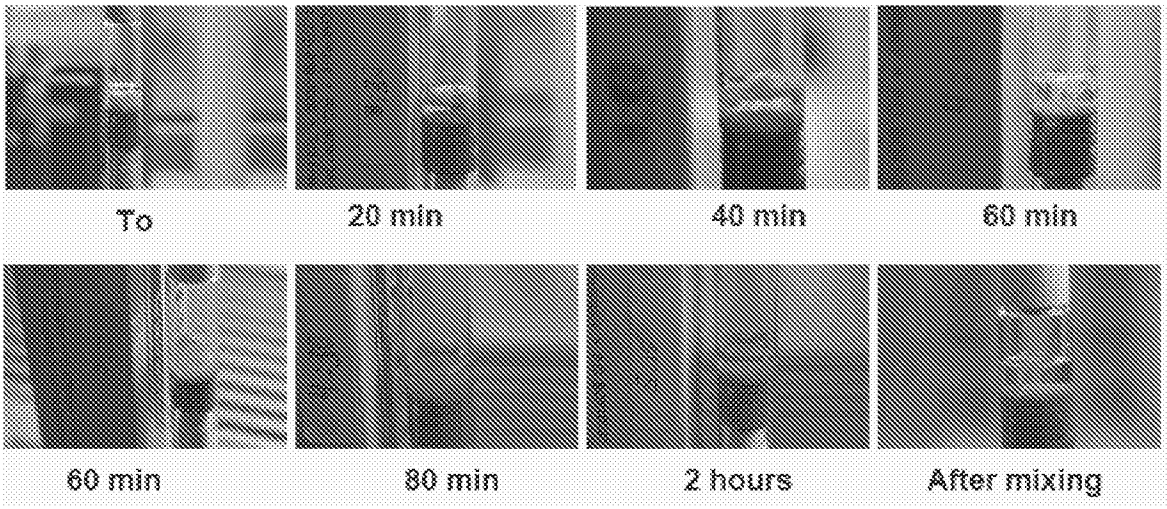


Figure 22

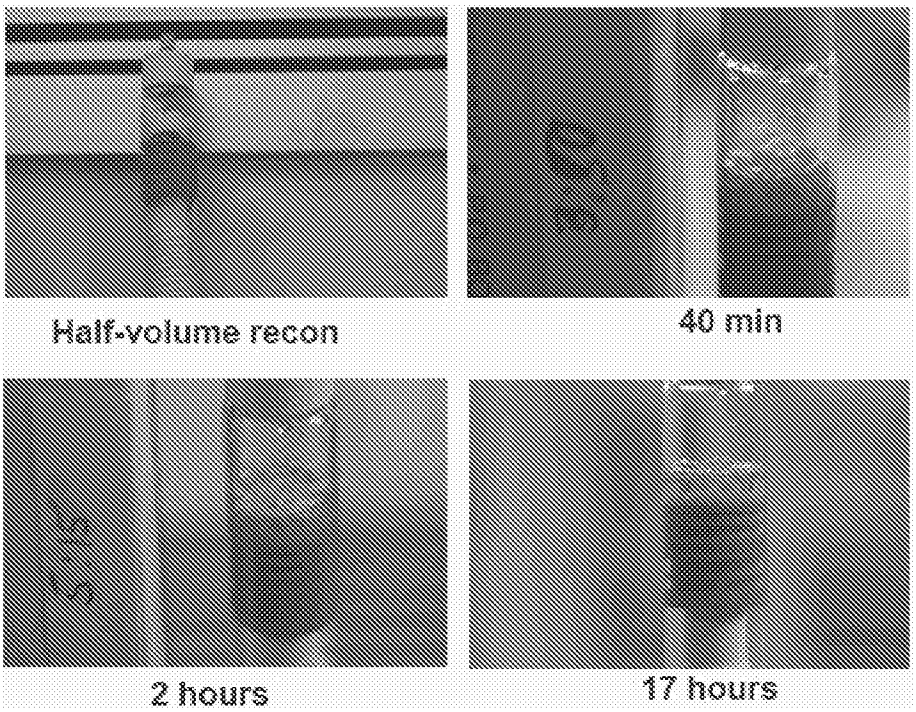


Figure 23

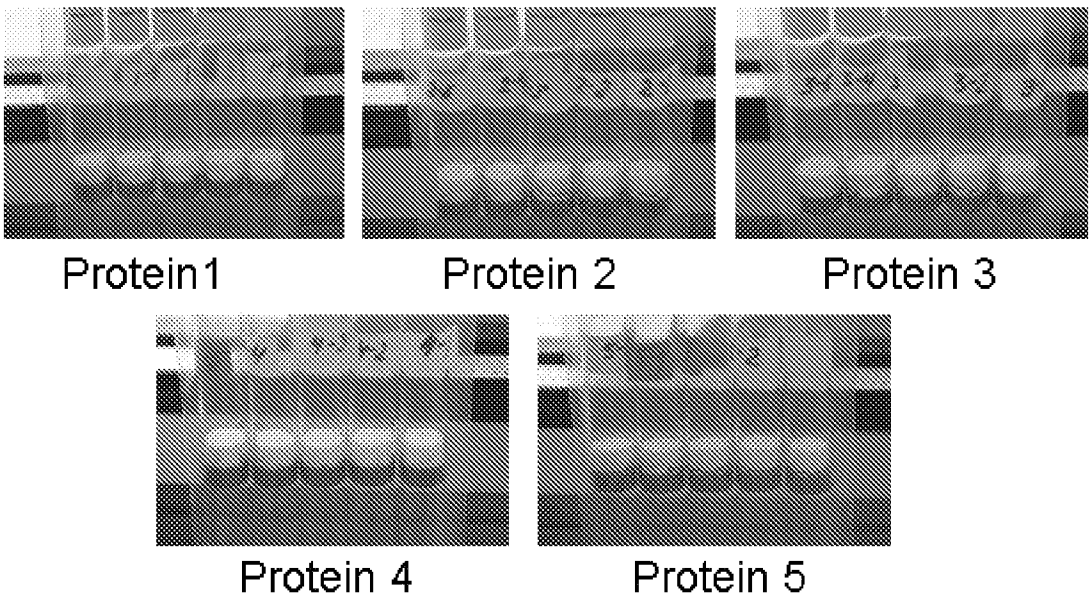


Figure 24

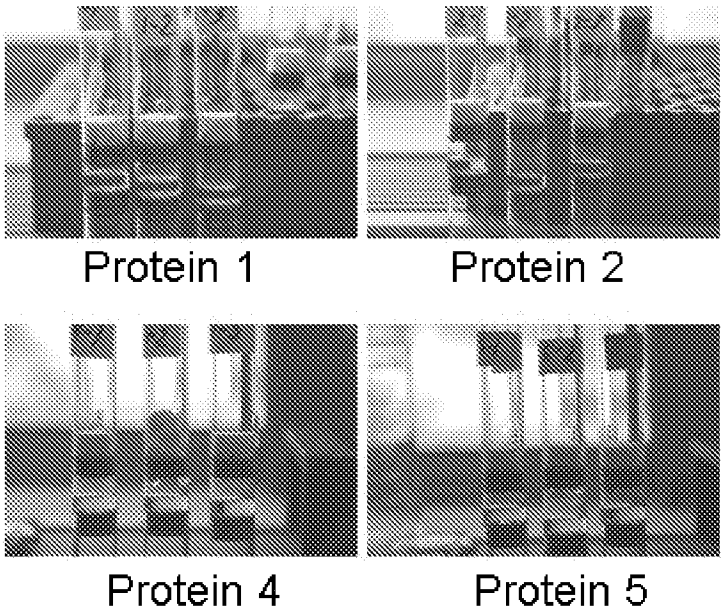


