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(54) Title: COMPOSITIONS OF PEPTIDE CRYSTALS

(57) Abstract: The present invention provides a pharmaceutical composition comprising crystals of a select peptide, glycine, an alcohol, zinc, a buffer and a pharmaceutically acceptable preservative at a pH of about 6.0 to about 8.5. The invention also provides for the use of the pharmaceutical composition for treating various diseases and conditions in a mammal. The invention further provides a process for preparing crystals of the peptide and a process for preparing pharmaceutical compositions thereof.

COMPOSITIONS OF PEPTIDE CRYSTALS

This application claims the benefit of U.S. Provisional Application Number 60/255,251, filed December 13, 2000.

This invention is in the field of human medicine. In particular, this invention relates to pharmaceutical compositions useful for treating diseases such as diabetes.

GLP-1, or glucagon-like peptide-1, is a 37 amino acid peptide that, in a glucose-dependent manner, stimulates insulin secretion from pancreatic β-cells, leading to glucose uptake by cells and thereby decreasing serum glucose levels. These and other effects make GLP-1 an attractive candidate for the treatment of Type 2 diabetes. For an extensive review of GLP-1 and related peptides, see Kieffer, T. J. *et al.*, Endocrine Reviews 20:876-913 (1999).

Analogs of GLP-1 have also been developed. One such analog, Val^8-GLP-1(7-37)OH, is disclosed in Galloway, *et al.*, U.S. Patent No. 5,705,483, issued 6 January 1998.

Techniques for crystallizing GLP-1 related peptides have been developed as a means of providing a sustained pharmacokinetic profile after subcutaneous administration. For example, Danley, D. E., *et al.* [EPO publication number 619 322 A2, 12 October 1994] disclosed methods of preparing compositions comprising amorphous, micro-crystalline or crystalline native GLP-1 peptides and their subcutaneous administration in rats. Hoffmann, J. A. *et al.* [WIPO publication WO 99/30731, 24 June 1999] disclosed processes for preparing individual plate-like crystals of GLP-1 related peptides, including Val^8-GLP-1(7-37)OH, and compositions thereof which exhibited an extended time action upon subcutaneous injection in dogs.

However, in order for compositions of crystals of a GLP-1 related peptide to be useful for the treatment of Type 2 diabetes or other diseases and conditions, many challenges must be addressed. Generally, a pharmaceutical product must be manufactured at a large scale. Furthermore, a process for manufacturing the pharmaceutical product must be developed that is reproducible and able to handle significant variation in the process steps leading to formation of the bulk product. See, for example, Shadle, P. J., Development of Recovery Processes for Recombinant Proteins and Peptides, Chapter 3 (pages 31-90) in Development of Biophamaceutical Parenteral Dosage Forms, Bontempo, J. A., ed., Marcel Dekker, Inc., New York (1997).

The production of therapeutic products comprising peptide crystals faces additional hurdles and challenges. For instance, impurities that are not sufficiently removed during purification of a bulk peptide can adversely impact crystal formation [see Caylor, C. L. *et al.*, Proteins: Structure, Function, and Genetics 36:270-281 (1999)].

GLP-1 related peptides themselves have properties that present problems and challenges in product manufacturing and formulation development. Certain GLP-1 peptides, including Val^8-GLP-1(7-37)OH, have exhibited conformation and racemization problems during large-scale processing. Furthermore, an α-helix to β-sheet conformational conversion can occur in GLP-1 related peptides during shear-induced process steps and in compositions comprising a preservative such as phenol. Thus, despite recent advances relating to Val^8-GLP-1(7-37)OH, there remains a need to develop improved pharmaceutical compositions.

The present invention provides novel pharmaceutical compositions comprising crystals of biologically active Val^8-GLP-1(7-37)OH. Such compositions exhibit satisfactory chemical and physical stability.

The present invention provides a pharmaceutical composition comprising crystals of a peptide of Formula I (SEQ ID NO: 1):

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

18 19 20 21 22 23 24 25 26 27 28
Ser-Tyr-Leu-Glu-Xaa-Gln-Ala-Ala-Lys-Xaa-Phe-

29 30 31 32 33 34 35 36 37
Ile-Xaa-Trp-Leu-Val-Lys-Gly-Arg-R

Formula I (SEQ ID NO: 1)

wherein, Xaa at position 8 is Val; Xaa at position 22 is Gly, Xaa at position 27 is Glu; Xaa at position 30 is Ala; and R is Gly;

glycine at a concentration of about 5 mM to about 100 mM;

an alcohol selected from the group consisting of ethanol and isopropanol at a concentration by volume of about 1% to about 10%;

zinc at a concentration of about 0.5 moles to about 2.5 moles per mole of the peptide;

a buffer selected from the group consisting of TRIS, maleate and succinate; and

a pharmaceutically acceptable preservative;

wherein the composition has a pH of about 6.0 to about 8.5.

The composition may also comprise a tonicity agent such as sodium chloride.

The invention also provides the use of the pharmaceutical composition in preparing a medicament for treating diabetes, hyperglycemia or obesity in a mammal.

The invention also provides a method of treating diabetes, hyperglycemia or obesity in a mammal, comprising administering to the mammal the pharmaceutical composition described herein.

The invention also provides a process for preparing crystals of the peptide and a process for preparing a pharmaceutical composition comprising crystals of the peptide.

The three-letter abbreviation code for amino acids used in this specification conforms with the list contained in Table 3 of Annex C, Appendix 2 of the PCT Administrative Instructions and with 37 CFR § 1.822(d)(1)(2000).

Glucagon-like peptide-1 (GLP-1) is a 37 amino acid peptide that is secreted by the L-cells of the intestine in response to food ingestion.

As accustomed in the art, the N-terminal residue of a GLP-1 related peptide is represented as position 7. Thus, the amino acid sequence of the naturally occurring human GLP-1 related peptide designated GLP-1(7-37)OH is:

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-

29 30 31 32 33 34 35 36 37
Ile-Ala-Trp-Leu-Val-Lys-Gly-Arg-Gly

(SEQ ID NO: 3)

Val^8-GLP-1(7-37)OH (SEQ ID NO: 2) is GLP-1(7-37)OH wherein Ala at position 8 has been substituted with Val. Alternatively, Val^8-GLP-1(7-37)OH (SEQ ID NO: 2) may be described as a peptide of Formula I (SEQ ID NO: 1):

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

18 19 20 21 22 23 24 25 26 27 28
Ser-Tyr-Leu-Glu-Xaa-Gln-Ala-Ala-Lys-Xaa-Phe-

29 30 31 32 33 34 35 36 37
Ile-Xaa-Trp-Leu-Val-Lys-Gly-Arg-R

Formula I (SEQ ID NO: 1)

wherein, Xaa at position 8 is Val; Xaa at position 22 is Gly; Xaa at position 27 is Glu; Xaa at position 30 is Ala; and R is Gly.

