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DESCRIPTION

BACKGROUND

[0001] Protein-based therapies can be more difficult to administer to patients than other pharmaceuticals. Because the efficacy of a protein is related to its conformation, therapeutic formulations cannot be subjected to conditions that contribute to the unfolding, or denaturing, of the protein. Special care is typically used in preparing, storing, and administering protein-based therapies. In addition to avoiding any denaturing of the protein, it is often desirable to control the amount of the protein administered to a patient over time. This helps to avoid protein concentrations within the patient that are undesirably high or that are too low to be effective. Controlled release protein-based therapies can be administered by a variety of methods, including oral delivery of tablets or capsules, inhalation of powders, and implantation of depots from which the protein is gradually released.

[0002] The preparation of these formulations typically includes mixing the protein with an organic solvent. For example, a powder formulation may be made by spraying a mixture of the protein and an organic solvent into liquid nitrogen. Alternatively, the protein may be mixed with a solution of a bioerodible polymer in an organic solvent, with formation of microparticles which contain the protein and the polymer by coagulation of the mixture. Furthermore, proteins, powdered formulations, or microparticles can be mixed with an organic solvent to produce a liquid or gel which may be injected into a patient. A drawback to the use of organic solvents is their tendency to cause protein denaturing.

[0003] Additives have been used to stabilize proteins in the presence of a denaturing organic solvent. These additives include surfactants (U.S. Pat. No. 5,096,885), amino acids (U.S. Pat. No. 4,297,344), polyols (U.S. Pat. No. 5,589,167), natural polymers (WO 8903671), synthetic polymers (Pharm. Res. 8:285-291, 1991), and metals (U.S. Pat. No. 6,191,107 B1).

[0004] There is a need for improved stabilization of proteins during the preparation, storage, and administration of protein-based therapies. Protein formulations which have good stability in organic solvents would be useful in a wide variety of controlled release applications.

BRIEF SUMMARY

[0005] In a first aspect, the present invention is a composition comprising a protein, a polyol, and zinc, as defined in the claims.

[0006] In a second aspect, the present invention concerns use of a composition for the preparation of formulation, as defined in the claims.

[0007] In a third aspect, the present invention is a method of making a sustained release composition, comprising mixing a complex and a liquid carrier to form said sustained release composition. The liquid carrier comprises sucrose acetate isobutyrate; and the complex comprises a protein, a polyol, and zinc, as defined in the claims.

[0008] In a fourth aspect, the present invention is a kit containing a container, a protein, a polyol, zinc, and a liquid carrier, as defined in the claims. The liquid carrier comprises sucrose acetate isobutyrate.

[0009] The present invention provides a composition, and use thereof, comprising a protein, an alcohol, and zinc, as defined in the claims. The alcohol is selected from the group consisting of a monosaccharide, a polysaccharide, glycerol, mannitol, sorbitol, inositol, and polyethylene glycol.

[0010] The present invention provides a method of making a sustained release composition, comprising mixing a complex and a liquid carrier to form said sustained release composition. The liquid carrier comprises sucrose acetate isobutyrate; and the complex comprises a protein, an alcohol, and zinc, as defined in the claims. The alcohol is selected from the group consisting of a monosaccharide, a polysaccharide, glycerol, mannitol, sorbitol, inositol, and polyethylene glycol.

[0011] The present invention provides a kit containing a container, a protein, an alcohol, zinc, and a liquid carrier as defined in the claims. The liquid carrier comprises sucrose acetate isobutyrate. The alcohol is selected from the group consisting of a monosaccharide, a polysaccharide, glycerol, mannitol, sorbitol, inositol, and polyethylene glycol.

BRIEF DESCRIPTION OF THE DRAWINGS
[0012] Various other objects, features and attendant advantages of the present invention will be more fully appreciated as the same becomes better understood from the following detailed description when considered in connection with the accompanying drawings in which like reference characters designate like or corresponding parts throughout the several views and wherein:

Figure 1 is a view of a vial containing an injectable composition.

