

Lyophilized Formulations

Field of the Invention

The present invention relates to formulations useful for reducing the reconstitution time of lyophilized biological molecules and methods of use.

5 Background of the invention

Lyophilization is a process which removes solvent from a solution to form solid or powder which is stable and easier to store at elevated temperature than the liquid. Lyophilization, also known as freeze drying, involves freezing followed by sublimation. The resulting lyophilized matter may be stored without refrigeration, reducing storage and transportation costs of the substance as well as the storage space required for the product. It also can reduce the weight of the product, which similarly reduces shipping and related costs. Lyophilization is particularly useful for preserving and storing various biological molecules, because it increases their shelf-life.

Biological molecules are more difficult to stabilize via formulation than small molecules due to the number of chemical groups and dependence of stability on maintaining native folding. For this reason, many commercial biological molecules are lyophilized. In general, lyophilization improves stability through (i) the removal of water (since most biologic chemical degradations are hydrolytic) and (ii) decreasing the overall mobility of the system (since dynamic movement of side chains and molecules is necessary for chemical and physical degradation events to occur).

Lyophilized biological molecules are reconstituted prior to use, often in the very containers in which they were lyophilized and stored. Short reconstitution time is preferable for both physicians and patients. If the reconstitution time of the lyophilized biological molecule is too long, it will increase the preparation time thus making it difficult to administer to many patients at the same. In addition, many biological molecules are designed to be administered by the patients themselves. A shorter reconstitution time ensures that patients will completely reconstitute the biological molecule before administration, thus improving safety and efficacy.

Previous efforts to reduce reconstitution time have focused primarily on the formulation of the reconstitution buffer. In contrast, the present invention is directed to the addition of a volatile additive to the formulation of the buffer used for lyophilization of a biological molecule. Thus, there is a need for methods and compositions for reducing the reconstitution time of a lyophilized biological molecule.

Summary of the Invention

The present invention is directed to a method for producing a polypeptide composition comprising: combining a polypeptide with a volatile additive to form a liquid mixture and lyophilizing the liquid mixture to obtain a lyophilized polypeptide composition.

The present invention is also directed to a method for reducing the reconstitution time of a lyophilized polypeptide composition comprising: a) lyophilizing a liquid mixture comprising the polypeptide, wherein the liquid mixture comprises a volatile additive and b) reconstituting the

lyophilized polypeptide with a sufficient amount of a pharmaceutically acceptable dispersing agent to the lyophilized polypeptide composition to yield a liquid polypeptide composition, wherein time for reconstituting the polypeptide lyophilized in the presence of the volatile additive is less than the time for reconstituting the same polypeptide lyophilized in the absence of the volatile additive.

The present invention is also directed to a method for producing a liquid polypeptide composition comprising: obtaining a lyophilized polypeptide produced by a method of the present invention, and reconstituting the lyophilized polypeptide with a sufficient amount of a pharmaceutically acceptable dispersing agent to yield a liquid polypeptide composition.

The present invention is also directed to a formulation suitable for lyophilization of a polypeptide.

The present invention is also directed to a dry polypeptide composition produced by a method of the present invention.

The present invention is also directed to a liquid polypeptide composition produced by a method of the present invention.

Brief Description of the Drawings

Figure 1. Plots of lyophile reconstitution times using shaking method.

Figure 2. Plots of lyophile reconstitution times using undisturbed method.

Figure 3. Plots of lyophile reconstitution times as a function of sample number. (A) Box plots summarizing the average recon times over all tests as a function of sample number. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentile. Outliers below 10th or above 90th percentile are plotted as symbols. (B) All lyophile recon times plotted as a function of sample number.

Figure 4. Plots of lyophile reconstitution times as a function of sample number, separated by analyst and reconstitution method.

Figure 5. Amino acid sequence of Albiglutide (SEQ ID NO:1).

Figure 6. Bar chart comparing reconstitution endpoints of samples, lyophilized either with or without the addition of 2%tBuOH. The bars represent the average over all tests, and whiskers one standard deviation over all tests, as a function of sample type.

Figure 7. Bar chart comparing reconstitution endpoints of samples, lyophilized either with or without the addition of 2%tBuOH. The bars represent the average over all tests, and whiskers one standard deviation over all tests, as a function of sample type.

Figure 8. Amino acid sequence of anti-NOGO mAb heavy (SEQ ID NO: 3) and light chains (SEQ ID NO: 4).

Figure 9. Amino acid sequence of anti-TNFR1 dAb (SEQ ID NO: 5).

Figure 10. Amino acid sequence of IL18 (SEQ ID NO: 6).

Figure 11. Amino acid sequence of anti-IL5 heavy (SEQ ID NO: 7) and light chains (SEQ ID NO: 8).

Figure 12. Amino acid sequence of anti-CD20 VH (SEQ ID NO: 9) and VL domains (SEQ ID NO: 10).

5 Detailed Description of the Invention

The present invention provides, inter alia, lyophilization formulations. The invention also provides, inter alia, methods of making and methods of using lyophilization formulations. It has been discovered by the inventors that when a volatile additive is added to a lyophilization formulation for a polypeptide reconstitution times for the lyophilized polypeptide are reduced.

10 The present invention is also directed to a dry polypeptide composition produced by a method of the present invention.

The invention provides a number of methods, reagents, and compounds that can be used for producing a polypeptide composition comprising: combining a polypeptide with a volatile additive to form a liquid mixture and lyophilizing the liquid mixture to obtain a lyophilized polypeptide composition.

15 Methods for lyophilizing polypeptides from liquid solutions, and products comprising lyophilized polypeptides obtained from such lyophilized liquid solutions, are provided herein. In certain embodiments, the volatile additive is dissolved in aqueous solution containing the polypeptide and lyophilized to provide solid compositions containing the lyophilized polypeptide. In certain
20 embodiments, these solid compositions containing the lyophilized polypeptide are stable and are suitable for storage, e.g., suitable for storage for long periods of time. Such storage may be at ambient conditions, may be under controlled temperature, may be under controlled humidity, or other condition or set of conditions; and may be stored in a sealed container (e.g., a bottle or jar with a removable lid, a tubes, a capsule, a caplet, a vial, syringe, dual-cartridge-syringe, or other
25 container), and may be in a sealed container under an inert gas (e.g., nitrogen, argon, helium, or other inert gas), or other container with or without other element or compound in the container.

It is to be understood that this invention is not limited to particular methods, reagents, compounds, compositions, or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular
30 embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a polypeptide" includes a combination of two or more polypeptides, and the like.

"About" as used herein when referring to a measurable value such as an amount, a temporal
35 duration, and the like, is meant to encompass variations of $\pm 20\%$ or $\pm 10\%$, including $\pm 5\%$, $\pm 1\%$, and $\pm 0.1\%$ from the specified value, as such variations are appropriate to perform the disclosed methods.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains.

Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

All "amino acid" residues identified herein are in the natural L-configuration. In keeping with standard polypeptide nomenclature, abbreviations for amino acid residues are as shown in the following table.

1 Letter	3 Letter	Amino Acid
Y	Tyr	L-tyrosine
G	Gly	L-glycine
F	Phe	L-phenylalanine
M	Met	L-methionine
A	Ala	L-alanine
S	Ser	L-serine
I	Ile	L-isoleucine
L	Leu	leucine
T	Thr	L-threonine
V	Val	L-valine
P	Pro	L-proline
K	Lys	L-lysine
H	His	L-histidine
Q	Gln	L-glutamine
E	Glu	L-glutamic acid
W	Trp	L-tryptophan
R	Arg	L-arginine
D	Asp	L-aspartic acid
N	Asn	L-asparagine
C	Cys	L-cysteine.

It should be noted that all amino acid residue sequences are represented herein by formulae whose left to right orientation is in the conventional direction of amino-terminus to carboxy-terminus.

In certain embodiments the volatile additive is an organic solvent. In one embodiment the organic solvent comprises a lower oxyhydrocarbon, a lower halohydrocarbon, a lower haloxyhydrocarbon, a lower sulfoxyhydrocarbon, a lower cyclohydrocarbon or combination thereof. In one embodiment the lower oxyhydrocarbon is methanol, ethanol, n-propanol, isopropanol, n-butanol, iso-butanol, 2-butanol, t-butanol, pentanol, iso-pentanol, 2-pentanol, 3-pentanol, t-pentanol, methylethylketone, benzylalcohol, acetic acid, methylethyl ketone, or a combination thereof.

In certain embodiments the volatile additive is acetonitrile, ammonium acetate, or ammonium carbonate. In one embodiment the amount of ammonium acetate or ammonium carbonate is about 50mM to about 300mM. In one embodiment the amount of ammonium acetate or ammonium carbonate is about 100mM or about 250mM.