The word "crystal" in the present specification refers to a solid material comprising a peptide in which the particles making up the solid have a definite form or structure. Particles lacking such form or structure are referred to as "amorphous." Words used in the present specification to describe crystals include, in order of increasing crystal quality; 1) "microcrystals," which are small crystals possessing a definite but essentially non-linear form or structure, 2) "stars" or "clusters," which are distinct crystals fused at or emanating from a central core and which may contain amorphous material in addition to crystalline material, and 3) "rods," "needles" or "plates," which are individual crystals possessing a distinctive form or structure consistent with their name.

The term "thin plate crystals" refers to individual peptide crystals having an apparent orthorhombic structure in which the three axes of the crystals have disparate lengths. By way of illustration, the thin plate crystals generated in the large-scale crystallization of Val^8-GLP-1(7-37)OH described in Example 4 are about 1 μm thick, about 4-6 μm wide and about 20-60 μm long. The thin plate crystals of the present invention generally have a thickness of about 0.5 μm to about 3.0 μm, a width of about 3 μm to

about 10 μm and a length of about 10 μm to about 100 μm. Under the microscope thin plate crystals may appear orthorhombic but the actual angles between the axes may or may not be 90°.

The word "stable" used in the present specification refers to a composition in which both the chemical stability and physical stability of the composition remain at an acceptable level over time. The word "chemical" used in conjunction with stability of a peptide composition refers to covalent modifications or alterations of the peptide. The word "physical" used in conjunction with stability of a peptide composition refers to the molecular conformation, solubility or solid form properties of the peptide. By way of illustration, peptide crystal compositions in which the crystals quickly clump into large aggregates or dissolve into the liquid medium exhibit unsatisfactory physical stability.

The word "pharmaceutical" used in the present specification in reference to a peptide composition means it contains a peptide useful for treating a disease or condition. For example, the peptide Val^8-GLP-1(7-37)OH described in the present invention is useful in treating humans and other mammals who have Type 2 diabetes.

The word "treating" refers to the management and care of a patient for the purpose of combating a disease, condition, or disorder and includes the administration of a pharmaceutical composition comprising crystals to prevent the onset of the symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition or disorder. Treating diabetes therefore includes the maintenance of physiologically desirable blood glucose levels in patients in need thereof.

For the convenience and safety of patients being treated, pharmaceutical compositions of the present

invention also contain a pharmaceutically acceptable preservative. A "pharmaceutically acceptable preservative" refers to a chemical that is compatible with and suitable for pharmaceutical use in humans and that is added to a peptide composition to prevent or inhibit the growth of micro-organisms. The term "phenolic preservative" as used herein refers to a pharmaceutically acceptable preservative containing a phenolic moiety and includes phenol, m-cresol, methylparaben and mixtures thereof. Utilizing a pharmaceutically acceptable preservative in a peptide composition allows a patient to conveniently make multiple withdrawals of the composition from the same container, such as a vial or cartridge, over an extended period of time.

The word "buffer" refers to a chemical compound in a composition that minimizes changes in hydrogen ion concentration that would otherwise occur as a result of a chemical reaction and includes TRIS, maleate, phosphate, succinate, glycylglycine and adipate.

The term "TRIS" refers to 2-amino-2-hydroxymethyl-1,3-propanediol, and any pharmaceutically acceptable salt thereof. The free base and the hydrochloride form are two common forms of TRIS. TRIS is also known in the art as trimethylol aminomethane, tromethamine and tris(hydroxymethyl)aminomethane.

The word "maleate" refers to maleic acid, which has the chemical formula HOOCCH:CHCOOH, and pharmaceutically acceptable salt forms such as sodium maleate and potassium maleate.

The word "succinate" refers to succinic acid, which has the chemical formula $CO_2H(CH_2)_2CO_2H$, and pharmaceutically acceptable salt forms such as sodium succinate and potassium succinate.

The word "adipate" refers to adipic acid, which has the chemical formula $CO_2H(CH_2)_4CO_2H$, and pharmaceutically

acceptable salt forms such as sodium adipate and potassium adipate.

The word "glycylglycine" refers to the dipeptide Gly-Gly, the free base form of Gly-Gly and pharmaceutically acceptable salt forms such as glycylglycine hydrochloride.

The word "ethanol" is synonymous with ethyl alcohol and refers to the chemical CH_3CH_2OH.

The word "isopropanol" is synonymous with isopropyl alcohol and refers to the chemical $(CH_3)_2CHOH$.

The term "tonicity agent" refers to a non-volatile chemical compound that modifies the osmotic pressure of a solution or suspension composition and includes sodium chloride, other salts, glycerin and mannitol.

"TCR" stands for "Temperature Cycling and Resuspension" and refers to the automated test of pharmaceutical compositions involving temperature cycling and physical resuspension described herein as Method 1. The "Modified TCR Test" refers to the test described herein as Method 2.

The symbol "%" is equivalent to the word "percent" and, as used herein in reference to a volume of a specified liquid within a larger liquid composition or added to a liquid composition, means the actual volume of the specified liquid divided by the total volume of the combined composition after the specified liquid is added, multiplied by 100.

As noted, the present invention provides a pharmaceutical composition comprising crystals of a peptide of Formula I (SEQ ID NO: 1):

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

18 19 20 21 22 23 24 25 26 27 28
Ser-Tyr-Leu-Glu-Xaa-Gln-Ala-Ala-Lys-Xaa-Phe-

29 30 31 32 33 34 35 36 37
Ile-Xaa-Trp-Leu-Val-Lys-Gly-Arg-R

Formula I (SEQ ID NO: 1)

wherein:

Xaa at position 8 is Val;

Xaa at position 22 is Gly;

Xaa at position 27 is Glu;

Xaa at position 30 is Ala;

R is Gly;

glycine at a concentration of about 5 mM to about 100 mM;

an alcohol selected from the group consisting of ethanol and isopropanol, at a concentration by volume of about 1% to about 10%;

zinc at a concentration of about 0.5 moles to about 2.5 moles per mole of the peptide;

a buffer selected from the group consisting of TRIS, maleate and succinate;

a pharmaceutically acceptable preservative; and

a pH of about 6.0 to about 8.5.