DETAILED DESCRIPTION

[0013] The present invention concerns stabilization of a protein with a polylol and a metal, protecting the protein from denaturing when in contact with an organic solvent. The degree of retention of the native protein conformation is a surprising and unexpected effect of the combination of a protein with both a polylol and a metal. The polylol and metal together provide a synergistic protection of the protein conformation and activity which is greater than what would be expected from the effect that either the polylol or the metal have separately.

[0014] The polylol is an alcohol, and may be any hydrocarbon containing two or more hydroxyl groups (-OH) bonded to carbon, where hydrocarbon refers to a compound containing carbon and hydrogen, which may also contain heteroatoms such as oxygen, nitrogen, sulfur, phosphorus, and halogen. The term polylol excludes those compounds which do not provide for a monomer recovery of at least 40%, according to the following test:

A mixture of recombinant human growth hormone (rHGH, GENENTECH, S. San Francisco, CA) in 25 mM sodium bicarbonate (25 mg rHGH/mL) is combined with zinc acetate to provide a 10:1 molar ratio of zinc to growth hormone. To this mixture is added 1 percent by weight (w%) of the polylol to be tested. A 1.0 mg sample of this mixture is then added to N-methyl pyrrolidone (NMP), providing a ratio of protein mass (mg) to volume of solvent (mL) of 5 mg/mL. The resulting mixture is homogenized for 2 min at 8,000 rpm with a shear homogenizer tip and then incubated at 37 °C for 24 hours. The rhGH is recovered by dilution into a 10-fold excess of a stabilizing buffer (5 mM EDTA, 50 mM HEPES, 0.01%NaNO3, pH 8.0). The amount and quality of the protein recovered in this step is then determined by size exclusion chromatography - high performance liquid chromatography (SEC-HPLC), using a 7.8 X 300 mm TSK 2000-SWXL column at room temperature, with a mobile phase of 50 mM Na2HPO4, 150 mM NaCl, pH 7.2, a flow rate of 1.0 mL/min, and a run time of 20 min. Protein (10 μg) is injected and the eluant monitored for absorbance at 214 nm.

[0015] Examples of polylols include monosaccharides, such as glucose, fructose, and ribose, including cyclic isomers; glycerol; mannitol; sorbitol; inositol; poly saccharides, including disaccharides such as sucrose, trehalose, lactose, maltose, and cellobiose, and trisaccharides such as 3-fucosyllactose and blood group B trisaccharide; and polyethers such as polyethylene glycols (PEG's). The term "polyether" means a hydrocarbon containing three or more ether bonds (C-O-C). The polylol may be substituted. "Substituted" means that the moiety contains at least one, preferably 1-3 substituent(s). Suitable substituents include ether (-O-C), amino (-NH2), oxy (-O-), carbonyl (-C=O), thiol, and the like. Preferably, the polylol is mannitol, trehalose, or a polyethylene glycol. Preferred polyethylene glycols have a molecular weight, as measured by size exclusion chromatography (SEC) from 400 kDa to 8,000 kDa. More preferably, the polyethylene glycol has a molecular weight from 400 kDa to 3,500 kDa. It is preferred that the polylol has molecular weight less than about 70,000 kDa.

[0016] The relative amounts of protein and polylol in a formulation may be chosen to minimize protein denaturing. For a given protein, the ideal ratio may vary depending on the polylol used. Preferably, the mass ratio of trehalose to protein is from 100:1 to 1:100. More preferably, the mass ratio of trehalose to protein is from 1:1 to 1:10. Even more preferably, the mass ratio of trehalose to protein is from 1:3 to 1:4. Preferably, the mass ratio of mannitol to protein is from 100:1 to 1:100. More preferably, the mass ratio of mannitol to protein is from 1:1 to 1:10. Even more preferably, the mass ratio of mannitol to protein is from 1:1 to 1:2. Preferably, the mass ratio of PEG to protein will be from 100:1 to 1:100. More preferably, the mass ratio of PEG to protein is from 1:1 to 1:10.