Figure 25

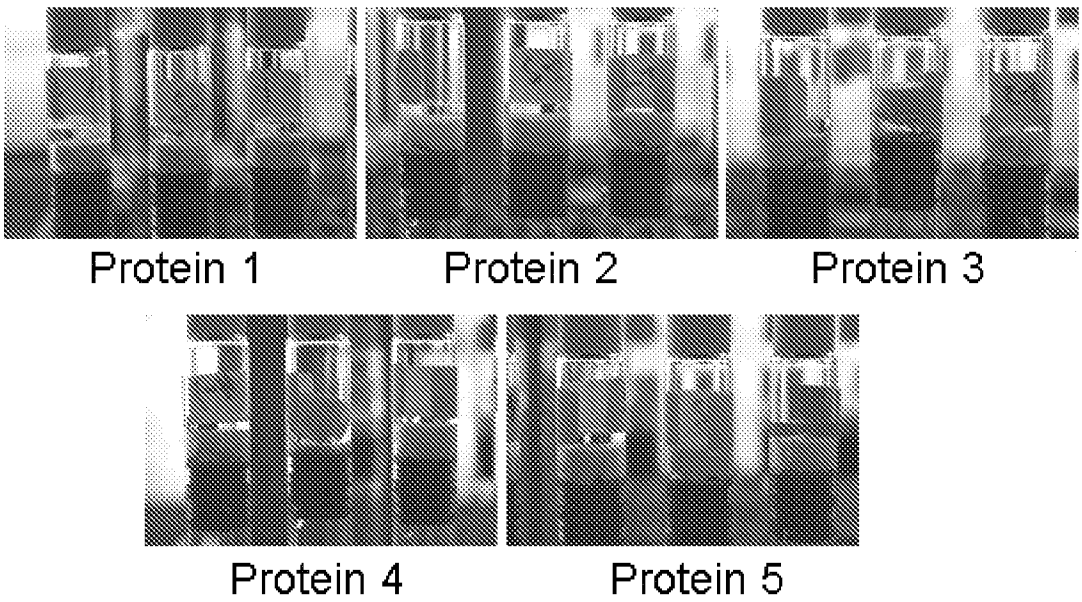


Figure 26

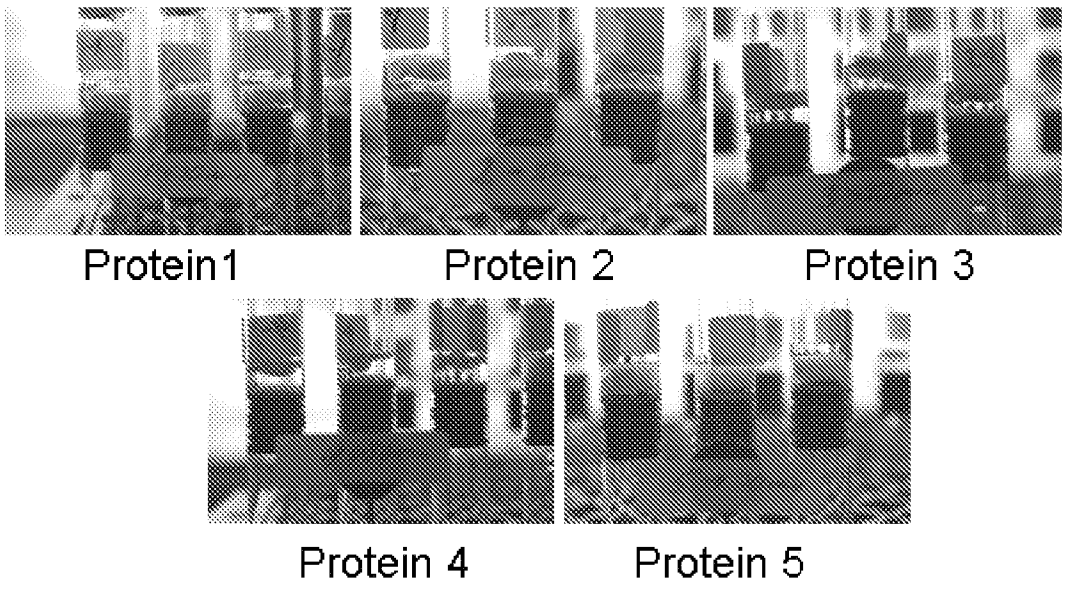


Figure 27

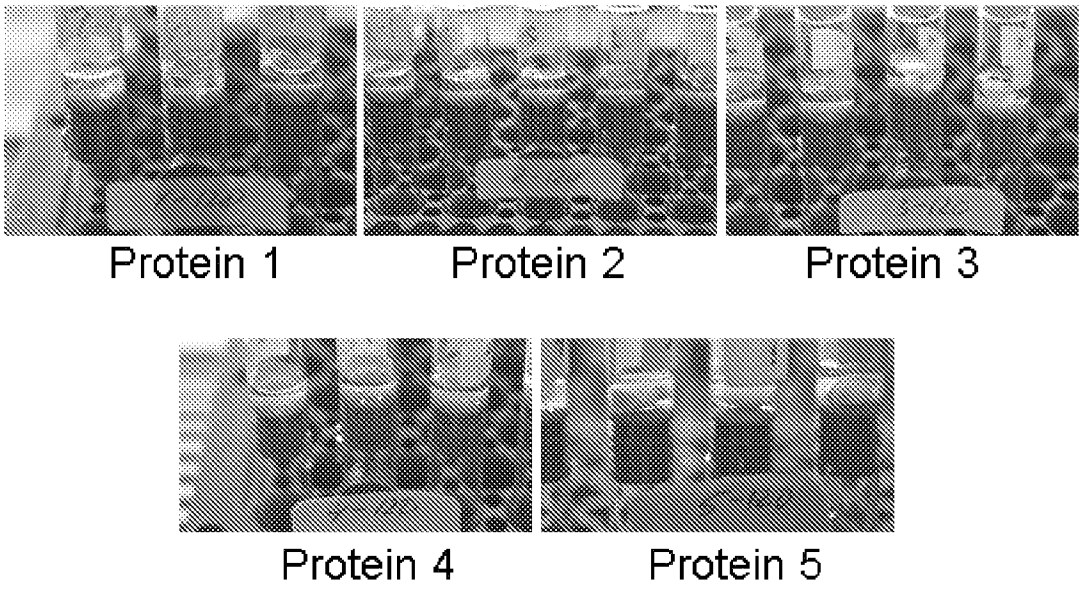


Figure 28

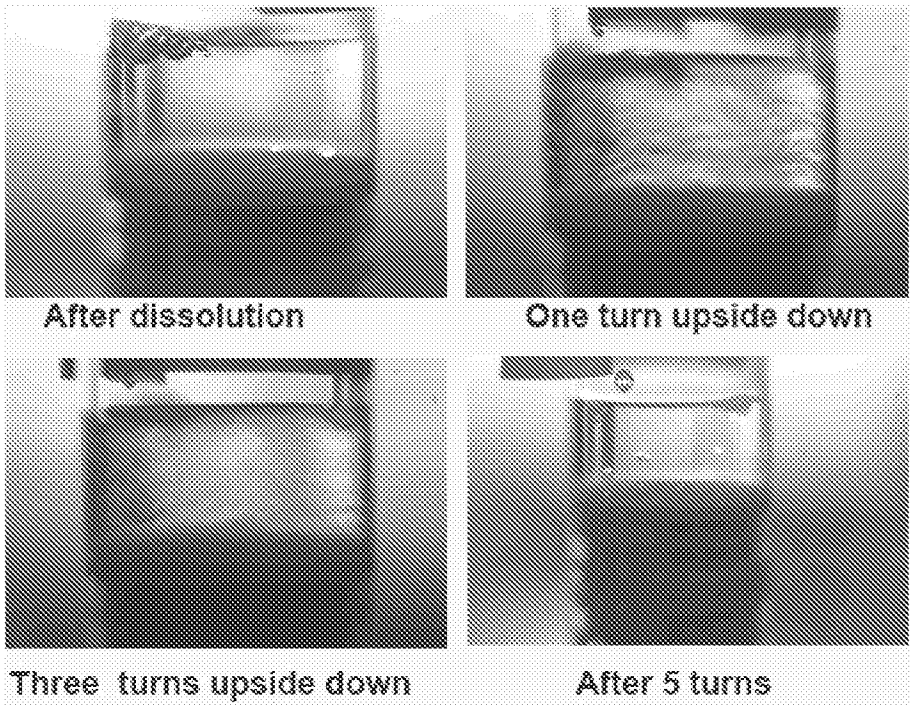