The invention is based on the observation that compositions of crystals of Val^8-GLP-1(7-37)OH comprising zinc, an alcohol, glycine and a preservative exhibit increased stability in the presence of a buffer selected from the group consisting of TRIS, maleate and succinate.

Thus, the invention provides an improved, stable pharmaceutical composition comprising crystals of a peptide of Formula I (SEQ ID NO: 1):

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

18 19 20 21 22 23 24 25 26 27 28
Ser-Tyr-Leu-Glu-Xaa-Gln-Ala-Ala-Lys-Xaa-Phe-

29 30 31 32 33 34 35 36 37
Ile-Xaa-Trp-Leu-Val-Lys-Gly-Arg-R

Formula I (SEQ ID NO: 1)

wherein, Xaa at position 8 is Val; Xaa at position 22 is Gly, Xaa at position 27 is Glu; Xaa at position 30 is Ala; and R is Gly;

glycine at a concentration of about 5 mM to about 100 mM;

an alcohol selected from the group consisting of ethanol and isopropanol, at a concentration by volume of about 1% to about 10%;

zinc at a concentration of about 0.5 moles to about 2.5 moles per mole of the peptide;

a buffer selected from the group consisting of TRIS, maleate and succinate;

a pharmaceutically acceptable preservative; and

a pH of about 6.0 to about 8.5.

The invention therefore provides improved zinc-alcohol-glycine-preservative formulations of crystals of Val^8-GLP-1(7-37)OH comprising buffer selected from the group consisting of TRIS, maleate and succinate.

The Val^8-GLP-1(7-37)OH peptide of the present invention may be chemically synthesized by solution-phase, solid-phase

or semi-synthetic methods. Alternatively, the peptide may be prepared biosynthetically using recombinant DNA technology in modified bacteria, yeast, mammalian cells or in transgenic plants or animals. See, for example, Galloway, *et al.*, U.S. Patent No. 5,705,483, issued 6 January 1998, herein incorporated by reference.

The preparation of pharmaceutical compositions of crystals of Val^8-GLP-1(7-37)OH and proof of their chemical and physical stability are provided in Examples 4 to 13. Additional pharmaceutically acceptable excipients, such as those described in Remington's Pharmaceutical Sciences (1985) may be included in the compositions of the present invention. Preferably, such additional excipients do not affect the novel and basic characteristics of the invention, so that the chemical and physical stability and the therapeutic benefit of the pharmaceutical compositions are retained.

Thin plate crystals described herein have the appearance of orthorhombic crystals in which the three axes have disparate lengths. Although the lengths of the axes are not to be construed as limited to specific dimensions, the thin plate crystals of the present invention generally have a thickness of about 0.5 μm to about 3.0 μm, a width of about 3 μm to about 10 μm and a length of about 10 μm to about 100 μm. Under the microscope thin plate crystals may appear orthorhombic but the actual angles between the axes may or may not be 90°.

In addition to their surface charge, the shape and dimensions of thin plate crystals are important properties that can provide a slow sedimentation rate in a pharmaceutical composition of the present invention. Preferably, the thickness, width and length of the peptide crystals are about 0.5-1.5 μm, about 3-8 μm, and about 15-80

μm, respectively. More preferably, the thickness, width and length of the crystals are about 0.8-1.2 μm, about 4-6 μm and about 20-60 μm, respectively. Most preferably, the thickness, width and length of the crystals are about 1 μm, about 5 μm and about 30-50 μm, respectively. Analysis of the sedimentation rates and volumes of pharmaceutical compositions comprising these peptide crystals is described in Example 7.

The compositions of the present invention comprise glycine at a concentration of about 5 mM to about 100 mM. Preferably, the glycine concentration is about 10 mM to about 50 mM. More preferably, the glycine concentration is about 20 mM to about 30 mM and more highly preferred is a glycine concentration of about 22 mM to about 24 mM. A glycine concentration of about 23 mM is most preferred.

The compositions of the present invention comprise an alcohol preferably selected from the group consisting of ethanol and isopropanol at a concentration, by total volume of the composition, of about 1% to about 10%. A preferred concentration of the alcohol in the compositions is about 2% to about 6% by volume. More preferred is an alcohol concentration of about 4%. A preferred alcohol is ethanol.

The compositions of the present invention comprise zinc at a concentration of about 0.5 moles to about 2.5 moles per mole of the peptide. The zinc present in the compositions is generally in the form of a zinc ion derived from zinc oxide or from zinc salts such as zinc chloride or zinc acetate. A preferred concentration of zinc is about 1.0 to about 2.25 moles per mole of the peptide in the composition. Other ranges of preferred zinc concentrations in the compositions are about 1.1 moles to about 2.0 moles per mole of the peptide and about 1.3 moles to about 1.7 moles per

mole of the peptide. A more preferred zinc concentration is about 1.5 moles per mole of the peptide.

The compositions of the present invention may comprise a tonicity agent such as sodium chloride. Other tonicity agents, such as glycerin, mannitol and salts other than sodium chloride, may also be incorporated into the compositions in addition to or in place of sodium chloride.

The quantities of sodium chloride (NaCl) noted in this specification refer to the quantities of sodium chloride added to a composition at a designated point in the preparation of the composition. The NaCl quantities noted in the specification do not include sodium chloride that may form from additions of acids and bases such as NaOH and HCl that may be used for pH adjustment at various stages in the preparation of the compositions. Also, it is appreciated that when sodium chloride is added to an aqueous composition a substantial portion will exist as sodium ions and chloride ions. For ease of measurement and understanding, however, the sodium and chloride ion concentrations of the compositions will not be considered, only the quantity of sodium chloride added.

When included in a composition of the present invention, a preferred concentration of sodium chloride in the composition is about 30 mM to about 200 mM. A more preferred quantity of sodium chloride is 50 mM to about 150 mM. Other ranges of preferred sodium chloride concentration are about 80 mM to about 120 mM, about 70 mM to about 130 mM, and about 90 mM to about 130 mM. A most preferred concentration of sodium chloride in the compositions of the present invention is about 110 mM.

The compositions of the present invention comprise a buffer selected from the group consisting of TRIS, maleate and succinate. A preferred buffer is selected from the group consisting of TRIS and maleate. The most preferred

buffer is TRIS. Combinations of buffer (including combinations of TRIS and maleate) may also be used.

A preferred range of concentration of TRIS in the compositions of the present invention, if TRIS is the selected buffer, is about 5 mM to about 40 mM. A more preferred range of concentration of TRIS is about 10 mM to about 20 mM. A most preferred concentration of TRIS is about 15 mM.