[0017] The metal is preferably divalent. More preferably, the metal is zinc. The metal may be added to the protein by mixing an aqueous solution of the protein with a metal complex. For example, a zinc complex such as zinc acetate, zinc oxide, or zinc carbonate may be added to a solution or suspension of the protein in a buffer. Preferably, the molar ratio of metal to protein is from 1:1 to 100:1. More preferably, the molar ratio of metal to protein is from 1:1 to 20:1. Even more preferably, the molar ratio of
metal to protein is from 1:1 to 10:1.

[0018] Proteins useful in the present invention include, for example, molecules such as cytokines and their receptors, as well as chimeric proteins comprising cytokines or their receptors, including, for example tumor necrosis factor alpha and beta, their receptors (TNFR-1; Gray et al., (1990) Proc. Natl. Acad. Sci. USA 87:7380-7384; and TNFR-2; Kohno et al., (1990) Proc. Natl. Acad. Sci. USA 87:8331-8335) and their derivatives; renin; growth hormones, including human growth hormone, bovine growth hormone, methionine-human growth hormone, des-phenylalanine human growth hormone, and porcine growth hormone; growth hormone releasing factor (GRF); parathyroid and pituitary hormones; thyroid stimulating hormone; human pancreas hormone releasing factor; lipoproteins; colchicine; prolactin; corticotrophin; thyrotrophic hormone; oxytocin; vasopressin; somatostatin; lypressin; pancroeyzmin; leuprolide; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; luteinizing hormone releasing hormone (LHRH); LHRH agonists and antagonists; glucagon; clotting factors such as factor VIIa, factor IX, factor X, factor XII, and von Willebrand factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator other than a tissue-type plasminogen activator (t-PA), for example aurokinase; bombesin; thrombin; homoeopotic growth factor; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin, human fibrinogen, an inhibiting substance; relaxin A-chain; relaxin B-chain; prorrelaxin; mouse gonadotropin-associated peptide; chorionic gonadotropin; gonadotropin releasing hormone; bovine somatotropin; porcine somatotropin; a microbial protein, such as beta-lactamase; DNase; actin; a vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; integrin; protein A or D; rheumatoid factors; a neurotrophic factor such as brain-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 aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TG) such as TGF-alpha and TGF-beta, 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(1-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins; CD proteins such as CD-3, CD-4, CD-8, and CD-19; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, -gamma, and consensus interferon; 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; viral antigen such as, for example, a portion of the HIV-1 envelope glycoprotein, gp120, gp160 or fragments thereof; transport proteins; homing receptors; adhesion; fertility inhibitors such as the progastaglandins; fertility promoters; regulatory proteins; antibodies and chimeric proteins, such as immunoadhesins; analogs and derivatives of these compounds, and pharmaceutically acceptable salts of these compounds, or their analogs or derivatives.

[0019] Preferably, the protein contains up to 120 amino acids per single chain. Preferably, the protein is capable of complexing with a metal. Protein-metal complexation has a dissociation constant (Kd) on the order of micromolar (μM) or smaller, as measured in water at physiological temperature and pH. The value Kd is defined as the product of the concentration of the unconjugated metal and the unconjugated protein, divided by the concentration of the protein-metal complex. A non-specific interaction between a protein and a metal under the same conditions has a Kd on the order of millimolar (mM). Preferably, the protein-metal complex has a Kd of 0.1 μM or smaller. More preferably, the protein-metal complex has a Kd of 0.01 μM or smaller.

[0020] More preferably, the protein is a growth hormone, such as human growth hormone (hGH), recombinant human growth hormone (rhGH), bovine growth hormone, methionine-human growth hormone, des-phenylalanine human growth hormone, and porcine growth hormone; insulin, insulin A-chain, insulin B-chain, and proinsulin; or a growth factor, such as vascular endothelial growth factor (VEGF), nerve growth factor (NGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor (TGF), and insulin-like growth factor-I and -II (IGF-I and IGF-II).