- 5 "Lower oxyhydrocarbons" as referred to herein means compounds possessing hydrocarbyl radicals and oxygen atoms having from 1 to 8 carbon atoms and from 1 to 4 oxygen atoms. Exemplary lower oxyhydrocarbons include, but are not limited to, lower alkanols, lower ketones, lower carboxylic acids, lower carboxylic esters, lower carbonates, and the like.

10 "Lower" as it refers to chemical compounds described herein refers to those compounds that have from 1 to 8 carbon atoms.

"Lower alkanol" refers to a saturated C₁-C₈ alkyl group which can be branched or straight-chained with from 1 to 4 hydroxyl groups. Exemplary lower alkanols having 1 hydroxyl group include, but are not limited to, methanol, ethanol, n-propanol, iso-propanol, n-butanol, iso-butanol, 2-butanol, t-butanol, pentanol, iso-pentanol, 2-pentanol, 3-pentanol, t-pentanol, and the like.

- 15 Exemplary "lower ketones" include, but are not limited to, acetone, methyl ethyl ketone, methyl propyl ketone, methyl isopropyl ketone, methyl butyl ketone, methyl iso-butyl ketone, methyl 2-butyl ketone, methyl t-butyl ketone, diethyl ketone, ethyl propyl ketone, ethyl isopropyl ketone, ethyl butyl ketone, ethyl iso-butyl ketone, ethyl t-butyl ketone, and the like.

20 Exemplary "lower carboxylic acids" include, but are not limited to, formic acid, acetic acid, propionic acid, butyric acid isobutyric acid and the like.

Exemplary "lower carboxylic esters" include, but are not limited to, methyl acetate, ethyl acetate, propyl acetate, isopropyl acetate, butyl acetate, isobutyl acetate, 2-butyl acetate, t-butyl acetate, and the like.

- 25 Exemplary "lower carbonates" include, but are not limited to, dimethyl carbonate, methyl ethyl carbonate, methyl propyl carbonate, methyl isopropyl carbonate, methyl butyl carbonate, methyl iso-butyl carbonate, methyl 2-butyl carbonate, methyl t-butyl carbonate, diethyl carbonate, ethyl propyl carbonate, ethyl isopropyl carbonate, ethyl butyl carbonate, ethyl iso-butyl carbonate, ethyl t-butyl carbonate, and the like.

30 "Lower halohydrocarbons" as referred to herein means compounds possessing hydrocarbyl radicals and halo atoms having from 1 to 8 carbon atoms and from 1 to 4 halo atoms. Preferably, the halo atoms are chloro, fluoro and bromo. Most preferably, the halo atoms are chloro atoms. Exemplary lower halohydrocarbons, include, but are not limited to, methyl chloride, methylene chloride, chloroform, carbon tetrachloride, and the like.

35 "Lower haloxyhydrocarbons" means oxyhydrocarbons as defined herein which are further substituted with from 1 to 4 halo atoms. An exemplary haloxyhydrocarbon includes, but is not limited to, hexafluoroacetone.

"Lower sulfoxyhydrocarbons" means oxyhydrocarbons as defined herein which also contain a sulfur atom. Exemplary lower sulfoxyhydrocarbons include, but are not limited to, dimethyl sulfoxide (DMSO) and dimethyl sulfone.

"Lower cyclohydrocarbons" refers to hydrocarbonyl radicals which are cyclized such as, for example, 3- to 8- member hydrocarbon rings. An exemplary cyclohydrocarbon includes, but is not limited to, cyclohexane.

"Polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. A polypeptide can be of natural (tissue-derived) origins, recombinant or natural expression from prokaryotic or eukaryotic cellular preparations, or produced chemically via synthetic methods. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid. Non-natural residues are well described in the scientific and patent literature; a few exemplary non-natural compositions useful as mimetics of natural amino acid residues and guidelines are described below. Mimetics of aromatic amino acids can be generated by replacing by, e.g., D- or L-naphthylalanine; D- or L-phenylglycine; D- or L-2-thienylalanine; D- or L-1, -2,3-, or 4-pyrenylalanine; D- or L-3 thienylalanine; D- or L-(2-pyridinyl)-alanine; D- or L-(3-pyridinyl)-alanine; D- or L-(2-pyrazinyl)-alanine; D- or L-(4-isopropyl)-phenylglycine; D-(trifluoromethyl)-phenylglycine; D-(trifluoromethyl)-phenylalanine; D-p-fluoro-phenylalanine; D- or L-p-biphenylphenylalanine; K- or L-p-methoxy-biphenylphenylalanine; D- or L-2-indole(alkyl)alanines; and, D- or L-alkylalanines, where alkyl can be substituted or unsubstituted methyl, ethyl, propyl, hexyl, butyl, pentyl, isopropyl, iso-butyl, sec-isotyl, iso-pentyl, or a non-acidic amino acids. Aromatic rings of a non-natural amino acid include, e.g., thiazolyl, thiophenyl, pyrazolyl, benzimidazolyl, naphthyl, furanyl, pyrrolyl, and pyridyl aromatic rings.

"Peptide" as used herein includes peptides which are conservative variations of those peptides specifically exemplified herein. "Conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include, but are not limited to, the substitution of one hydrophobic residue such as isoleucine, valine, leucine, alanine, cysteine, glycine, phenylalanine, proline, tryptophan, tyrosine, norleucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. Neutral hydrophilic amino acids which can be substituted for one another include asparagine, glutamine, serine and threonine. "Conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide. Such conservative substitutions are within the definition of the classes of the peptides of the invention. "Cationic" as used herein refers to any peptide that possesses a net positive charge at pH 7.4. The biological activity of the peptides can be determined by standard methods known to those of skill in the art and described herein.

"Recombinant" when used with reference to a protein indicates that the protein has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein.

As used "reconstitution time" and grammatical variations thereof means the amount of time necessary for a lyophilized molecule to be dissolved and/or suspended in a liquid form. For instance, reconstitution time includes, but is not limited to the time required for a dried pellet of polypeptide to become suspended in water or a buffer after lyophilization. Accordingly, a "reduction" or "reducing" reconstitution time and grammatical variations thereof, means less time is required for polypeptide dried under a first formulation and/or condition to suspend in a liquid compared with the same polypeptide dried under a second formulation and/or condition and suspended in the same liquid.

The present invention is also directed to a method for reducing the reconstitution time of a lyophilized polypeptide composition comprising: a) lyophilizing a liquid mixture comprising the polypeptide, wherein the liquid mixture comprises a volatile additive and b) reconstituting the lyophilized polypeptide with a sufficient amount of a pharmaceutically acceptable dispersing agent to the lyophilized polypeptide composition to yield a liquid polypeptide composition, wherein time for reconstituting the polypeptide lyophilized in the presence of the volatile additive is less than the time for reconstituting the same polypeptide lyophilized in the absence of the volatile additive.

The present invention is also directed to a method for producing a liquid polypeptide composition comprising: obtaining a lyophilized polypeptide produced by a method of the present invention, and reconstituting the lyophilized polypeptide with a sufficient amount of a pharmaceutically acceptable dispersing agent to yield a liquid polypeptide composition. The present invention is also directed to a liquid polypeptide composition produced by a method of the present invention.

In certain exemplary embodiments, the lyophilized polypeptide readily reconstitutes once contacted with a sufficient amount of a pharmaceutically acceptable dispersing agent. For example, in certain embodiments, the lyophilized polypeptide is mixed, e.g., shaken for about 5 seconds and then allowed to stand for about 5 to about 30 minutes, with a dispersing agent to provide a liquid polypeptide composition. The dispersing agent is preferably sterile water or "water for injection" (WFI). The liquid polypeptide can be further diluted with isotonic saline or other excipients to produce a desirable concentration prior to administration. According to the present invention, "excipients" includes, but is not limited to, stabilizers, for example, human serum albumin (hsa), bovine serum albumin (bsa), α -casein, globulins, α -lactalbumin, LDH, lysozyme, myoglobin, ovalbumin, RNase A; buffering agents, for example, citric acid, HEPES, histidine, potassium acetate, potassium citrate, potassium phosphate (KH_2PO_4), sodium acetate, sodium bicarbonate, sodium citrate, sodium phosphate (NaH_2PO_4), Tris base, and Tris-HCl; amino acids/metabolites, for example, glycine, alanine (α -alanine, β -alanine), arginine, betaine, leucine, lysine, glutamic acid, aspartic acid, histidine, proline, 4-hydroxyproline, sarcosine, γ -aminobutyric acid (GABA), opines (alanopine, octopine, stramine), and trimethylamine N-oxide (TMAO); surfactants, for example, polysorbate 20 and 80, and poloxamer 407; fatty acids, for example, phosphatidyl choline, ethanolamine, and acetyltryptophanate; polymers, for example,