A preferred range of concentration of maleate in the compositions of the present invention, if maleate is the selected buffer, is about 2 mM to about 20 mM. A more preferred range of concentration of maleate is about 5 mM to about 15 mM. A most preferred concentration of maleate is about 9 mM.

The compositions of the present invention comprise a pharmaceutically acceptable preservative. Preservatives provide safety and convenience to patients using pharmaceutical compositions. Antimicrobial agents may be added to a product formulation to protect the product from accidental microbial contamination during its manufacture, shelf life and use. This protection is also important when vials or cartridges containing a composition are provided that allow multiple withdrawals of the product. Selection and efficacy of pharmaceutically acceptable preservatives may also be guided by national regulatory agencies.

For the compositions of the present invention, pharmaceutically acceptable phenolic preservatives and benzyl alcohol are preferred. Examples of such phenolic preservatives include phenol, chlorocresol, m-cresol, o-cresol, p-cresol, ethylparaben, methylparaben, propylparaben, butylparaben and thymol, and mixtures thereof. More preferred preservatives are benzyl alcohol, m-cresol, phenol, methylparaben and mixtures thereof. A most preferred preservative is m-cresol.

A preferred concentration of preservative in the compositions of the present invention is about 1.0 mg/mL to about 20.0 mg/mL. Ranges of more preferred concentrations of preservative are about 2.0 mg/mL to about 8.0 mg/mL, about 2.5 mg/mL to about 4.5 mg/mL and about 2.0 mg/mL to about 4.0 mg/mL. A most preferred concentration of preservative is about 3.0 mg/mL.

The compositions of the present invention comprise the peptide Val^8-GLP-1(7-37)OH. Preferably, the total peptide concentration is about 1.0 mg/mL to about 50 mg/mL. More preferably, the peptide concentration is about 2.0 mg/mL to about 30 mg/mL. Other ranges of preferred concentrations of the peptide are about 5.0 to about 20.0 mg/mL, about 5.0 to about 10.0 mg/mL and about 2.0 mg/mL to about 8.0 mg/mL. A most preferred peptide concentration is about 6.0 mg/mL.

The compositions of the present invention comprise a hydrogen ion concentration, or pH, which is about 6.0 to about 8.5. A preferred pH range is about pH 6.5 to about pH 8.0.

The preferred pH range of the pharmaceutical compositions will also depend to some extent upon the selected buffer. With TRIS as the buffer, a preferred range of composition pH is about 6.5 to about 8.5. More preferred ranges of pH are about 7.0 to about 7.8, about 7.2 to about 7.8, about 7.5 to about 8.5, and about 7.0 to about 8.0. With TRIS as the buffer, a most preferred pH is about 7.5. With maleate as the buffer, a preferred range of composition pH is about 6.0 to about 7.5. More preferred ranges of pH are about 6.4 to about 7.5, about 6.4 to about 7.0, and about 6.0 to about 7.0. With maleate as the buffer, a most preferred pH is about 6.5.

A preferred composition of the present invention comprises; thin plate crystals of Val^8-GLP-1(7-37)OH at a concentration of 5.5 mg/mL to 6.5 mg/mL, glycine at a

concentration of 20 mM to 26 mM, ethanol at a concentration by volume of 3% to 5%, zinc at a concentration of 1.1 to 1.7 moles per mole of Val^8-GLP-1(7-37)OH, sodium chloride at a concentration of 100 mM to 120 mM, TRIS at a concentration of 10 mM to 20 mM, and m-cresol at a concentration of 2.5 mg/mL to 3.5 mg/mL. This preferred composition has a pH of 7.2 to 7.8.

Another preferred composition of the present invention comprises; thin plate crystals of Val^8-GLP-1(7-37)OH at a concentration of about 6 mg/mL, glycine at a concentration of about 23 mM, ethanol at a concentration of about 4% by volume, zinc at a concentration of about 0.2 mg/mL, sodium chloride at a concentration of about 110 mM, TRIS at a concentration of about 15 mM, m-cresol at a concentration of about 3 mg/mL and a pH of about 7.5.

As illustrated in Examples 6 to 13, pharmaceutical compositions of the present invention are stable. As illustrated in Example 14, pharmaceutical compositions of the present invention are suitable for use as a medicament for treating diabetes, hyperglycemia, obesity, or related conditions in mammals.

In order to provide safety, convenience and precise dosing in administering a suspension composition, suspended material should settle very slowly in the liquid composition, it should not compact tightly at the bottom of the container upon storage and settled material should resuspend readily with minimal swirling or agitation. Example 7 herein describes a peptide crystal composition of the present invention that exhibits a very slow crystal sedimentation rate and a sedimentation volume of greater than 50% after 24 hours of settling at ambient temperature. Also, there was no evidence of tight compaction of the crystals at the bottom of the cartridges upon storage.

In order to provide accurate dosing and safety to patients administering a suspension composition, the suspended material of the composition should not agglomerate or clump irreversibly after normal patient use and storage for a period of time as specified by the product label. Such agglomerated or clumped material may clog the orifice of a syringe needle or other device used to administer the composition, thereby reducing the quantity of peptide delivered. Examples 8 and 9 herein describe crystal compositions of the present invention comprising TRIS or maleate buffer that, by visual examination, exhibit satisfactory physical stability for at least 14 days in the TCR Test and for at least 28 days in the Modified TCR Test. The TCR Test especially exaggerates storage and agitation conditions beyond that expected by normal patient use. Example 10 herein describes particle size evaluations of the suspended material in the suspension compositions. These results showed the crystal particles in the compositions comprising TRIS or maleate buffer maintained their small size, that is, about 5 μm to about 10 μm (mean volume percent distribution), for at least 28 days in the Modified TCR Test.

Chemical degradation may also occur in peptide compositions leading to formation of compounds which have reduced potency and/or unknown side effects. Thus, in order to provide safety and full dosing to patients administering peptide pharmaceutical compositions, the level of chemical degradation occurring in the composition during patient use and storage should be kept to a minimum. Example 11 herein describes HPLC evaluations of Val^8-GLP-1(7-37)OH peptide compositions that underwent the TCR and Modified TCR Tests. After 14 days in the TCR Test or 28 days in the Modified TCR Test, the analyses showed less than about 2% of the peptide was chemically altered.