[0021] Formulations of proteins which are stabilized with a polyl and a metal may also contain other ingredients. These ingredients include, for example, preservatives, antioxidants, bulking agents, surfactants, chelating agents, emulsifying agents, and other excipients. The term "excipient" refers to a non-therapeutic agent added to a pharmaceutical composition to provide a desired consistency or stabilizing effect. Preservatives include, for example, phenol, benzyl alcohol, metacresol, methyl paraben, propyl paraben, benzoic acid, and benzethonium chloride. Surfactants include, for example, POLYSORBATE 20 and 80.

[0022] The protein, polyl, and metal, together with any other ingredients, may be combined in a single step or in two or more steps. Preferably, the protein is complexed with the metal before addition of the polyl. For example, the protein, polyl, metal, and optional ingredients may be mixed in an aqueous buffer to form a solution, emulsion, or suspension. Useful buffers include, for example, phosphate, Tris, citrate, succinate, acetate, and histidine buffers. Typically, the buffer is in the range of about 2 mM to about 100 mM. Preferred buffers include sodium succinate and potassium phosphate buffers.

[0023] The aqueous formulation of protein, polyl, and metal may be used to administer the protein-based therapy, or the
formulation may be further processed. For example, the formulation may be converted into a solid by lyophilization or freeze-drying, or it may be incorporated into a biodegradable polymer. A biodegradable polymer decomposes when placed inside an organism, as measured by a decline in the molecular weight of the polymer over time. Polymer molecular weights can be determined by a variety of methods including size exclusion chromatography (SEC), and are generally expressed as weight averages or number averages. A polymer is biodegradable if, when in phosphate buffered saline (PBS) of pH 7.4 and a temperature of 37°C, its weight-average molecular weight is reduced by at least 25% over a period of 6 months as measured by SEC. Useful biodegradable polymers include polyesters, such as poly(caprolactone), poly(γ-caprolactone), poly(lactic acid), poly(polyhydroxybutyrate); poly(α-hydroxy acids), such as poly(ε-caprolactone) and poly(trimethylene carbonate); poly(alkylene oxide) copolymers; polycarbonates; polyesters; polyacetylenes; and polyamides. The biodegradation of the polymer can be enhanced by combining it with a delivery system that is designed to facilitate degradation. Such systems include the use of enzymes, such as enzymes that break down the polymer, or the use of a degradation-promoting matrix, such as a polymer that releases a degradation factor.

[0024] The composition may also include a carrier liquid. Preferably, the formulation of protein, polyl, and metal may be mixed with a carrier liquid to be injected into a patient; however, any order of mixing these ingredients is possible. Solid formulations and microparticles may also be injected when mixed with a liquid carrier. It is preferred that the volume of the liquid carrier to the combined mass of the protein and the metal is from 99:1 to 70:30 w/v. More preferably, the ratio of the liquid carrier to the protein and the metal is from 95:5 to 85:15 w/v. For an implant administered by injection, the liquid mixture preferably transforms into a depot upon contact with the fluid in the body. This depot is characterized by its phase separation from the physiological fluid and its increased viscosity relative to the original liquid composition. It is this depot that serves to controllably release the protein.

[0025] The liquid carrier may be a biodegradable polymer which solidifies upon administration. Alternatively, the liquid carrier may be an agent which provides a viscosity increase upon administration. Examples of these agents include hyaluronic acid, as well as succrose octate isobutyrate (SAIB) as used in the SABER system (SOUTHERN BIOSYSTEMS, Birmingham, AL). The SABER system is an injectable drug delivery system which is composed of a non-polymeric liquid material and an organic solvent (U.S. Pat. No. 5,747,055; Smith and Tipton (1996) Pharmaceutical Research 13(3):300). SABER is injected as a low viscosity liquid that increases rapidly in viscosity after injection. The resulting high viscosity matrix is adhesive, biodegradable, and biocompatible.