polyethylene glycol (PEG), and polyvinylpyrrolidone (PVP) 10, 24, 40; low molecular weight excipients, for example, arabinose, cellobiose, ethylene glycol, fructose, fucose, galactose, glycerin/glycerol, glucose, inositol, lactose, mannitol, maltose, maltotriose, mannose, melibiose, 2-methyl-2,4-pentanediol, octulose, propylene glycol, raffinose, ribose, sorbitol, sucrose, trehalose, xylitol, and xylose; and high molecular weight excipients, for example, cellulose, β -cyclodextrin, dextran (10 kd), dextran (40 kd), dextran (70 kd), ficoll, gelatin, hydroxypropylmethyl-cellulose, hydroxyethyl starch, maltodextrin, methocel, peg (6 kd), polydextrose, polyvinylpyrrolidone (PVP) k15 (10 kd), PVP (40 kd), PVP k30 (40 kd), PVP k90 (1000 kd), sephadex G 200, and starch; antioxidants, for example, ascorbic acid, cysteine HCl, thioglycerol, thioglycolic acid, thiosorbitol, and glutathione; reducing agents, for example, cysteine HCl, dithiothreitol, and other thiol or thiophenes; chelating agents, for example, EDTA, EGTA, glutamic acid, and aspartic acid; inorganic salts/metals, for example, Ca^{2+} , Ni^{2+} , Mg^{2+} , Mn^{2+} , Na_2SO_4 , $(\text{NH}_4)_2\text{SO}_4$, $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$, $\text{K}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$, MgSO_4 , and NaF; organic salts, for example, Na acetate, Na polyethylene, Na caprylate (Na octanoate), propionate, lactate, succinate, and citrate; organic solvents, for example, acetonitrile, dimethylsulfoxide (dms), and ethanol.

In exemplary embodiments of the present invention, the liquid polypeptide compositions that are produced exhibit desirable characteristics, such as desirable viscosity and surface tension characteristics.

The term "surface tension" refers to the attractive force exerted by the molecules below the surface upon those at the surface/air interface, resulting from the high molecular concentration of a liquid compared to the low molecular concentration of the gas. Liquids with low values of surface tension, such as nonpolar liquids, flow more readily than water. Typically, values of surface tensions are expressed in newtons/meters or dynes/centimeters.

"Dynamic surface tension" as referred to herein is the surface/air interface and the dynamic interfacial tension to the surface/surface interface. There are a number of alternative methods for measuring dynamic surface tension, for example, captive bubble surface tensionometry or pulsating bubble surface tensionometry.

The term "viscosity" refers to the internal resistance to flow exhibited by a fluid at a specified temperature; the ratio of shearing stress to rate of shear. A liquid has a viscosity of one poise if a force of 1 dyne/square centimeter causes two parallel liquid surfaces one square centimeter in area and one square centimeter apart to move past one another at a velocity of 1 cm/second. One poise equals one hundred centipoise.

When referring to apparent viscosity, it is understood that the value of viscosity is dependent on the conditions under which the measurement was taken, such as temperature, the rate of shear and the shear stress employed. The apparent viscosity is defined as the ratio of the shear stress to the rate of shear applied. There are a number of alternative methods for measuring apparent viscosity. For example, viscosity can be tested by a suitable cone and plate, parallel plate or other type of viscometer or rheometer.

The present invention is also directed to a formulation suitable for lyophilization of a polypeptide. In certain embodiments the liquid mixture comprises from about 0.1% by volume to about 10% by volume of volatile additive, from about 0.25% by volume to about 5% by volume of volatile additive, or about 2% by volume of volatile additive. In one embodiment the formulation comprises about 10mM sodium phosphate, pH 7.2, about 117mM trehalose, about 153mM mannitol, and about 0.01% (w/v) polysorbate-80. In another embodiment, the formulation comprises 26 mM histidine, 150 mM trehalose, 0.02% polysorbate 80 (PS80), pH 6.0. In certain embodiments the polypeptide comprises albiglutide (SEQ ID NO:1). In other embodiments the polypeptide comprises IL18 (SEQ ID NO: 6). In one embodiment, the present invention is directed to a liquid formulation comprising about 2% t-butanol. In one embodiment the liquid formulation comprises about 2% t-butanol, about 10mM sodium phosphate, pH 7.2, about 117mM trehalose, about 153mM mannitol, and about 0.01% (w/v) polysorbate-80. In another embodiment, the formulation comprises about 2% t-butanol, about 26 mM histidine, pH 6.0, about 150 mM trehalose, and about 0.02% polysorbate 80 (PS80).

As used herein a "therapeutic protein" refers to any protein and/or polypeptide that can be administered to a mammal to elicit a biological or medical response of a tissue, system, animal or human that is being sought, for instance, by a researcher or clinician. A therapeutic protein may elicit more than one biological or medical response. Furthermore, the term "therapeutically effective amount" means any amount which, as compared to a corresponding subject who has not received such amount, results in, but is not limited to, healing, prevention, or amelioration of a disease, disorder, or side effect, or a decrease in the rate of advancement of a disease or disorder. The term also includes within its scope amounts effective to enhance normal physiological function as well as amounts effective to cause a physiological function in a patient which enhances or aids in the therapeutic effect of a second pharmaceutical agent.

In one aspect, the present invention is directed to a composition comprising: a therapeutic polypeptide, a buffer, a surfactant, at least one excipient, and at least one volatile additive. In one embodiment, the therapeutic polypeptide comprises GLP-1 or a fragment and/or variant thereof. In one embodiment, the therapeutic polypeptide comprises at least two GLP-1(7-36(A8G)) polypeptides genetically fused to the N-terminus of human serum albumin. In one embodiment, the therapeutic polypeptide comprises SEQ ID NO:1. In one embodiment, the therapeutic protein comprises a GLP-1 agonist. In one embodiment, the therapeutic protein comprises human serum albumin.

In one aspect, the present invention is directed to use of any one of the compositions of claims of the present invention for making a medicament comprising SEQ ID NO:1.

In one embodiment, the buffer is sodium phosphate. In one embodiment the buffer is histidine. In one embodiment, the surfactant is polysorbate-80. In one embodiment, the at least one excipient is selected from: trehalose, maltose, sucrose, mannose, lactose, mannitol, sorbitol, glycerol and dextrose. In one embodiment, the excipient comprises: trehalose and mannitol.

"GLP-1 agonist" as used herein means any compound or composition capable of simulating insulin production and/or having at least one GLP-1 activity including, but not limited to an

incretin hormone and/or fragment, variant and/or conjugate thereof and an incretin mimetic and/or fragment, variant and/or conjugate thereof.

“Incretin hormone” as used herein means any hormone that potentiates insulin secretion or otherwise raises the level of insulin. One example of an incretin hormone is GLP-1. GLP-1 is an incretin secreted by intestinal L cells in response to ingestion of food. In a healthy individual, GLP-1 plays an important role regulating post-prandial blood glucose levels by stimulating glucose-dependent insulin secretion by the pancreas resulting in increased glucose absorption in the periphery. GLP-1 also suppresses glucagon secretion, leading to reduced hepatic glucose output. In addition, GLP-1 delays gastric emptying time and slows small bowel motility delaying food absorption. GLP-1 promotes continued beta cell competence by stimulating transcription of genes involved in glucose dependent insulin secretion and by promoting beta-cell neogenesis (Meier, et al. Biodrugs 2003; 17 (2): 93-102).

“GLP-1 activity” as used herein means one or more of the activities of naturally occurring human GLP-1, including but not limited to, reducing blood and/or plasma glucose, stimulating glucose-dependent insulin secretion or otherwise raising the level of insulin, suppressing glucagon secretion, reducing fructosamine, increases glucose delivery and metabolism to the brain, delaying gastric emptying, and promoting beta cell competence, and/or neogenesis. Any of these activities and other activity associated with GLP-1 activity may be caused directly or indirectly by a composition having GLP-1 activity or a GLP-1 agonist. By way of example, a composition having GLP-1 activity may directly or indirectly stimulate glucose-dependent insulin production while the stimulation of insulin production may indirectly reduce plasma glucose levels in a mammal.

An “incretin mimetic” as used herein is a compound capable of potentiating insulin secretion or otherwise raise the level of insulin. An incretin mimetic may be capable of stimulating insulin secretion, increasing beta cell neogenesis, inhibiting beta cell apoptosis, inhibiting glucagon secretion, delaying gastric emptying and inducing satiety in a mammal. An incretin mimetic may include, but is not limited to, any polypeptide which has GLP-1 activity, including but not limited to, exendin 3 and exendin 4, including any fragments and/or variants and/or conjugates thereof.