The optimal clinical benefits of a peptide composition of the present invention are realized when reproducible, prolonged absorption is achieved. As a corollary to this goal, adverse events resulting from dose dumping that may occur soon after administration of a composition may be avoided if the quantity of soluble peptide in the composition, which tends to be absorbed very quickly, is minimized during the typical use and storage conditions of the patient. Example 12 herein describes HPLC evaluations of the soluble portion of suspension compositions of the present invention that comprised about 3 mg/mL of total Val^8-GLP-1(7-37)OH peptide. For the compositions comprising TRIS, maleate or succinate buffer, the concentration of soluble peptide was 8 μg/mL or less after 14 days in the TCR Test or after 28 days in the Modified TCR Test. This means that less than 0.3% of the peptide became solubilized during the course of these tests.

For pharmaceutical compositions of peptide crystals, another aspect of physical stability is maintenance of the proper molecular conformation of the peptide. This proper conformation may be critical to delivering a molecule capable of interacting with its receptor and eliciting the desired biological response. For GLP-1 related peptides such as Val^8-GLP-1(7-37)OH, a predominantly α-helix conformation is believed to be important in providing a soluble and, therefore, bioavailable peptide while a mostly β-sheet form is believed to be essentially insoluble and, therefore, not bioavailable. Example 13 herein describes pharmaceutical compositions of the present invention for which the conformation of the peptide was evaluated by FTIR analysis after 14 days in the TCR Test. The analyses showed the peptide compositions comprising TRIS buffer (with or without NaCl) or comprising maleate buffer (without NaCl)

retained the peptide predominantly in an α-helix conformation throughout the 14-day test. Therefore, the Val^8-GLP-1(7-37)OH peptide in these compositions will be maximally bioavailable after administration to a mammal.

The clinical benefits of the Val^8-GLP-1(7-37)OH compositions of the present invention are realized when the administered peptide is present in the mammal being treated for a prolonged period of time. Example 14 herein describes evaluation of a pharmaceutical composition of the present invention comprising suspended Val^8-GLP-1(7-37)OH crystals that was administered subcutaneously to dogs. The results showed that elevated plasma levels of the peptide were maintained for at least 24 hours.

The crystals of Val^8-GLP-1(7-37)OH and compositions thereof according to the present invention may be used as a medicament or in preparing a medicament for the treatment of diabetes, hyperglycemia, obesity, irritable bowel syndrome or related conditions in mammals such as humans. The present invention also provides a method of treating diabetes, hyperglycemia, obesity, irritable bowel syndrome or related conditions in mammals such as humans, which comprises administering to the mammal crystals of Val^8-GLP-1(7-37)OH or a pharmaceutical composition thereof.

A dose of 0.001 to 5 mg of Val^8-GLP-1(7-37)OH in crystals or compositions thereof per kg of body weight of a mammal may be administered parenterally to the mammal in need of such treatment. One skilled in the art will recognize that smaller or larger doses may also be operable, depending on the patient, their condition and the manner of administration. Preferred dose ranges include 0.001 to 1 mg/kg, 0.002 to 3 mg/kg, 0.005 to 2 mg/kg, 0.01 to 1 mg/kg, 0.01 to 0.1 mg/kg, 0.2 to 0.8 mg/kg, 0.8 to 3 mg/kg and 0.2 to 3 mg/kg. More preferred dose ranges include 0.001 to 1

mg/kg, 0.005 to 2 mg/kg, 0.01 to 1 mg/kg, and 0.01 to 0.1 mg/kg.

A total dose of 0.01 to 20 mg, based on the mass of the crystals or the mass of the crystals in the compositions of the present invention, may be administered parenterally to a mammal, such as a human, in need of such treatment. One skilled in the art will recognize that a smaller or larger total dose may also be operable, depending on the patient, their condition and the manner of administration. Preferred total dose ranges include 0.01 mg to 10 mg, 0.1 to 10 mg, 1 to 8 mg and 2 to 6 mg. More preferred total dose ranges are 1 to 8 mg and 2 to 6 mg.

Pharmaceutical compositions of the present invention may be administered parenterally to patients in need thereof more than once per day, once per day, once every two days, twice per week, once per week, or in other dosing regimens known to those skilled in the art. A preferred dosing regimen is administration once per day.

The claimed compositions may be administered to a patient in need thereof by a variety of parenteral delivery methods appreciated by the skilled artisan. Preferred methods include subcutaneous injection, intramuscular injection, pulmonary administration, buccal, nasal, or transdermal delivery, and delivery by internal or external pump. More preferred delivery methods are subcutaneous and intramuscular injection. When injected, syringes or cartridges employing needles or needle-less devices well known in the art may be employed.

The present invention also provides a process for preparing crystals of a peptide of Formula I (SEQ ID NO: 1):

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

18 19 20 21 22 23 24 25 26 27 28
Ser-Tyr-Leu-Glu-Xaa-Gln-Ala-Ala-Lys-Xaa-Phe-

29 30 31 32 33 34 35 36 37
Ile-Xaa-Trp-Leu-Val-Lys-Gly-Arg-R

Formula I (SEQ ID NO: 1)

wherein:

Xaa at position 8 is Val;

Xaa at position 22 is Gly;

Xaa at position 27 is Glu;

Xaa at position 30 is Ala;

R is Gly;

comprising the steps;

(a) preparing a glycine-free solution of the peptide at a pH of about 9.0 to about 12.0,

(b) adding glycine to a concentration between about 5 mM and about 250 mM,

(c) adding about 2% to about 20% by volume of an alcohol selected from the group consisting of ethanol and isopropanol, and about 0.2 to about 2.5 moles of zinc per mole of the peptide,

(d) adjusting the pH of the solution to between about pH 7.5 and about pH 10.5, and

(e) allowing crystals of the peptide to form.

The first step in the process is preparing a glycine-free solution of the peptide at a pH of about 9.0 to about 12.0. This "alkaline normalization" step appears to reduce

the content of β-sheet conformation in the peptide and enhance the α-helix conformation that is important for solubility and bioavailability. This step also serves to maintain the peptide in a preferred α-helix conformation prior to the subsequent process step. This key step thus "normalizes" variation in bulk lots of the peptide into a more reproducible, homogenous solution.

Preferably, the Val^8-GLP-1(7-37)OH peptide concentration in the alkaline normalization solution is greater than 5 mg/mL. More preferably, the peptide concentration is about 10 mg/mL to about 30 mg/mL. Other ranges of preferred concentration of dissolved peptide are about 5 mg/mL to about 25 mg/mL, about 8 mg/mL to about 25 mg/mL and about 10 mg/mL to about 20 mg/mL. The most preferred peptide concentration is about 15 mg/mL.