After dissolution
One turn upside down
Three turns upside down
After 5 turns

Figure 29

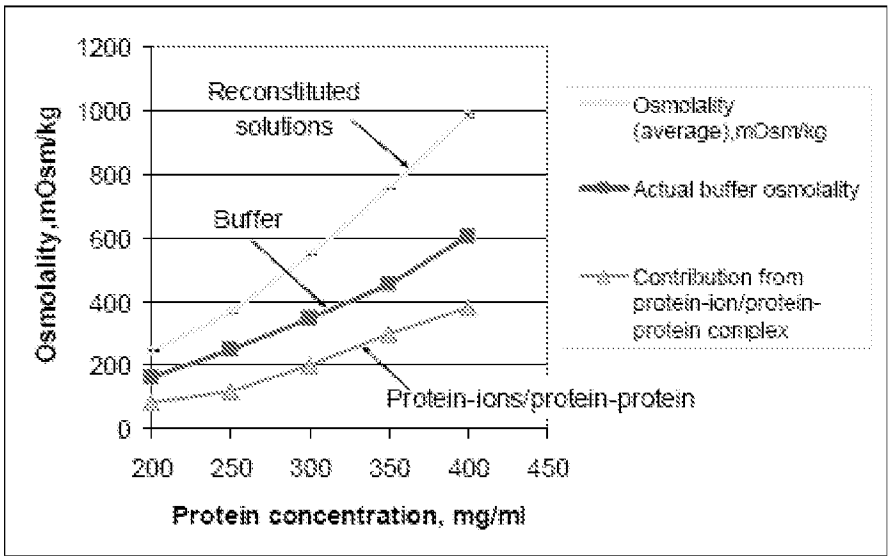

1200
1000
800
600
400
200
0
Osmolality,mOsm/kg
Reconstituted solutions
Buffer
Protein-ions/protein-protein
200
250
300
350
400
450
Protein concentration, mg/ml
Osmolality (average),mOsm/kg
Actual buffer osmolality
Contribution from protein-ion/protein-protein complex