As used herein “conjugate” or “conjugated” and grammatical variations thereof refer to two molecules that are bound to each other. For example, a first polypeptide may be covalently or non-covalently bound to a second polypeptide. The first polypeptide may be covalently bound by a chemical linker or may be genetically fused to the second polypeptide, wherein the first and second polypeptide share a common polypeptide backbone.

As used herein “fragment,” when used in reference to a polypeptide, is a polypeptide having an amino acid sequence that is the same as part but not all of the amino acid sequence of the entire naturally occurring polypeptide. Fragments may be “free-standing” or comprised within a larger polypeptide of which they form a part or region as a single continuous region in a single larger polypeptide. By way of example, a fragment of naturally occurring GLP-1 would include amino acids 7 to 36 of naturally occurring amino acids 1 to 36. Furthermore, fragments of a polypeptide may also be variants of the naturally occurring partial sequence. For instance, a fragment of GLP-

1 comprising amino acids 7-30 of naturally occurring GLP-1 may also be a variant having amino acid substitutions within its partial sequence.

“Variant” as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical
5 variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a
10 polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by
15 the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis. Variants may also include, but are not limited to, polypeptides or fragments thereof having chemical modification of one or more of its amino acid side groups. A
20 chemical modification includes, but is not limited to, adding chemical moieties, creating new bonds, and removing chemical moieties. Modifications at amino acid side groups include, without limitation, acylation of lysine-ε-amino groups, N-alkylation of arginine, histidine, or lysine, alkylation of glutamic or aspartic carboxylic acid groups, and deamidation of glutamine or asparagine. Modifications of the terminal amino group include, without limitation, the des-
25 amino, N-lower alkyl, N-di-lower alkyl, and N-acyl modifications. Modifications of the terminal carboxy group include, without limitation, the amide, lower alkyl amide, dialkyl amide, and lower alkyl ester modifications. Furthermore, one or more side groups, or terminal groups, may be protected by protective groups known to the ordinarily-skilled protein chemist.

In one embodiment of the present invention, the polypeptide is a GLP-1 polypeptide. “GLP-1
30 polypeptide” includes, but is not limited to, GLP-1 or a fragment, variant, and/or conjugate thereof. GLP-1 fragments and/or variants and/or conjugates of the present invention typically have at least one GLP-1 activity. A GLP-1 or a fragment, variant, and/or conjugate thereof may comprise human serum albumin. Human serum albumin may be conjugated to the GLP-1 or fragment and/or variant thereof. Human serum albumin may be conjugated to an incretin
35 hormone (such as GLP-1) and/or incretin mimetic (such as exendin 3 and exendin 4) and/or fragments and/or variants thereof through a chemical linker prior to injection or may be chemically linked to naturally occurring human serum albumin *in vivo* (see for instance, U.S. Patent No. 6,593,295 and U.S. Patent No. 6,329,336, herein incorporated by reference in their entirety). Alternatively, human serum albumin may be genetically fused to a GLP-1 and/or
40 fragment and/or variant thereof or other GLP-1 agonist such as exendin-3 or exendin-4 and/or fragments and/or variants thereof. Examples of GLP-1 and fragments and/or variants thereof genetically fused with human serum albumin are provided in the following PCT applications: WO

2003/060071, WO 2003/59934, WO 2005/003296, WO 2005/077042 (herein incorporated by reference in their entirety).

Polypeptides having GLP-1 activity may comprise at least one fragment and/or variant of human GLP-1. The two naturally occurring fragments of human GLP-1 are represented in SEQ ID NO: 2.

5 7 8 9 10 11 12 13 14 15 16 17
 His-Ala-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-

 18 19 20 21 22 23 24 25 26 27 28
 Ser-Tyr-Leu-Glu-Gly-Gln-Ala-Ala-Lys-Glu-Phe-
 10 29 30 31 32 33 34 35 36 37
 Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Xaa (SEQ ID NO.: 2)

15 wherein: Xaa at position 37 is Gly (hereinafter designated as "GLP-1(7-37)"), or -NH₂ (hereinafter designated as "GLP-1(7-36)"). GLP-1 fragments may include, but are not limited to, molecules of GLP-1 comprising, or alternatively consisting of, amino acids 7 to 36 of human GLP-1 (GLP-1(7-36)). Variants of GLP-1 or fragments thereof may include, but are not limited to, one, two, three, four, five or more amino acid substitutions in wild type GLP-1 or in the naturally occurring fragments of GLP-1 shown in SEQ ID NO.: 2. Variants GLP-1 or fragments of GLP-1 may include,
 20 but are not limited to, substitutions of an alanine residue analogous to alanine 8 of wild type GLP-1, such alanine being mutated to a glycine (hereinafter designated as "A8G") (See for example, the mutants disclosed in U.S. Pat. No. 5,545,618, herein incorporated by reference in its entirety).

25 In some aspects, at least one fragment and variant of GLP-1 comprises GLP-1(7-36(A8G)) and is genetically fused to human serum albumin. In a further embodiment, polypeptides of the invention comprise one, two, three, four, five, or more tandemly oriented molecules of GLP-1 and/or fragments and/or variants thereof fused to the N- or C-terminus of human serum albumin or variant thereof. Other embodiments have such A8G polypeptides fused to the N- or C-terminus of albumin or variant thereof. An example of two tandemly oriented GLP-1(7-36)(A8G) fragments and/or variants fused to the N-terminus of human serum albumin comprises SEQ ID
 30 NO:1, which is presented in Figure 3. In another aspect, at least one fragment and variant of GLP-1 comprises at least two GLP-1(7-36(A8G)) tandemly and genetically fused to the human serum albumin. In one aspect, at least two GLP-1(7-36(A8G)) are genetically fused at the N-terminus of the human serum albumin. At least one polypeptide having GLP-1 activity can comprise SEQ ID No.: 1.

35 Variants of GLP-1(7-37) may be denoted for example as Glu²²-GLP-1(7-37)OH which designates a GLP-1 variant in which the glycine normally found at position 22 of GLP-1(7-37)OH has been replaced with glutamic acid; Val⁸-Glu²²-GLP-1(7-37)OH designates a GLP-1 compound in which alanine normally found at position 8 and glycine normally found at position 22 of GLP-1(7-37)OH have been replaced with valine and glutamic acid, respectively. Examples of variants of GLP-1
 40 include, but are not limited to,

