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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 polyol 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 polyol and a metal. The polyol 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 polyol or the metal have separately.

**[0014]** The polyol 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 polyol 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 (wt%) of the polyol 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%NaN<sub>3</sub>, 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 50mM NaH<sub>2</sub>PO<sub>4</sub>, 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 eluent monitored for absorbance at 214 nm.

**[0015]** Examples of polyols include monosaccharides, such as glucose, fructose, and ribose, including cyclic isomers; glycerol; mannitol; sorbitol; inositol; polysaccharides, 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 polyol may be substituted. "Substituted" means that the moiety contains at least one, preferably 1-3 substituent(s). Suitable substituents include ether (-O-C), amino (-NH<sub>2</sub>), oxy (-O-), carbonyl (>C=O), thiol, and the like. Preferably, the polyol 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 polyol has molecular weight less than about 70,000 kDa.

**[0016]** The relative amounts of protein and polyol in a formulation may be chosen to minimize protein denaturing. For a given protein, the ideal ratio may vary depending on the polyol 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; thyrotropic hormone; oxytocin; vasopressin; somatostatin; lypressin; pancreozymin; 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 VIIIc, factor IX, tissue factor, and von Willebrands 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 a urokinase; bombesin; thrombin; hemopoietic 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; mullerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; chorionic gonadotropin; gonadotropin releasing hormone; bovine somatotropin; porcine somatotropin; a microbial protein, such as beta-lactamase; DNase; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; 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- $\beta$ ; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor (TGF) such as TGF-alpha and TGF-beta, including TGF- $\beta$ 1, TGF- $\beta$ 2, TGF- $\beta$ 3, TGF- $\beta$ 4, or TGF- $\beta$ 5; 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; addressins; fertility inhibitors such as the prostaglandins; 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 ( $K_D$ ) on the order of micromolar ( $\mu\text{M}$ ) or smaller, as measured in water at physiological temperature and pH. The value  $K_D$  is defined as the product of the concentration of the uncomplexed metal and the uncomplexed 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  $K_D$  on the order of millimolar (mM). Preferably, the protein-metal complex has a  $K_D$  of 0.1  $\mu\text{M}$  or smaller. More preferably, the protein-metal complex has a  $K_D$  of 0.01  $\mu\text{M}$  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 polyol 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, benzalconium chloride, and benzethonium chloride. Surfactants include, for example, POLYSORBATE 20 and 80.

**[0022]** The protein, polyol, 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 polyol. For example, the protein, polyol, 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, polyol, 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 bioerodible polymer. A bioerodible 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 bioerodible 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 bioerodible polymers include polyesters, such as poly(caprolactone), poly(glycolic acid), poly(lactic acid), and poly(hydroxybutyrate); polyanhydrides, such as poly(adipic anhydride) and poly(maleic anhydride); polydioxanone; polyamines; polyamides; polyurethanes; polyesteramides; polyorthoesters; polyacetals; polyketals; polycarbonates; polyorthocarbonates; polyphosphazenes; poly(malic acid); poly(amino acids); polyvinylpyrrolidone; poly(methyl vinyl ether); poly(alkylene oxalate); poly(alkylene succinate); polyhydroxycellulose; chitin; chitosan; and copolymers and mixtures thereof. Proteins may be incorporated into bioerodible polymers by formation of monolithic implants, which are surgically implanted, or by formation of microparticles of the bioerodible polymer containing the protein.

**[0024]** The composition may also include a carrier liquid. Preferably, the formulation of protein, polyol, 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 ratio of 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 bioerodible 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 sucrose acetate 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. 5,747,058; 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-FENSKE 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 stearate esters, stearate amides, long-chain fatty acid amides, long-chain fatty alcohols, long-chain esters, and disaccharide esters. Preferably, the liquid material is acetylated sucrose distearate, 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, miglyol, 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 (ie. 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, polyol and metal may be in one container, and the liquid carrier may be in another container. The protein, polyol and metal may be provided together as a powder, or the protein, polyol and metal may be provided together as a tablet or capsule. The protein, polyol and metal may be combined with the liquid carrier, and the final composition administered by injection. In another example, the polyol 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 polyol 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 polyol 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 polyol. In formulation 1, no polyol 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%NaN<sub>3</sub>, 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 2000-SWXL column at room temperature, with a mobile phase of 50mM NaH<sub>2</sub>PO<sub>4</sub>, 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.

[0036] The presence of a polyol and zinc provide superior protection against protein denaturing after exposure to ethanol and NMP. Formulations containing both a polyol and zinc yielded higher recovery of monomer than formulations stabilized with only a polyol or only zinc.

Table 1. Effect of organic solvents on rhGH stability

Formulation	Polyol Concentration (wt%)		Zinc: rhGH (molar ratio)	% Monomer Recovered (Mean±SD)		% Monomer Recovered (Control)
	Mannitol	Trehalose		EtOH	NMP	
1	0	0	10:1	31±8	37±3	98
2	1	0	10:1	36±1	44±1	98
3	1	0	0	22±1	19±1	98
4	0	5	0	65±1	88±1	99
5	0	5	10:1	72±1	89±0	99

### Example 2 - Controlled release formulation

[0037] Solid formulations of growth hormone were produced by combining rhGH with zinc and/or a polyol. 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 polyol 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 polyol. In formulations 6-11, the polyol was added to the rhGH mixture without the addition of zinc.

[0038] The effect of polyol 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.