Preferably, an aqueous alkaline solution comprising only water and a base such as NaOH, KOH or ammonium hydroxide is employed to dissolve the peptide. A more preferred base is NaOH. Optionally, the Val^8-GLP-1(7-37)OH peptide is first dissolved in a glycine-free solution at an acidic pH, then adjusted with a base such as NaOH, KOH or ammonium hydroxide to achieve a glycine-free solution of the peptide at a pH of about 9.0 to about 12.0.

Preferably, the pH of the alkaline normalization step is about 9.5 to about 11.5. More preferred ranges of the pH of the alkaline normalization step are about 9.5 to about 10.5 and about 10.0 to about 11.0. Most preferably, the pH is about 10.5. The alkaline solution comprising the dissolved Val^8-GLP-1(7-37)OH peptide may be allowed to sit quiescently for a period of about 5 minutes to about 3 hours at ambient temperature, which, although it is not to be construed as a limitation, is generally between about 20°C and about 25°C. The alkaline solution may also be gently

stirred. More preferably, the dissolved alkaline peptide solution will sit quiescently for about 1 hour at ambient temperature. One skilled in the art will recognize that combinations of pH, time, temperature and stirring conditions for this step can be readily established for each peptide that ensures "normalization" of the peptide conformation is complete yet avoids or minimizes racemization or chemical degradation that may occur to the peptide.

The next step in the process for preparing crystals of Val^8-GLP-1(7-37)OH is the addition of glycine. Amino acids such as glycine bind zinc ions which also bind very tightly to the histidine residue(s) in a peptide. Thus, competition for zinc binding may play a role in the formation of Val^8-GLP-1(7-37)OH peptide crystals, as well as in the stability of subsequent crystalline compositions. The glycine added to the alkaline peptide solution may be in a solid form or in a stock solution. Preferably, glycine is added as a solid. Preferably, the added glycine is in free-base form. Preferably, the resulting concentration of glycine in the alkaline peptide solution is about 5 mM to about 250 mM. Ranges of more preferred glycine concentration are about 10 mM to about 150 mM, about 20 mM to about 100 mM, about 40 mM to about 80 mM and about 55 mM to about 65 mM. Most preferably, the glycine concentration is about 60 mM.

Optionally, the pH of the alkaline peptide solution may be readjusted after the addition of the glycine. If the pH is adjusted, it is preferably adjusted to a pH between about 9.0 and about 11.0. More preferably, it is adjusted to a pH between about 9.2 and about 9.8. Most preferably, it is adjusted to about pH 9.5.

Optionally, the alkaline peptide solution with added glycine may be filtered. Filtration is recommended if any evidence of undissolved particles, dust or lint is apparent

in the solution. If desired, this is also a good place in the process at which the solution can be sterilized by performing an aseptic filtration step. Preferably, the filtration will be conducted using a sterile non-pyrogenic filter having low-protein binding and a pore size of 0.45 μm or less. More preferably, the filter is a sterile non-pyrogenic, low-protein binding filter of pore size 0.22 μm or less. Preferably, the filter is a sterile polysulfone filter or a sterile polyvinylidene fluoride filter.

The next step in the process is addition to the alkaline peptide solution of about 2% to about 20% of the total final volume of an alcohol selected from the group consisting of ethanol and isopropanol, and about 0.2 moles to about 2.5 moles of zinc per mole of the peptide. Generally, if a lower glycine concentration is used in the crystallization process, then zinc levels at the lower end of the range may be more successful in generating crystals. See, in particular, Example 3 herein. The zinc and ethanol may be added in a single aqueous stock solution or may be added separately in one or more steps in any order. Preferably, the alcohol is added before the zinc is added.

Preferably, the added alcohol represents, by volume, about 2% to about 20% of the total final volume of the alkaline peptide-zinc-alcohol solution. More preferably, the alcohol represents about 5% to about 15% of the total final volume. More preferably, the alcohol represents about 6% to about 12% of the total final volume. Most preferably, the alcohol represents about 9% of the total final volume. Preferably, the alcohol is ethanol.

The zinc added at this stage refers to the zinc ion. The zinc may be added in a variety of forms, but a zinc oxide solution acidified with dilute HCl and salt forms such as zinc acetate or zinc chloride are preferred. More

preferred is a zinc oxide solution acidified with dilute HCl.

Preferably, about 0.5 moles to about 2.5 moles of zinc per mole of the Val^8-GLP-1(7-37)OH peptide is added in this process step. Other preferred ranges of zinc addition include 1.0 moles to about 2.25 moles of zinc per mole of the peptide, 1.1 to 2.0 moles of zinc per mole of the peptide, 1.3 to 1.7 moles per mole of peptide, and 1.4 to 1.6 moles per mole of peptide. Most preferably, about 1.5 moles of zinc per mole of peptide is added.

Preferably, the solution comprising zinc that is added to the Val^8-GLP-1(7-37)OH peptide solution is added slowly and/or in small increments, which minimizes the localized precipitation of peptide and/or zinc complexes that may form at the site of addition. More preferably, glycine is also a component of the solution comprising zinc that is being added at this step. Any glycine added in combination with the zinc at this step is in addition to the glycine that is introduced into the alkaline normalization solution as described above. For example, a zinc-glycine solution may be prepared by dissolving zinc oxide in dilute HCl to a pH of about 1.6 and then adding solid glycine. A sufficient quantity of glycine is added to raise the pH of the solution to between about pH 2 and about pH 3. The pH of the zinc-glycine solution may be raised further using, for example, dilute NaOH. A preferred pH range of the zinc-glycine solution is about pH 4.0 to about pH 6.0. A more preferred pH range of the zinc-glycine solution is about pH 5.0 to about pH 5.5. As noted earlier, glycine has a binding affinity for zinc that may compete with zinc binding to the peptide. Thus, the presence of glycine in the solution comprising zinc that is being added to the composition allows the zinc solution to be added more quickly because localized precipitation problems are minimized. In

addition, having a zinc-glycine solution above pH 2.0, and preferably about pH 4.0 to about pH 6.0, allows the solution to be sterile filtered using filters that are rated by their manufacturers to handle, for example, pH 2-10 solutions, prior to its introduction into a sterile peptide composition. Preferably, the zinc-glycine solution comprises about 50 mM to about 70 mM glycine and about 20 mM to about 200 mM zinc.

The last steps in the initial crystallization of Val^8-GLP-1(7-37)OH are adjusting the pH of the solution to between about pH 7.5 and about pH 10.5 and allowing crystals of the peptide to form. Preferred reagent solutions useful for adjusting the pH of the solution include dilute HCl, dilute acetic acid and dilute NaOH.