[0026] The non-polymeric liquid material in the SABER system is a non-water soluble liquid having a viscosity of at least 5,000 centipoise (cP) at 37°C which does not crystallize neat under ambient physiological conditions. The viscosity of the liquid can be measured using a CANON-FENSKI viscometer at a temperature of 25°C. The kinematic viscosity of the SABER composition, including the liquid material and the solvent, is preferably less than 1000 cP at room temperature. More preferably, the kinematic viscosity of the SABER composition is less than 200 cP at room temperature. Suitable liquid materials include stearyl esters, stearyl amides, long-chain fatty acid amides, long-chain fatty alcohols, long-chain esters, and disaccharide esters. Preferably, the liquid material is acetylated sucrose diesterate, disaccharide acetate butyrate, or SAIB. The weight ratio of liquid material to solvent is preferably from 50:50 to 85:15 w/w. More preferably, the weight ratio of liquid material to solvent is from 50:50 to 70:30 w/w.

[0027] These formulations typically also include one or more organic solvents, such as methylene chloride, ethyl acetate, dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), dimethylformamide (DMF), ethanol (EtOH), N-methyl pyrrolidone (NMP), benzyl benzoate, benzyl alcohol, and propylene carbonate. The stability of a protein in the presence of an organic solvent is measured by recovering the protein from the solvent and measuring the percentage of the protein which is intact (i.e. not denatured). Protein that is not denatured is referred to as monomer, since denatured proteins tend to aggregate together. The percentage of monomer can be measured by HPLC-SEC. Preferably, the percentage of monomer recovered is from 35% to 100%. More preferably, the percentage of monomer recovered is from 70% to 100%. Even more preferably, the percentage of monomer recovered is from 90% to 100%. Even more preferably, the percentage of monomer recovered is from 95% to 100%. Even more preferably, the percentage of monomer recovered is from 99% to 100%.

[0028] When the composition is administered in vivo, preferably less than 10% of the protein is released from the depot within 24 hours of administration, more preferably less than 5% of the protein is released from the depot within 24 hours of administration, even more preferably less than 1% of the protein is released from the depot within 24 hours of administration, even more preferably less than 0.2% of the protein is released from the depot within 24 hours of administration, even more preferably less than 0.01% of the protein is released from the depot within 24 hours of administration. The organism to which the composition is administered can be, for example, a rat or a human.

[0029] The release of the protein preferably occurs over a period of days, weeks, or months. It is preferred that at least 25% of
the total amount of protein is released within one year of administration, more preferably at least 25% of the total amount of the protein is released within one month of administration, most preferably at least 25% of the total amount of the protein is released within one week of administration. Alternatively, it is preferred that at least 20% of the total amount of protein is released within one year of administration, more preferably at least 20% of the total amount of the protein is released within one month of administration, most preferably at least 20% of the total amount of the protein is released within one week of administration. The desired length of the release period will vary according to the physiological treatment desired. It is preferred that the amount of protein released within a 24 hour period is from 0.01% to 5%. More preferably, the amount of protein released within a 24 hour period is from 0.05% to 3%. Even more preferably, the amount of protein released within a 24 hour period is from 1% to 3%.

[0030] The composition may be conveniently packaged in a sterile container, such as the vial 10 illustrated in Figure 1. This container may be part of a kit which may optionally contain a sterile syringe and needle. The vial 10 may be sealed with a septum 12. This septum seals the liquid 14 and may be pierced by a needle and syringe to permit withdrawal of the mixture. The vial may contain all the ingredients necessary for the controlled release of the protein. The liquid composition in the vial preferably contains a unit dosage of the protein. It is preferred that the end user of the mixture not be required to add further ingredients or to measure the dosage prior to administration. The liquid composition may be contained in a syringe such that it can be directly administered by injection.

[0031] Alternatively, the composition may be packaged in more than one container. For example, a liquid carrier may be in one vial, and a mixture of the protein in a solvent or solvent mixture may be in another vial. The solvents and/or solvent mixture may be the same as or different from the liquid carrier. The contents of the vials may be combined and mixed, and the final composition administered by injection. In another example, the formulation of protein, polyl and metal may be in one container, and the liquid carrier may be in another container. The protein, polyl and metal may be provided together as a powder, or the protein, polyl and metal may be provided together as a tablet or capsule. The protein, polyl and metal may be combined with the liquid carrier, and the final composition administered by injection. In another example, the polyl and metal may be provided as a mixture in the liquid carrier in a vial, and the protein may be provided in a separate container. Alternatively, the protein may be provided as a mixture in the liquid carrier in a vial, and the polyl and metal may be provided in a separate container. The contents of the containers may be combined such that a liquid formulation is formed, and the final composition administered by injection.