[0039] Formulations containing only a polyol or only zinc allowed for significant protein denaturing, with a maximum of 91.5% monomer recovery. The presence of both a polyol and zinc effectively protected the protein from denaturing. These formulations provided approximately 99% monomer recovery. The combination of polyol and zinc also allows for acceptable release rates of the protein from the depot, with 24-hour releases between 10 and 15%.

Table 2

Formulation	Polyol Concentration		Zinc: rhGH molar ratio	% Released		% Monomer Recovered
	Mannitol	Trehalose		1 Day	SD	
6	1	0	0	17	5.9	85.4
7	5	0	0	8	0.1	89.7
8	10	0	0	36	0.04	74.7
9	0	1	0	15	0.2	83.4
10	0	5	0	6	0.7	90.5
11	0	10	0	6	0.2	91.5
12	1	0	10:1	12	0.6	98.8
13	1*	0	10:1	10	3.2	99.1
14	5	0	10:1	12	1.0	99.2
15	10	0	10:1	10	0.3	99.2
16	0	1	10:1	10	0.01	98.9
17	0	1*	10:1	10	0.9	98.9
18	0	5	10:1	15	2.2	99.2



Formulation	Polyol Concentration		Zinc: rhGH molar ratio	% Released		% Monomer Recovered
19	0	10	10:1	14	1.4	99.2
20	0	0	10:1	12	1.2	83.9
21	0	0	0	10	5.3	91.1

\* 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

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**Patentkrav**

1. Sammensætning omfattende:  
et protein, hvor proteinet er rekombinant humant væksthormon;  
5 en polyol; og  
en metalkation, hvor metalkationen er divalent zink;  
hvor masseforholdet mellem polyol og protein er fra 1:1 til 1:100, og molforholdet mellem zink og protein er fra 1:1 til 100:1, og hvor polyolen og zinket  
10 tilsammen tilvejebringer en synergistisk beskyttelse af proteinkonformation og -aktivitet, og yderligere omfattende et organisk opløsningsmiddel.
2. Sammensætning ifølge krav 1, hvor:  
(a) polyolen er udvalgt fra gruppen bestående af et monosaccharid, et polysaccharid, glycerol, mannitol, sorbitol, inositol og polyethylenglycol; eller  
15 (b) polyolen er udvalgt fra gruppen bestående af mannitol, trehalose og polyethylenglycol.
3. Sammensætning ifølge krav 1 eller krav 2, hvor masseforholdet mellem polyol og protein er fra 1:1 til 1:10.  
20
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.
- 25
5. Sammensætning ifølge et hvilket som helst af de foregående krav, yderligere 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 krystalliseres i ren tilstand under omgivende fysiologiske forhold.  
30
6. Sammensætning ifølge krav 5, hvor det flydende materiale er en stearat-ester, en stearatamid, en langkædet fedtsyreamid, et langkædet fedtalkohol, en langkædet ester eller en disaccharidester.
- 35
7. Sammensætning ifølge krav 5, hvor det flydende materiale er acetyleret saccharosedistearat, eller det flydende materiale er disaccharidacetatbutyrat,

eller det flydende materiale er saccharoseacetatisobutytrat.

**8.** Sammensætning ifølge et hvilket som helst af de foregående krav, hvor:

5

(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.

10

**9.** Anvendelse af en sammensætning til fremstilling af en formulering yderligere omfattende et organisk opløsningsmiddel, hvor sammensætningen omfatter:

et protein, hvor proteinet er rekombinant humant væksthormon;

en polyol; og

en metalkation, hvor metalkationen er divalent zink;

15

hvor masseforholdet mellem polyol og protein er fra 1:1 til 1:100, og molforholdet 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.

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**10.** Anvendelse ifølge krav 9, hvor:

(a) polyolen er udvalgt fra gruppen bestående af et monosaccharid, et polysaccharid, glycerol, mannitol, sorbitol, inositol og polyethylenglycol; eller

(b) polyolen er udvalgt fra gruppen bestående af mannitol, trehalose og polyethylenglycol.

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**11.** Anvendelse ifølge krav 9 eller krav 10, hvor masseforholdet mellem polyol og protein er fra 1:1 til 1:10.

30

**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.

35

**13.** Anvendelse ifølge et hvilket som helst af kravene 9 til 12, yderligere 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.

- 5           **14.** Anvendelse ifølge krav 13, hvor det flydende materiale er en stearatester, en stearatamid, en langkædet fedtsyreamid, et langkædet fedtalkohol, en langkædet ester eller en disaccharidester.
- 10           **15.** Anvendelse ifølge krav 13, hvor det flydende materiale er acetyleret saccharosedistearat, eller det flydende materiale er disaccharidacetatbutyrat, eller det flydende materiale er saccharoseacetatisobutytrat.
- 15           **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.
- 20           **17.** Anvendelse af en sammensætning ifølge et hvilket som helst af kravene 5 til 7 til fremstilling af en injicerbar depotformulering, hvor polyolen og zinket tilsammen tilvejebringer en synergistisk beskyttelse af proteinkonformation og -aktivitet.
- 25           **18.** Anvendelse af en sammensætning ifølge krav 17, hvor mellem 10 og 15 % af proteinet frigives inden for 24 timer efter indgivelse.
- 30           **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 saccharoseacetatisobutytrat.
- 35           **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:

5 (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.

10 **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.

15

**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.

20

**26.** Kit ifølge et hvilket som helst af kravene 23 til 25, yderligere omfattende en sprøjte.

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

25

DRAWINGS

***FIG. 1***