Val ⁸ -GLP-1(7-37)OH	Gly ⁸ -GLP-1(7-37)OH	Glu ²² -GLP-1(7-37)O- H
Asp ²² -GLP-1(7-37)OH	Arg ²² -GLP-1(7-37)OH	Lys ²² -GLP-1(7-37)OH
Cys ²² -GLP-1(7-37)OH	Val ⁸ -Glu ²² -GLP-1(7-37)OH	Val ⁸ -Asp ²² -GLP-1(7-37)OH
Val ⁸ -Arg ²² -GLP-1(7-37)OH	Val ⁸ -Lys ²² -GLP-1(7-37)OH	Val ⁸ -Cys ²² -GLP-1(7-37)OH
Gly ⁸ -Glu ²² -GLP-1(7-37)OH	Gly ⁸ -Asp ²² -GLP-1(7-37)OH	Gly ⁸ -Arg ²² -GLP-1(7-37)OH
Gly ⁸ -Lys ²² -GLP-1(7-37)OH	Gly ⁸ -Cys ²² -GLP-1(7-37)OH	Glu ²² -GLP-1(7-36)OH
Asp ²² -GLP-1(7-36)OH	Arg ²² -GLP-1(7-36)OH	Lys ²² -GLP-1(7-36)OH
Cys ²² -GLP-1(7-36)OH	Val ⁸ -Glu ²² -GLP-1(7-36)OH	Val ⁸ -Asp ²² -GLP-1(7-36)OH
Val ⁸ -Arg ²² -GLP-1(7-36)OH	Val ⁸ -Lys ²² -GLP-1(7-36)OH	Val ⁸ -Cys ²² -GLP-1(7-36)OH
Gly ⁸ -Glu ²² -GLP-1(7-36)OH	Gly ⁸ -Asp ²² -GLP-1(7-36)OH	Gly ⁸ -Arg ²² -GLP-1(7-36)OH
Gly ⁸ -Lys ²² -GLP-1(7-36)OH	Gly ⁸ -Cys ²² -GLP-1(7-36)OH	Lys ²³ -GLP-1(7-37)OH
Val ⁸ -Lys ²³ -GLP-1(7-37)OH	Gly ⁸ -Lys ²³ -GLP-1(7-37)OH	His ²⁴ -GLP-1(7-37)OH
Val ⁸ -His ²⁴ -GLP-1(7-37)OH	Gly ⁸ -His ²⁴ -GLP-1(7-37)OH	Lys ²⁴ -GLP-1(7-37)OH
Val ⁸ -Lys ²⁴ -GLP-1(7-37)OH	Gly ⁸ -Lys ²³ -GLP-1(7-37)OH	Glu ³⁰ -GLP-1(7-37)OH
Val ⁸ -Glu ³⁰ -GLP-1(7-37)OH	Gly ⁸ -Glu ³⁰ -GLP-1(7-37)OH	Asp ³⁰ -GLP-1(7-37)OH
Val ⁸ -Asp ³⁰ -GLP-1(7-37)OH	Gly ⁸ -Asp ³⁰ -GLP-1(7-37)OH	Gln ³⁰ -GLP-1(7-37)OH
Val ⁸ -Gln ³⁰ -GLP-1(7-37)OH	Gly ⁸ -Gln ³⁰ -GLP-1(7-37)OH	Tyr ³⁰ -GLP-1(7-37)OH
Val ⁸ -Tyr ³⁰ -GLP-1(7-37)OH	Gly ⁸ -Tyr ³⁰ -GLP-1(7-37)OH	Ser ³⁰ -GLP-1(7-37)OH
Val ⁸ -Ser ³⁰ -GLP-1(7-37)OH	Gly ⁸ -Ser ³⁰ -GLP-1(7-37)OH	His ³⁰ -GLP-1(7-37)OH
Val ⁸ -His ³⁰ -GLP-1(7-37)OH	Gly ⁸ -His ³⁰ -GLP-1(7-37)OH	Glu ³⁴ -GLP-1(7-37)OH
Val ⁸ -Glu ³⁴ -GLP-1(7-37)OH	Gly ⁸ -Glu ³⁴ -GLP-1(7-37)OH	Ala ³⁴ -GLP-1(7-37)OH
Val ⁸ -Ala ³⁴ -GLP-1(7-37)OH	Gly ⁸ -Ala ³⁴ -GLP-1(7-37)OH	Gly ³⁴ -GLP-1(7-37)OH
Val ⁸ -Gly ³⁴ -GLP-1(7-37)OH	Gly ⁸ -Gly ³⁴ -GLP-1(7-37)OH	Ala ³⁵ -GLP-1(7-37)OH
Val ⁸ -Ala ³⁵ -GLP-1(7-37)OH	Gly ⁸ -Ala ³⁵ -GLP-1(7-37)OH	Lys ³⁵ -GLP-1(7-37)OH
Val ⁸ -Lys ³⁵ -GLP-1(7-37)OH	Gly ⁸ -Lys ³⁵ -GLP-1(7-37)OH	His ³⁵ -GLP-1(7-37)OH
Val ⁸ -His ³⁵ -GLP-1(7-37)OH	Gly ⁸ -His ³⁵ -GLP-1(7-37)OH	Pro ³⁵ -GLP-1(7-37)OH
Val ⁸ -Pro ³⁵ -GLP-1(7-37)OH	Gly ⁸ -Pro ³⁵ -GLP-1(7-37)OH	Glu ³⁵ -GLP-1(7-37)OH
Gly ⁸ -Glu ³⁵ -GLP-1(7-37)OH	Val ⁸ -Ala ²⁷ -GLP-1(7-37)OH	Val ⁸ -His ³⁷ -GLP-1(7-37)OH
Val ⁸ -Glu ²² -Lys ²³ -GLP-1(7-37)OH	Val ⁸ -Glu ²² -Glu ²³ -GLP-1(7-37)OH	Val ⁸ -Glu ²² -Ala ²⁷ -GLP-1(7-37)OH
Val ⁸ -Gly ³⁴ -Lys ³⁵ -GLP-1(7-37)OH	Val ⁸ -His ³⁷ -GLP-1(7-37)OH	Gly ⁸ -His ³⁷ -GLP-1(7-37)OH
Val ⁸ -Glu ²² -Ala ²⁷ -GLP-1(7-37)OH	Gly ⁸ -Glu ²² -Ala ²⁷ -GLP-1(7-37)OH	Val ⁸ -Lys ²² -Glu ²³ -GLP-1(7-37)OH
Gly ⁸ -Lys ²² -Glu ²³ -GLP-1(7-37)OH.	Val ⁸ -Glu ³⁵ -GLP-1(7-37)OH	

Variants of GLP-1 may also include, but are not limited to, GLP-1 or GLP-1 fragments having chemical modification of one or more of its amino acid side groups. A chemical modification includes, but is not limited to, adding chemical moieties, creating new bonds, and removing chemical moieties. Modifications at amino acid side groups include, without limitation, acylation

of lysine- ϵ -amino groups, N-alkylation of arginine, histidine, or lysine, alkylation of glutamic or aspartic carboxylic acid groups, and deamidation of glutamine or asparagine. Modifications of the terminal amino group include, without limitation, the des-amino, N-lower alkyl, N-di-lower alkyl, and N-acyl modifications. Modifications of the terminal carboxy group include, without
5 limitation, the amide, lower alkyl amide, dialkyl amide, and lower alkyl ester modifications. Furthermore, one or more side groups, or terminal groups, may be protected by protective groups known to the ordinarily-skilled protein chemist.

GLP-1 fragments or variants may also include polypeptides in which one or more amino acids have been added to the N-terminus and/or C-terminus of GLP-1(7-37)OH of the fragment or
10 variant. The amino acids in GLP-1 in which amino acids have been added to the N-terminus or C-terminus are denoted by the same number as the corresponding amino acid in GLP-1(7-37)OH. For example, the N-terminus amino acid of a GLP-1 compound obtained by adding two amino acids to the N-terminus of GLP-1(7-37)OH is at position 5; and the C-terminus amino acid of a
15 GLP-1 compound obtained by adding one amino acid to the C-terminus of GLP-1(7-37)OH is at position 38. Thus, position 12 is occupied by phenylalanine and position 22 is occupied by glycine in both of these GLP-1 compounds, as in GLP-1(7-37)OH. Amino acids 1-6 of a GLP-1 with amino acids added to the N-terminus may be the same as or a conservative substitution of the amino acid at the corresponding position of GLP-1(1-37)OH. Amino acids 38-45 of a GLP-1 with amino
20 acids added to the C-terminus may be the same as or a conservative substitution of the amino acid at the corresponding position of glucagon or exendin-4.

Albiglutide is a novel analogue of GLP-1 synthesized through genetic fusion of a DPP-IV resistant form of the peptide as a dimer to human albumin, which provides a long-lasting GLP-1 activity with a half- life of about 5 to 7 days. The primary amino acid sequence of albiglutide is SEQ ID NO.:1.

25 In another aspect of the present invention, the composition comprising at least one polypeptide having GLP-1 activity is administered to a human from once daily to once every month and can be administered once daily, once every two days, once every three days, once every seven days, once every fourteen days, once every four weeks and/or once every month. In another aspect, a
30 first dose and a second dose of a composition comprising at least one polypeptide having GLP-1 activity is administered to a human. The first and the second dose can be the same or can be different. Each dose of least one polypeptide having GLP-1 activity can comprise about 0.25 μ g to about 1000 mg of the at least one polypeptide having GLP-1 activity. Doses can include, but are
35 not limited to, 0.25 μ g, 0.25 mg, 1 mg, 3 mg, 6 mg, 16 mg, 24 mg, 48 mg, 60 mg, 80 mg, 104 mg, 20 mg, 400 mg, 800, mg up to about 1000 mg of the at least one polypeptide having GLP-1 activity.

In one embodiment, compositions of the present invention comprise about 15 mg, 30 mg, 50 mg or 100 mg of SEQ ID NO:1.

In another embodiment the polypeptide is an antigen binding polypeptide. In one embodiment the antigen binding polypeptide is selected from the group consisting of a soluble receptor,

antibody, antibody fragment, immunoglobulin single variable domain, Fab, F(ab')₂, Fv, disulphide linked Fv, scFv, closed conformation multispecific antibody, disulphide-linked scFv, or diabody.

The term "antigen binding polypeptide" as used herein refers to antibodies, antibody fragments and other protein constructs which are capable of binding to an antigen.

- 5 In one embodiment the antigen binding polypeptide is an anti-NOGO mAb. In one embodiment the antigen binding polypeptide comprises heavy chain of SEQ ID NO: 3 and the light chain of SEQ ID NO: 4.

- 10 In one embodiment the antigen binding polypeptide is an anti-IL5 mAb. In one embodiment the antigen binding polypeptide comprises heavy chain of SEQ ID NO: 7 and the light chain of SEQ ID NO: 8.

- In one embodiment the antigen binding polypeptide is an anti-CD20 mAb. In one embodiment the antigen binding polypeptide comprises heavy chain variable region of SEQ ID NO: 9 and the light chain variable region of SEQ ID NO: 10. In one embodiment the antigen binding polypeptide is an immunoglobulin single variable domain. In one embodiment the immunoglobulin single variable domain is an anti-TNFR1 dAb. In one embodiment the immunoglobulin single variable domain comprises SEQ ID NO: 5.