Figure 30

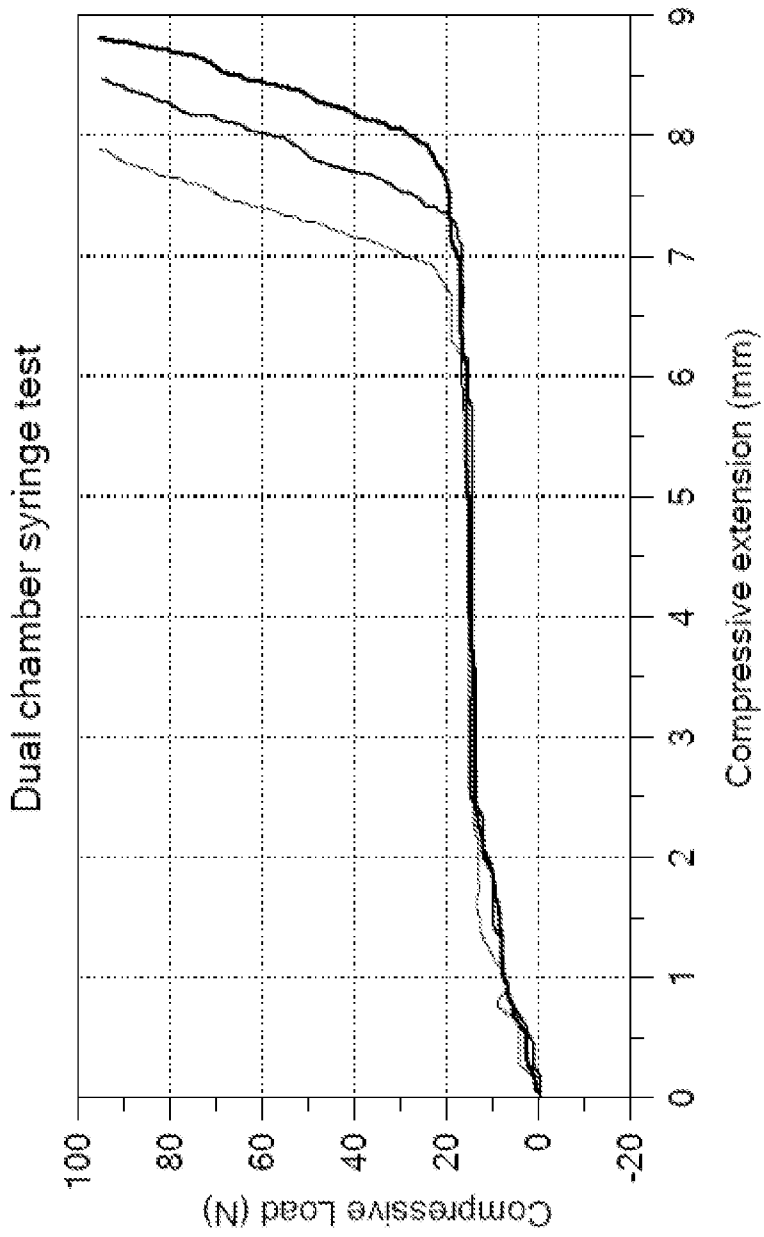

Dual chamber syringe test
Compressive Load (N)
100
80
60
40
20
0
-20
0
1
2
3
4
5
6
7
8
9
Compressive extension (mm)

Figure 31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/39205

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395 (2010.01)
USPC - 424/130.1
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)
IPC(8): A61K 39/395 (2010.01)
USPC - 424/130.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/130.1; 514/2

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
USPTO WEST (PGPB, USPT, USOC, EPAB, JPAB); Google
lyophilized, protein, antibody, growth factor, clotting factor, cytokine, fusion protein, vaccine, enzyme, SMIP, mg/ml, aggregated, isotonicity, amorphous, reconstitute, reconstituted, reconstitution, diluent, container, agitation

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2006/0275306 A1 (ANDYA, et al.) 07 December 2006 (07.12.2006), especially, para [0008]-[0010], [0016], [0017], [0041], [0123]	1-4, 34-40
Y	US 2009/0062205 A1 (BALASUBRAMANIAN, et al.) 05 March 2009 (05.03.2009), especially, para [0017]	1-4, 34-40
Y	US 2006/0029599 A1 (KAISHEVA, et al.) 09 February 2006 (09.02.2006), especially, para [0030]	4

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 August 2010 (11.08.2010)	30 AUG 2010
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. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 10/39205

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-33, 41-49
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.