Preferred pH ranges for crystallization of Val^8-GLP-1(7-37)OH include about pH 8.0 to about pH 10.0, about pH 7.5 to about pH 9.5, about pH 8.5 to about pH 9.2, about pH 9.0 to about pH 9.5, about pH 7.5 to about pH 8.5, about pH 8.7 to about pH 9.5, and about pH 9.2 to about pH 10.0.

One skilled in the art will recognize that the preferred pH of crystallization will depend on many factors, including the concentration of the peptide, the alcohol concentration, the zinc concentration, the ionic strength of the solution and the temperature of crystallization.

The skilled artisan will further recognize that, for a given set of conditions, a preferred manner of determining the optimal pH of crystallization is to determine it empirically, that is, to slowly add the acidification solution, preferably dilute HCl or dilute acetic acid, in small increments, and observe what happens after each increment is added. Generally, small quantities of localized zones of precipitation will occur at the spot of addition of the acidic solution. When gentle swirling takes increasingly longer periods of time to completely redissolve

the precipitation, that is the best time to stop adding the acid and allow crystallization from the clear or slightly cloudy solution to proceed.

The skilled artisan will further recognize that the pH and temperature that one selects for crystallization will have an impact on the speed at which the crystallization proceeds, the crystallization yield, and the size and homogeneity of the crystals formed. Preferably, the pH of crystallization for Val^8-GLP-1(7-37)OH is about pH 8.0 to about pH 10. More preferably, the pH is about 8.7 to about 9.5. Other ranges of preferred pH of crystallization are about 8.8 to about 9.3, about 9.0 to about 9.5, and about 8.5 to about 9.3. Most preferably, the crystallization is conducted at about pH 9.1.

Preferably, the temperature of crystallization is about 10°C to about 30°C. More preferably, the temperature of crystallization is about 15°C to about 28°C. Most preferably, the temperature of crystallization is ambient temperature, or about 20°C to about 25°C.

Preferably, the crystallization step described above is complete, that is, 90% or more of the peptide is precipitated in predominantly crystalline form, in about 3 hours to about 72 hours. More preferably, the crystallization is complete in about 10 hours to about 48 hours. Most preferably, the crystallization is complete in about 16 hours to about 26 hours. Completion of crystallization may be determined by a variety of means, including HPLC analysis of the peptide present in an aliquot of the composition. Method 5 herein describes one such protocol that may be employed.

Preferably, the Val^8-GLP-1(7-37)OH crystals produced according to the steps of the process described above are thin plate crystals. The crystals produced by the process may be examined by microscopy.

Pharmaceutical compositions comprising crystals of Val^8-GLP-1(7-37)OH prepared as described above may be prepared by adding suitable, pharmaceutically acceptable excipients to the crystal suspension in the original mother liquor. Alternatively, the crystals may be isolated by filtration, gentle centrifugation or other means of phase separation, and used in a variety of ways to prepare pharmaceutically acceptable compositions. The skilled artisan will recognize suitable procedures and excipients useful for preparing such pharmaceutical compositions. The compositions of the present invention are preferably prepared as described herein.

To prepare a stable pharmaceutical composition of crystals of Val^8-GLP-1(7-37)OH, the pH of the suspension of crystals in their complete original mother liquor, or portion thereof, is lowered to a pH value at which 97% or more of the peptide becomes insoluble. Preferably, this part of the process begins within a few hours after the initial crystallization is determined to be complete. Preferably, the pH is lowered using a dilute solution of HCl or acetic acid wherein the acidic solution is added slowly and in incremental portions. The skilled artisan will recognize that the preferred pH at which this second stage of crystallization should occur will depend on many factors, including the nature of the peptide and its concentration, the alcohol concentration, the zinc concentration, the ionic strength of the suspension and the temperature of crystallization. Preferably, the pH is about 0.2 to 2.0 pH units lower than the pH at which the initial crystallization proceeded. More preferably, the pH is about 0.5 to about 1.5 pH units lower, and most preferably, the pH is about 0.8 to 1.3 pH units lower than the pH at which the initial crystallization proceeded. The temperature of this second stage of crystallization is preferably ambient temperature,

or about 20°C to about 25°C. For the peptide Val^8-GLP-1(7-37)OH, a preferred pH is about 7.5 to about 8.5. A more preferred pH is about 7.8 to about 8.2.

Preferably, the pH of a suspension of Val^8-GLP-1(7-37)OH peptide crystals is lowered to a pH at which 98% or more, and more preferably at which 99% or more of the peptide becomes insoluble in the composition. The additional precipitation formed in this second stage of crystallization comprises crystals. Preferably, the additional precipitation formed in this second stage of crystallization will be predominantly crystals of comparable morphology and size distribution as those formed in the first stage of crystallization.

Preferably, the second stage of crystallization is complete enough, that is, 97% or more of the peptide is insoluble, to allow the following step to begin within 30 hours, more preferably within 18 hours, more preferably within 6 hours and most preferably within 2 hours of when the second stage of crystallization started. Quantitation of precipitation yield may be determined by a variety of means, including HPLC analysis of the peptide present in an aliquot of the composition. Method 5 herein describes one such protocol that may be employed.

The next step in the process to prepare a stable pharmaceutical composition of crystals of Val^8-GLP-1(7-37)OH is to add a pharmaceutically acceptable preservative and buffer selected from the group consisting of TRIS, maleate and succinate. Optionally, one or more tonicity agents such as sodium chloride, other salts, glycerin or mannitol may also be added. These components may be added as a single solution, as combination solutions or individually in any order. It is preferred that the preservative is added last. Of these components, a preferred buffer is selected from the group consisting of TRIS, maleate and succinate, a preferred

preservative is m-cresol and a preferred tonicity agent is sodium chloride. A more preferred buffer is selected from the group consisting of TRIS and maleate. A most preferred buffer is TRIS.

A preferred quantity of TRIS to add to the crystalline peptide suspension, if TRIS is the selected buffer, is such that the TRIS concentration in the final composition is about 5 mM to about 40 mM. A more preferred range of TRIS concentration in the final composition is about 10 mM to about 20 mM. A most preferred concentration of TRIS in the final composition is about 15 mM.

A preferred quantity of maleate to add to the crystalline peptide suspension, if maleate is the selected buffer, is such that the maleate concentration in the final composition is about 2 mM to about 20 mM. A more preferred range of maleate concentration in the final composition is about 5 mM to about 15 mM. A most preferred concentration of maleate in the final composition is about 9 mM.