The terms Fv, Fc, Fd, Fab, or F(ab)₂ are used with their standard meanings (see, e.g., Harlow et al., *Antibodies A Laboratory Manual*, Cold Spring Harbor Laboratory, (1988)).

- 20 A "chimeric antibody" refers to a type of engineered antibody which contains a naturally-occurring variable region (light chain and heavy chains) derived from a donor antibody in association with light and heavy chain constant regions derived from an acceptor antibody.

- A "humanized antibody" refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity (see, e.g., Queen et al., *Proc. Natl. Acad Sci USA*, 86:10029-10032 (1989), Hodgson et al., *Bio/Technology*, 9:421 (1991)). A suitable human acceptor antibody may be one selected from a conventional database, e.g., the KABAT.RTM. database, Los Alamos database, and Swiss Protein database, by homology to the nucleotide and amino acid sequences of the donor antibody. A human antibody characterized by a homology to the framework regions of the donor antibody (on an amino acid basis) may be suitable to provide a heavy chain constant region and/or a heavy chain variable framework region for insertion of the donor CDRs. A suitable acceptor antibody capable of donating light chain constant or variable framework regions may be selected in a similar manner. It should be noted that the acceptor antibody heavy and light chains are not required to originate from the same acceptor antibody.
- 35 The prior art describes several ways of producing such humanized antibodies--see for example EP-A-0239400 and EP-A-054951.

The term "donor antibody" refers to an antibody (monoclonal, and/or recombinant) which contributes the amino acid sequences of its variable regions, CDRs, or other functional fragments

or analogs thereof to a first immunoglobulin partner, so as to provide the altered immunoglobulin coding region and resulting expressed altered antibody with the antigenic specificity and neutralizing activity characteristic of the donor antibody.

The term "acceptor antibody" refers to an antibody (monoclonal and/or recombinant) heterologous to the donor antibody, which contributes all (or any portion, but in some embodiments all) of the amino acid sequences encoding its heavy and/or light chain framework regions and/or its heavy and/or light chain constant regions to the first immunoglobulin partner. In certain embodiments a human antibody is the acceptor antibody.

"CDRs" are defined as the complementarity determining region amino acid sequences of an antibody which are the hypervariable regions of immunoglobulin heavy and light chains. See, e.g., Kabat et al., Sequences of Proteins of Immunological Interest, 4th Ed., U.S. Department of Health and Human Services, National Institutes of Health (1987). There are three heavy chain and three light chain CDRs (or CDR regions) in the variable portion of an immunoglobulin. Thus, "CDRs" as used herein refers to all three heavy chain CDRs, or all three light chain CDRs (or both all heavy and all light chain CDRs, if appropriate). The structure and protein folding of the antibody may mean that other residues are considered part of the antigen binding region and would be understood to be so by a skilled person. See for example Chothia et al., (1989) Conformations of immunoglobulin hypervariable regions; Nature 342, p 877-883.

As used herein the term "domain" refers to a folded protein structure which has tertiary structure independent of the rest of the protein. Generally, domains are responsible for discrete functional properties of proteins and in many cases may be added, removed or transferred to other proteins without loss of function of the remainder of the protein and/or of the domain. An "antibody single variable domain" is a folded polypeptide domain comprising sequences characteristic of antibody variable domains. It therefore includes complete antibody variable domains and modified variable domains, for example, in which one or more loops have been replaced by sequences which are not characteristic of antibody variable domains, or antibody variable domains which have been truncated or comprise N- or C-terminal extensions, as well as folded fragments of variable domains which retain at least the binding activity and specificity of the full-length domain.

The phrase "immunoglobulin single variable domain" refers to an antibody variable domain (V_H , V_{HH} , V) that specifically binds an antigen or epitope independently of a different V region or domain. An immunoglobulin single variable domain can be present in a format (e.g., homo- or hetero-multimer) with other, different variable regions or variable domains where the other regions or domains are not required for antigen binding by the single immunoglobulin variable domain (i.e., where the immunoglobulin single variable domain binds antigen independently of the additional variable domains). A "domain antibody" or "dAb" is the same as an "immunoglobulin single variable domain" which is capable of binding to an antigen as the term is used herein. An immunoglobulin single variable domain may be a human antibody variable domain, but also includes single antibody variable domains from other species such as rodent (for example, as disclosed in WO 00/29004), nurse shark and Camelid V_{HH} dAbs (nanobodies). Camelid V_{HH} are immunoglobulin single variable domain polypeptides that are derived from species

including camel, llama, alpaca, dromedary, and guanaco, which produce heavy chain antibodies naturally devoid of light chains. Such V_{HH} domains may be humanized according to standard techniques available in the art, and such domains are still considered to be "domain antibodies" according to the invention. As used herein " V_H " includes camelid V_{HH} domains. NARV are another
5 type of immunoglobulin single variable domain which were identified in cartilaginous fish including the nurse shark. These domains are also known as Novel Antigen Receptor variable region (commonly abbreviated to V(NAR) or NARV). For further details see Mol. Immunol. 44, 656-665 (2006) and US20050043519A.

10 The term "Epitope-binding domain" refers to a domain that specifically binds an antigen or epitope independently of a different V region or domain, this may be a domain antibody (dAb), for example a human, camelid or shark immunoglobulin single variable domain.

As used herein, the term "antigen-binding site" refers to a site on a protein which is capable of specifically binding to antigen, this may be a single domain, for example an epitope-binding domain, or it may be paired V_H/V_L domains as can be found on a standard antibody. In some
15 aspects of the invention single-chain Fv (ScFv) domains can provide antigen-binding sites.

The terms "mAbdAb" and dAbmAb" are used herein to refer to antigen-binding proteins of the present invention. The two terms can be used interchangeably, and are intended to have the same meaning as used herein.

20 Examples

The present invention may be further understood by reference to the following non-limiting examples.

25 Example 1—Lyophilization of albiglutide in the presence of t-butanol, ethanol, acetonitrile, NH_4 Acetate or NH_4HCO_3

Samples of albiglutide were prepared for lyophilization according to the analytical preparation matrix described in Table I. The lyophiles were prepared by lyophilizing 0.750mL of solution containing 50mg of albiglutide in 10mM sodium phosphate, pH 7.2, with 117mM trehalose, 153mM mannitol, 0.01% (w/v) polysorbate-80 in vials (2mL 13mm) containing the appropriate
30 concentration of t-butanol, ethanol, and acetonitrile (1, 5, and 10% w/v); and the appropriate concentration of NH_4 Acetate and NH_4HCO_3 (100mM and 250 mM).

Table 1. Albiglutide conditions as a function of sample number.

#	Conditions	Shake/Rest	Undisturbed		
		Recon (min)	Fold-change	Recon (min)	Fold-change
1:	Form. Buffer	7.18 (+/-0.75)	N.A.	18.09 (+/-4.55)	
2:	1% tButanol	3.23 (+/-0.43)	0.45	6.01 (+/-1.56)	0.33
3:	5% tButanol	5.38 (+/-0.44)	0.75	9.18 (+/-4.57)	0.51
4:	10% tButanol	4.06 (+/-0.56)	0.57	4.80 (+/-0.10)	0.27
5:	1% Ethanol	6.17 (+/-0.67)	0.86	14.89 (+/-2.83)	0.82
6:	5% Ethanol	6.85 (+/-0.61)	0.95	13.47 (+/-0.56)	0.74
7:	10% Ethanol	5.78 (+/-0.55)	0.81	11.01 (+/-0.83)	0.61
8:	1% Acetonitrile	5.90 (+/-0.55)	0.82	13.22 (+/-0.60)	0.73
9:	5% Acetonitrile	6.05 (+/-0.53)	0.84	12.60 (+/-1.64)	0.70
10:	10% Acetonitrile	5.82 (+/-0.51)	0.81	10.27 (+/-0.11)	0.57
11:	100 mM NH4Acetate	7.81 (+/-0.51)	1.09	10.73 (+/-0.59)	0.59
12:	250 mM NH4Acetate	6.65 (+/-0.90)	0.93	10.98 (+/-3.22)	0.61
13:	100mM NH4HCO3	6.55 (+/-0.56)	0.91	10.63 (+/-2.26)	0.59
14:	250mM NH4HCO3	6.72 (+/-0.20)	0.94	9.95 (+/-1.85)	0.55
		(n = 5)		(n = 3)	

The samples were lyophilized using a LyoStar freeze dryer under the following cycling conditions:

74hr Cycle			
Stage	Time (min)	Temp	Pressure
Load	0	5°C	Atmospheric
	60	5°C	Atmospheric
Ramp to Freeze	180	-55°C	Atmospheric
Freeze	300	-55°C	Atmospheric
Ramp to Anneal	360	-15°C	Atmospheric
Anneal	660	-15°C	Atmospheric
Ramp to 1° Dry	780	-55°C	Atmospheric
Refreeze	900	-55°C	Atmospheric
Pull Vacuum	930	-55°C	100um Hg
1° Dry	1050	-55°C	100um Hg
Ramp to 1° Dry	1170	-25°C	100um Hg
1° Dry	3570	-25°C	100um Hg
Ramp to 2° Dry	3930	40°C	100um Hg
2° Dry	4350	40°C	100um Hg
2hr Ramp to RT	4470	25°C	100um Hg
Hold at RT	4650	25°C	100um Hg
74.5			

Example 2—Reconstitution of lyophilized albiglutide

- 5 The lyophiles were reconstituted with 0.675mL water for injection (WFI) via a 1mL syringe and 25G(5/8") needle through the lyophilized vial stopper (tuberculin syringes, Becton-Dickinson# 309626). The volume of 0.675mL corrects for the volume of the dry components resulting in a final volume of 0.75mL. The reconstitution was performed using either a "shaking method" or an "undisturbed method". For the shaking method, the sample was shaken for 10 seconds, followed by 15 seconds of rest. For the undisturbed method, the sample was rocked back and forth for 5 seconds and then left undisturbed. The samples were considered to have been reconstituted when no particles were visible in the vials. Results for both reconstitution methods are shown in Table 1 and also in Figures 1 and 2.

15 Example 3—Lyophilization of albiglutide in the presence of t-butanol

- Samples of albiglutide were prepared for lyophilization according to the analytical preparation matrix described in Table I. The lyophiles were prepared by lyophilizing 0.750mL of solution containing 50mg of albiglutide in 10mM sodium phosphate, pH 7.2, with 117mM trehalose, 153mM mannitol, 0.01% (w/v) polysorbate-80 in vials (2mL 13mm) containing the appropriate concentration of t-butanol (between 34 and 202 mM, or 0.25 to 2% w/v).

Table 2. Albiglutide conditions as a function of sample number.

Sample #	6 Conditions:	Vials/Cond.	Vol. of 100mg/mL BDS (mL)
1	albiglutide control	52	45.0 (0.75mL/vial)
2	2% t-Butanol	42	37.5(0.76mL/vial)
3	1.5% t-Butanol	42	37.5(0.76mL/vial)
4	1% t-Butanol	52	45.0(0.76mL/vial)
5	0.5% t-Butanol	42	37.5(0.75mL/vial)
6	0.25% t-Butanol	42	37.5(0.75mL/vial)

The samples were lyophilized using a LyoStar freeze dryer under the following cycling conditions:

74hr Cycle			
Stage	Time (min)	Temp	Pressure
Load	0	5°C	Atmospheric
	60	5°C	Atmospheric
Ramp to Freeze	180	-55°C	Atmospheric
Freeze	300	-55°C	Atmospheric
Ramp to Anneal	360	-15°C	Atmospheric
Anneal	660	-15°C	Atmospheric
Ramp to 1° Dry	780	-55°C	Atmospheric
Refreeze	900	-55°C	Atmospheric
Pull Vacuum	930	-55°C	100um Hg
1° Dry	1050	-55°C	100um Hg
Ramp to 1° Dry	1170	-25°C	100um Hg
1° Dry	3570	-25°C	100um Hg
Ramp to 2° Dry	3930	40°C	100um Hg
2° Dry	4350	40°C	100um Hg
2hr Ramp to RT	4470	25°C	100um Hg
Hold at RT	4650	25°C	100um Hg
74.5			

Example 4—Reconstitution of lyophilized albiglutide

- 5 The lyophiles were reconstituted with 0.675mL water for injection (WFI) via a 1mL syringe and 25G(5/8") needle through the lyophilized vial stopper (tuberculin syringes, Becton-Dickinson# 309626). The volume of 0.675mL corrects for the volume of the dry components resulting in a final volume of 0.75mL. The reconstitution was performed using an "undisturbed method" (addition of the water was followed by immediate light mixing followed by allowing the vial to stand undisturbed). Addition of the water was performed with four variations based on analyst (three different people performing the reconstitution step) and pipetting method:
- 10

Analyst 1: reconstitution via syringe through the stopper.

Analyst 2: reconstitution via syringe through the stopper.

Analyst 3: reconstitution via syringe through the stopper.

Analyst 1: reconstitution via traditional P1000 Rainin pipetting method..

Individual reconstitution times are summarized in Table 4, and graphically in Figure 3. There is a clear effect of t-butanol added prior to lyophilization on the resulting reconstitution time endpoint values. The control samples were lyophilized in the presence of formulation buffer only (no volatile additive) and gave an average of $t = 17.87 (\pm 2.94)$ min ($n = 26$); the high and low values were $t = 12.25$ min and $t = 26.53$ min, respectively. The 0.25% t-Butanol samples were virtually identical to control samples, with a reconstitution endpoint average time of $t = 19.35 (\pm 4.08)$ min ($n = 20$); the high and low values were $t = 12.42$ min and $t = 28.90$ min, respectively.

At concentrations of t-butanol at 0.5% or above, there is a clear acceleration of reconstitution rates with endpoint values decreasing with increasing t-butanol concentrations. At 0.5% t-butanol, the average reconstitution time endpoint over all results was approximately 65% of the control group, with $t = 11.84 (\pm 2.94)$ min ($n = 19$); the high and low values were $t = 0.58$ min and $t = 17.93$, respectively. Note that one sample in the 0.5% group gave > 37 min. In this case the lyophile stuck to the side of the vial above the solvent meniscus and failed to go into solution, thus the data point was excluded from averaging.

Comparing 1% t-butanol, 1.5% t-butanol, and 2% t-butanol groups, there is a marked acceleration in reconstitution times with endpoints of $t = 7.71 (\pm 2.44)$ min ($n = 30$) at 1% tButanol, $t = 7.16 (\pm 2.65)$ min ($n = 20$) at 1.5% tButanol, and $t = 4.74 (\pm 1.75)$ min ($n = 20$) at 2% tButanol. High and Low reconstitution endpoint values were $t = 1.37$ min and $t = 11.67$ min at 1% tButanol, $t = 3.90$ min and $t = 15.50$ min at 1.5% tButanol, and $t = 2.75$ min and $t = 9.25$ min at 2% tButanol. These range values would suggest that reconstitution times for albiglutide lyophilized in the presence of 2% tButanol in formulation buffer could be 3-fold faster, on the order of 10 min, than is currently recommended for albiglutide in formulation buffer, 30 min. In summary, the 2% tButanol samples are approximately 1.5 fold faster than the 1% tButanol samples, and is ~3.75-fold faster than the control sample group.

Table 4. Average reconstitution time endpoints for albiglutide lyophiles, grouped according to sample type.

Grouping	Ctrl	2% tBu	1.5% tBu	1% tBu	0.5% tBu	0.25% tBu
All tests - Average	17.87	4.74	7.16	7.71	11.84	19.35
(std dev)	2.94	1.75	2.65	2.44	5.18	4.08
(n)	26	20	20	30	19	20
Analyst 1 (syringe) - Average	17.06	4.61	6.51	6.97	13.53	17.37
(std dev)	1.42	1.57	1.27	1.45	0.71	4.34
(n)	6	3	3	6	3	3
Analyst 2 (syringe) - Average	17.95	4.90	8.63	7.16	9.23	21.24
(std dev)	3.27	2.62	3.81	3.99	7.76	4.49
(n)	7	6	6	9	5	6
Analyst 3 (syringe) - Average	18.01	4.60	6.39	8.00	11.48	18.80
(std dev)	4.55	0.87	1.64	1.26	5.05	3.99
(n)	7	6	6	9	6	6
Analyst 1 (pipette) - Average	18.40	4.78	6.71	8.84	13.86	18.94
(std dev)	1.54	1.93	2.54	1.24	3.56	3.93
(n)	6	5	5	6	5	5

Results of lyophile recon times were also separated as a function of analyst, and comparing the method involving injection of WFI through the stopper (syringe) versus the traditional method of uncapping the vial and dispensing via Rainin pipette, summarized in Table 4 and graphically in Figure 4. The distribution of reconstitution time endpoints within each sample group represents the dominant source of variability, with individual analyst and/or reconstitution methods having minimal influence on the average values or standard deviations for each sample type. Exceptions are for 0.5% tButanol (sample 5) and 0.25% tButanol (sample 6) which showed a wider variability of average reconstitution times and standard deviations as compared to the other samples.