[0032] Preferably, the packaging of the composition or its components is disposable, more preferably recyclable. It is preferred that the composition and its packaging are sterile.

EXAMPLES

Example 1 - Stabilized protein formulations

[0033] A solid formulation of growth hormone was produced by combining rhGH (GENENTECH, S. San Francisco, CA) in 25 mM sodium bicarbonate (25 mg rhGH/mL) with zinc acetate to provide a 10:1 molar ratio of zinc to growth hormone. In formulations 2 and 5, the polyl was added to this mixture at the concentration indicated. In formulations 3 and 4, no zinc was added either before or after the addition of the polyl. In formulation 1, no polyl was added to the mixture of rhGH and zinc.

[0034] The effect of organic solvents on protein stability was determined by adding rhGH solid formulation (1.0 mg) to either absolute ethanol (EtOH) or N-methyl pyrrolidine (NMP). The ratio of protein mass (mg) to volume of solvent (mL) was 5 mg/mL. After protein addition, the samples were homogenized for 2 min at 8,000 rpm with a shear homogenizer tip. The resulting suspensions were allowed to incubate at 37°C for 24 hours. The rhGH was recovered by dilution into a 10-fold excess of a stabilizing buffer (5 mM EDTA, 50 mM HEPES, 0.01% NaN₃, pH 8.0). The amount and quality of the protein recovered in this step was determined by size exclusion chromatography - high performance liquid chromatography (SEC-HPLC), and the results are shown in Table 1. The SEC-HPLC was run using a 7.8 X 300 mm TSK 2000SWXL column at room temperature, with a mobile phase of 50 mM Na₂HPO₄, 150 mM NaCl, pH 7.2. The flow rate was 1.0 ml/min, and the run time was 20 min. Protein (10 μg) was injected and the eluent monitored for absorbance at 214 nm.

[0035] The criteria for a stable formulation were maximum recovery of monomeric rhGH without formation of aggregates. Control samples of each formulation were analyzed by incubation in the buffer without exposure to an organic solvent, and the results indicate that the starting material for each formulation did not contain significant amounts of aggregate.
The presence of a polyl and zinc provide superior protection against protein denaturing after exposure to ethanol and NMP. Formulations containing both a polyl and zinc yielded higher recovery of monomer than formulations stabilized with only a polyl or only zinc.

Table 1. Effect of organic solvents on rhGH stability

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Polyl Concentration (wt%)</th>
<th>Zinc: rhGH (molar ratio)</th>
<th>% Monomer Recovered (Mean±SD)</th>
<th>% Monomer Recovered (Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mannitol</td>
<td>Trehalose</td>
<td>EtOH</td>
<td>NMP</td>
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<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>10:1</td>
<td>31±8</td>
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<td>2</td>
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<td>10:1</td>
<td>72±1</td>
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</table>

Example 2: Controlled release formulation

Solid formulations of growth hormone were produced by combining rhGH with zinc and/or a polyl. In formulations 12-20, rhGH in 25 mM sodium bicarbonate (20 mg rhGH/mL) was combined with zinc acetate to provide a 10:1 molar ratio of zinc to growth hormone. In formulations 12-19, the polyl was then added to the mixture at the concentration indicated. Formulations 13 and 17 additionally included 0.02% POLYSORBATE 20, which was added with the polyl. In formulations 6-11, the polyl was added to the rhGH mixture without the addition of zinc.

The effect of polyl and zinc on protein stability in a controlled release system was determined by adding rhGH formulation in SABER (100 µL) into 2 mL of release buffer. The formulation contained a 5% load of rhGH in a 80:20 mixture of SAIB and benzyl alcohol. The buffer was 50 mM HEPES, 95 mM KCl, pH 7.2. The resulting suspensions were stored at 37°C for 24 hours. The amount and quality of the protein recovered in this step was determined by SEC-HPLC, and the results are given in Table 2. The entire release medium was analyzed by SEC-HPLC to determine total protein content and percentage of non-aggregated protein present, using a method similar to that described in Maa et al., J. Pharm Sci. 2(87) 152-159, 1998.

Table 2

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Polyl Concentration</th>
<th>Zinc: rhGH molar ratio</th>
<th>% Released</th>
<th>% Monomer Recovered</th>
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<td></td>
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<td>1</td>
<td>10:1</td>
<td>10 0.01</td>
</tr>
<tr>
<td>17</td>
<td>0</td>
<td>1*</td>
<td>10:1</td>
<td>10 0.9</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
<td>5</td>
<td>10:1</td>
<td>15 2.2</td>
</tr>
<tr>
<td>Formulation</td>
<td>Polyol Concentration</td>
<td>Zinc: rhGH molar ratio</td>
<td>% Released</td>
<td>% Monomer Recovered</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------------</td>
<td>------------------------</td>
<td>------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>19</td>
<td>0</td>
<td>10:1</td>
<td>14</td>
<td>1.4</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
<td>0</td>
<td>10:1</td>
<td>12</td>
</tr>
<tr>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

* Additionally formulated with 0.02% POLYSORBATE 20 surfactant

[0040] Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein in the Examples.

**REFERENCES CITED IN THE DESCRIPTION**

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**Patent documents cited in the description**

- US5296885A [0063]
- US4297344A [0063]
- US5559167A [0063]
- WO8903671A [0063]
- US516110781 [0063]
- US5747058A [0025]

**Non-patent literature cited in the description**

Patentkrav

1. Sammensætning omfattende:
et protein, hvor proteinet er rekombinant humant vækst hormon;
en polyol; og
en metalkation, hvor metalkationen er divalent zink;
hvor masseforholdet mellem polyol og protein er fra 1:1 til 1:100, og molfor-
holdet mellem zink og protein er fra 1:1 til 100:1, og hvor polyolen og zinket
tilsammen tilvejebringer en synergistisk beskyttelse af proteinkonformation
og -aktivitet, og yderligere omfattende et organisk oplosningsmiddel.

2. Sammensætning ifølge krav 1, hvor:
(a) polyolen er udvalgt fra gruppen bestående af et monosaccharid, et po-
lysaccharid, glycerol, mannitol, sorbitol, inositol og polyethylenglycol; eller
(b) polyolen er udvalgt fra gruppen bestående af mannitol, trehalose og poly-
ethylenglycol.

3. Sammensætning ifølge krav 1 eller krav 2, hvor masseforholdet mellem
polyol og protein er fra 1:1 til 1:10.

4. Sammensætning ifølge et hvilket som helst af kravene 1 til 3, hvor:
(a) molforholdet mellem zink og protein er fra 1:1 til 20:1; eller
(b) molforholdet mellem zink og protein er fra 1:1 til 10:1.

5. Sammensætning ifølge et hvilket som helst af de foregående krav, yderli-
gere omfattende et bæremateriale;
hvor bærematerialet omfatter et ikke-polymert, ikke-vandopløseligt flydende
materiale med en viskositet på mindst 5.000 cP ved 37 °C, som ikke krystalli-
seres i ren tilstand under omgivende fysiologiske forhold.

6. Sammensætning ifølge krav 5, hvor det flydende materiale er en stearat-
ester, en stearatamid, en langkædet fedtsyramid, et langkædet fedt alkohol,
en langkædet ester eller en disaccharidester.

7. Sammensætning ifølge krav 5, hvor det flydende materiale er acetyleret
saccharosedi stearat, eller det flydende materiale er disaccharidacetatbutyrat,
eller det flydende materiale er saccharoseacetatisobutyrat.

8. Sammensætning ifølge et hvilket som helst af de foregående krav, hvor:
   (a) sammensætningen har en viskositet på mindre end 1.000 cP ved rum-
   temperatur; eller
   (b) sammensætningen har en viskositet på mindre end 200 cP ved rumtem-
   peratur.