Example 5—Reconstitution of lyophilized mAbs, dAbs, and IL18

Anti-NOGO mAb (SEQ ID NOS: 3 and 4), anti-TNFR1 dAb (SEQ ID NO: 5), IL18 (SEQ ID NO: 6), anti-IL5 (SEQ ID NOS: 7 and 8), and anti-CD20 (variable domains SEQ ID NOS: 9 and 10) were concentrated to the concentrations shown below in a formulation comprising 26 mM histidine, 150 mM trehalose, 0.02% polysorbate 80 (PS80), pH 6.0, in a volume of about 0.75 mL, and then lyophilized in a 2 mL vial.

79.3 mg anti-IL5 mAb
 77.9 mg anti-NOGO mAb
 78.2 mg anti-CD20 mAb
 29.1 mg anti-TNFR1 dAb

19.0 mg IL-18

The lyophiles were reconstituted with 0.675mL water for injection (WFI) via a 1mL syringe and 25G(5/8") needle through the lyophilized vial stopper (tuberculin syringes, Becton-Dickinson# 309626). The volume of 0.675mL corrects for the volume of the dry components resulting in a final volume of 0.75mL. The reconstitution was performed using an "undisturbed method" (addition of the water was followed by immediate light mixing followed by allowing the vial to stand undisturbed). Addition of the water was performed by three different analysts (three different people performing the reconstitution step) via syringe through stopper. Individual reconstitution times are summarized in Table 5, and graphically in Figures 6 and 7.

Across all 5 proteins tested, those with 2% t-butanol spiked in during lyophilization had faster reconstitution times than the control groups. This is evident not only for the mAbs tested, but also can be seen for the small molecules tested; anti-TNFR1 dAb and IL-18, which also appeared to have faster reconstitution times in the presence of t-butanol.

Table 5.

Note record time minutes M (Seconds/60)

	replicate	Anti-IL5 mAb	Anti-IL5 mAb w/ 2%	Anti-TNFR1 dAb	Anti-TNFR1 dAb w/ 2%	IL18	IL18 w/ 2%	Anti-NOGO mAb	Anti-NOGO mAb w/ 2%	Anti-CD20 mAb	Anti-CD20 mAb w/ 2%
		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
Analyst 1	1	26.73	2.10	1.40	0.38	0.20	0.05	34.45	6.35	46.30	7.50
	2	20.52	5.07	0.78	0.22	0.20	0.13	39.63	3.37	55.70	7.32
	3	24.22	7.08	0.68	0.43	0.15	0.10	28.55	4.78	54.62	6.35
	4	30.15	4.83	1.02	0.20	0.17	0.08	29.87	2.92	46.48	6.23
Analyst 2	1	23.08	4.72	0.35	0.43	0.35	0.27	58.30	4.95	35.83	6.08
	2	34.25	6.27	0.30	0.42	0.37	0.27	53.60	3.93	32.48	7.67
	3	30.02	5.78	0.40	0.37	0.28	0.30	63.55	3.68	39.73	10.48
	4	34.92	8.20	0.40	0.40	0.33	0.30	46.77	3.13	25.58	8.83
Analyst 3	1	40.47	5.97	0.78	0.30	0.27	0.18	58.52	4.48	49.28	4.43
	2	34.20	6.83	0.60	0.37	0.23	0.13	41.65	2.53	29.77	5.20
	3	31.52	8.50	0.62	0.35	0.23	0.15	46.35	3.30	33.32	4.00
	4	41.12	7.57	0.67	0.48	0.25	0.13	61.25	3.52	48.02	4.42
Total samples		12	12	12	12	12	12	12	12	12	12
Average		30.93	6.13	0.67	0.35	0.25	0.17	46.87	3.91	41.43	6.54
STDEV		6.51	1.79	0.31	0.08	0.07	0.09	12.27	1.06	10.00	1.93

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. Such equivalents are intended to be encompassed by the following claims. The entire contents of all references, patents, and published patent applications cited throughout this application are hereby incorporated by reference.

We claim:

1. A method for producing a polypeptide composition comprising: combining a polypeptide with a volatile additive to form a liquid mixture and lyophilizing the liquid mixture to obtain a
 5 lyophilized polypeptide composition, wherein the liquid mixture comprises about 2% by volume of volatile additive, and the volatile additive is t-butanol.
2. The method of claim 1 wherein the polypeptide is an antigen binding polypeptide.
3. The method of claim 1 wherein the polypeptide comprises at least two GLP-1 polypeptides genetically fused to the N-terminus of human serum albumin.
- 10 4. The method of claim 1 wherein the antigen binding polypeptide is selected from the group consisting of soluble receptor, antibody, antibody fragment, immunoglobulin single variable domain, Fab, F(ab')₂, Fv, disulphide linked Fv, scFv, closed conformation multispecific antibody, disulphide-linked scFv, and diabody.
5. A dry polypeptide composition produced by the method of claim 1.
- 15 6. The polypeptide composition of claim 5 that comprises at least two GLP-1 polypeptides genetically fused to the N-terminus of human serum albumin.
7. A method for producing a liquid polypeptide composition comprising: obtaining a lyophilized polypeptide produced by the method of claim 1, and reconstituting the lyophilized polypeptide with a sufficient amount of a pharmaceutically acceptable dispersing agent to yield a liquid
 20 polypeptide composition.
8. The method of claim 7 wherein the polypeptide is an antigen binding polypeptide.
9. The method of claim 7 wherein the polypeptide comprises at least two GLP-1 polypeptides genetically fused to the N-terminus of human serum albumin.
10. The method of claim 8 wherein the antigen binding polypeptide is selected from the group
 25 consisting of soluble receptor, antibody, antibody fragment, immunoglobulin single variable domain, Fab, F(ab')₂, Fv, disulphide linked Fv, scFv, closed conformation multispecific antibody, disulphide-linked scFv, and diabody.
11. A liquid polypeptide composition produced by the method of claim 7.
12. A method for reducing the reconstitution time of a lyophilized polypeptide composition
 30 comprising:
 - a) lyophilizing a liquid mixture comprising the polypeptide, wherein the liquid mixture comprises about 2% by volume of volatile additive, wherein the volatile additive is t-butanol and
 - b) reconstituting the lyophilized polypeptide with a sufficient amount of a pharmaceutically acceptable dispersing agent to the lyophilized polypeptide composition to yield a liquid
 35 polypeptide composition,

wherein time for reconstituting the polypeptide lyophilized in the presence of the volatile additive is less than the time for reconstituting the same polypeptide lyophilized in the absence of the volatile additive.

13. The method of claim 12 wherein the polypeptide is an antigen binding polypeptide.

5 14. The method of claim 12 wherein the polypeptide comprises at least two GLP-1 polypeptides genetically fused to the N-terminus of human serum albumin.

15. The method of claim 12 wherein the antigen binding polypeptide is selected from the group consisting of soluble receptor, antibody, antibody fragment, immunoglobulin single variable domain, Fab, F(ab')₂, Fv, disulphide linked Fv, scFv, closed conformation multispecific antibody,
10 disulphide-linked scFv, and diabody.

16. A composition comprising: a therapeutic polypeptide, a buffer, a surfactant, at least one excipient, and at least one volatile additive.

17. The composition of claim 16 wherein the therapeutic polypeptide comprises GLP-1 or a fragment and/or variant thereof.

15 18. The composition of claim 16 wherein the therapeutic polypeptide comprises at least two GLP-1(7-36(A8G)) polypeptides genetically fused to the N-terminus of human serum albumin.

19. The composition of any one of claims 16 to 18 wherein the therapeutic polypeptide comprises SEQ ID NO:1.

20. The composition of either one of 16 or 19 wherein the buffer is sodium phosphate.

20 21. The composition of any one of claims 16 to 20 wherein the surfactant is polysorbate-80.

22. The composition of any one of claims 16 to 21 wherein the at least one excipient is selected from: trehalose, maltose, sucrose, mannose, lactose, mannitol, sorbitol, glycerol and dextrose.

23. The composition of any one of claims 16 to 22 wherein the excipient comprises: trehalose and mannitol.

25 24. The composition of any one of claims 16 to 23 wherein the volatile additive is an oxyhydrocarbon.

25. The composition of claim 24 wherein the oxyhydrocarbon is selected from: methanol, ethanol, n-propanol, iso-propanol, n-butanol, iso-butanol, 2- butanol, t-butanol, pentanol, iso-pentanol, 2-pentanol, 3-pentanol, t-pentanol, methylethylketone, benzylalcohol, acetic acid,
30 methylethyl ketone, or a combination thereof.

26. The composition of any one of claims 16 to 25 wherein the volatile additive is selected from t-butanol, ethanol, acetonitrile, NH₄Acetate and NH₄HCO₃ or a combination thereof.

27. A composition comprising: a polypeptide comprising SEQ ID NO:1, sodium phosphate, trehalose, mannitol, polysorbate-80 and t-butanol.

28. The composition of any one of claims 16 to 25 wherein the volatile additive is t-butanol and the t-butanol is present at about 2% by volume.