9. Anvendelse af en sammmensætning til fremstilling af en formulering yderli-
   gere omfattende et organisk opløsningsmiddel, hvor sammensætningen om-
   fatter:
   et protein, hvor proteinet er rekombinant humant væksthormon;
   en polyol; og
   en metalkation, hvor metalkationen er divalent zink;
   hvor masseforholdet mellem polyol og protein er fra 1:1 til 1:100, og molfor-
   holdet mellem zink og protein er fra 1:1 til 100:1, og hvor polyolen og zinket
   tilsammen tilvejebringer en synergistisk beskyttelse af proteinkonformation
   og -aktivitet.

10. Anvendelse ifølge krav 9, hvor:
   (a) polyolen er udvalgt fra gruppen bestående af et monosaccharid, et po-
   lysaccharid, glycerol, mannitol, sorbitol, inositol og polyethylenglycol; eller
   (b) polyolen er udvalgt fra gruppen bestående af mannitol, trehalose og poly-
   ethylenglycol.

11. Anvendelse ifølge krav 9 eller krav 10, hvor masseforholdet mellem poly-
    ol og protein er fra 1:1 til 1:10.

12. Anvendelse ifølge et hvilket som helst af kravene 9 til 11, hvor:
    (a) molforholdet mellem zink og protein er fra 1:1 til 20:1; eller
    (b) molforholdet mellem zink og protein er fra 1:1 til 10:1.

13. Anvendelse ifølge et hvilket som helst af kravene 9 til 12, yderligere om-
    fattende et bæremateriale;
    hvor bærematerialet omfatter et ikke-polymert, ikke-vandopløseligt flydende
    materiale med en viskositet på mindst 5.000 cP ved 37 °C, som ikke krystalli-
seres i ren tilstand under omgivende fysiologiske forhold.


15. Anvendelse ifølge krav 13, hvor det flydende materiale er acetyleret saccharosedistearat, eller det flydende materiale er disaccharidacetatbutyrat, eller det flydende materiale er saccharoseacetatisobutyrat.

16. Anvendelse ifølge et hvilket som helst af kravene 9 til 15, hvor:
(a) sammensætningen har en viskositet på mindre end 1.000 cP ved rumtemperatur; eller
(b) sammensætningen har en viskositet på mindre end 200 cP ved rumtemperatur.


18. Anvendelse af en sammensætning ifølge krav 17, hvor mellem 10 og 15 % af proteinet frigives inden for 24 timer efter indgivelse.

19. Fremgangsmåde til fremstilling af en depotsammensætning, omfattende: at blande en sammensætning ifølge et hvilket som helst af kravene 1 til 4 og et flydende bærestof til dannelse af depotsammensætningen; hvor det flydende bærestof omfatter saccharoseacetatisobutyrat.

20. Fremgangsmåde ifølge krav 19, hvor:
(a) depotsammensætningen har en viskositet på mindre end 1.000 cP ved rumtemperatur; eller
(b) depotsammensætningen har en viskositet på mindre end 200 cP ved rumtemperatur.
21. Fremgangsmåde ifølge krav 19 eller 20, hvor opløsningsmidlet er ethanol, benzylbenzoat, miglyol, propylencarbonat eller benzylalkohol.

22. Fremgangsmåde ifølge krav 21, hvor:
   (a) forholdet mellem saccharoseacetatisobutyrat og opløsningsmiddel er fra 50:50 w/w til 85:15 w/w; eller
   (b) forholdet mellem saccharoseacetatisobutyrat og opløsningsmiddel er fra 50:50 w/w til 70:30 w/w.

23. Kit, der omfatter:
   en beholder;
   en sammensætning ifølge et hvilket som helst af kravene 1 til 4; og
   et flydende bærestof;
   hvor det flydende bærestof omfatter saccharoseacetatisobutyrat.

24. Kit ifølge krav 23, omfattende en enhedsdosering af proteinet.

25. Kit ifølge krav 23 eller krav 24, hvor polyolen, metalkationen og det flydende bærestof er sterile.


27. Kit ifølge et hvilket som helst af kravene 23 til 26, hvor beholderen omfatter en septum.