If sodium chloride is selected to be a component of a peptide composition of the present invention, a preferred quantity to add to the crystalline peptide suspension is such that the added sodium chloride in the final composition is about 30 mM to about 200 mM. A more preferred concentration of added sodium chloride in the final composition is 50 mM to about 150 mM. Other ranges of preferred sodium chloride concentration are about 80 mM to about 120 mM, about 70 mM to about 130 mM, and about 90 mM to about 130 mM. A most preferred quantity of added sodium chloride in a pharmaceutical composition of the present invention is about 110 mM.

Although any pharmaceutically acceptable preservative may be added to the crystalline peptide suspension at this point in the process, for a composition of the present invention a phenolic preservative or benzyl alcohol is

preferred. Examples of phenolic preservatives include phenol, chlorocresol, m-cresol, o-cresol, p-cresol, ethylparaben, methylparaben, propylparaben, butylparaben, thymol or mixtures thereof. More preferred preservatives are benzyl alcohol, m-cresol, phenol, methylparaben and mixtures thereof. A most preferred pharmaceutically acceptable preservative is m-cresol.

A preferred quantity of a pharmaceutically acceptable preservative to add to a crystalline Val^8-GLP-1(7-37)OH composition at this point in the process is an amount such that the preservative concentration in the final composition is about 1.0 mg/mL to about 20.0 mg/mL. More preferred ranges of concentration of preservative in the final composition are about 2.0 mg/mL to about 8.0 mg/mL, about 2.5 mg/mL to about 4.5 mg/mL and about 2.0 mg/mL to about 4.0 mg/mL. A most preferred concentration of preservative in the final composition is about 3.0 mg/mL.

The final step in the process of preparing a stable pharmaceutical composition of crystals of Val^8-GLP-1(7-37)OH is an adjustment to a final pH between about 6.0 and about 8.5, and preferably between about pH 6.5 and about pH 8.0. Although any of a wide variety of acidification and/or alkalization reagent solutions may be employed for this pH adjustment, dilute HCl, dilute NaOH and dilute acetic acid are preferred. More preferred reagent solutions are dilute HCl and dilute NaOH. The preferred pH to which the composition is adjusted will depend to some extent upon the peptide concentration, the proposed route of administration and the selected buffer.

Preferably, with TRIS as the selected buffer, the pH will be adjusted to a pH between about 6.5 and about 8.5. More preferably, the pH will be adjusted to a pH between about 7.0 and about 7.8, between about 7.2 and about 7.8, between about 7.5 and about 8.5, or between about 7.0 and

about 8.0. A most preferred pH to which the composition is adjusted when TRIS is the selected buffer is about 7.5. With maleate as the selected buffer, the pH will be adjusted to a pH between about 6.0 and about 7.5. More preferably, the pH will be adjusted to a pH between about 6.4 and about 7.5, between about 6.4 and about 7.0, or between about 6.0 and about 7.0. A most preferred pH to which the composition is adjusted when maleate is the selected buffer is about 6.5.

Example 5 illustrates an embodiment of the present invention in which stable pharmaceutical compositions of thin plate crystals of the peptide Val^8-GLP-1(7-37)OH were prepared.

The present invention further provides a pharmaceutical composition prepared by the process described in the preceding paragraphs.

Method 1

TCR Test

The TCR (temperature cycling and resuspension) Test is an automated procedure combining temperature cycling from 25°C to 37°C and mechanical agitation that evaluates formulations under conditions more extreme than would be expected for patient usage. This test was described for insulin suspensions and solutions by Shnek, D. R. *et al.*, in J. Pharmaceutical Sciences 87:1459-1465 (1998).

Briefly, the TCR Test employs temperature cycling between 25°C and 37°C in an incubator unit combined with resuspensions conducted twice daily on a mechanical device outside the incubator unit. The 3-mL glass cartridges containing a 1 mm glass bead and rubber plunger are filled with peptide suspensions and capped with a disk seal. The samples are held in a horizontal position for 10 hours per

day at 25°C and 10 hours per day at 37°C, with 2-hour temperature ramping steps between them. The mechanical resuspension of the test cartridges performs three sets of 10 lateral rolls plus 10 cartridge inversions twice daily. The resuspensions are conducted during the 25°C temperature cycle. The entire test lasts up to 28 days. For additional details of the TCR Test see Shnek, D. R. *et al.*, *supra*.

Method 2

Modified TCR Test

The Modified TCR Test is similar to the TCR Test described in Method 1 except that the temperature cycles are 5°C and 25°C. The resuspensions are conducted during the 25°C cycle.

Method 3

Particle Size Measurements

Particle size measurements were performed using a Coulter Model LS230 Particle Size Analyzer (Beckman Coulter, Inc., Fullerton, CA, USA). An aliquot (about 100 µL) of the test suspension was diluted about 100-fold to about 300-fold by pipetting it into a diluent containing 2.4 mM zinc, 150 mM NaCl and 50 mM sodium acetate at pH 5. Particle size data were collected for about 120 seconds, and the resulting distribution was analyzed with Coulter particle size statistics software which assumed a spherical shape. The mean volume percent distribution of particle sizes was obtained and reported.

Method 4

Visual Assessment of Peptide Compositions

Cartridges of peptide suspension compositions being evaluated in the TCR Test and in the Modified TCR Test were

examined by trained operators on various test days. The test compositions were checked for visual changes compared to quiescently refrigerated control samples. Visual changes included the presence of large aggregates (also designated as clumps) and/or material that adhered to the cartridge wall (also designated as frosting). For the evaluation of test results, a milky white suspension or the presence of grainy material that resuspends upon swirling is reported as PASS while visual changes involving large aggregates, clumps, frosting, or grainy material that does not resuspend upon swirling is reported as FAILED. Additional details of these evaluation criteria are described in Shnek, D. R. *et al.*, *supra*.

Method 5

HPLC Analysis of Peptides

Peptides in the aqueous compositions of the present invention were analyzed on a 25 cm X 4.6 mm Zorbax 300 SB (C-8) HPLC column (Mac-Mod Analytical Inc., Chadds Ford, PA, USA) with UV detection at 214 nm. A gradient made from a first solution (0.1% TFA in water) and a second solution (0.1% TFA in acetonitrile) was used to effect elution of the peptides and related impurities.

Method 5 was used to determine the purity of peptides in suspensions undergoing the TCR Test (Method 1) and the Modified TCR Test (Method 2). For these analyses, an aliquot of the suspended composition was diluted about 10-fold or greater with a 2 M guanidine-0.01 N HCl solution to solubilize the peptide crystals prior to injection.

To quantitate the soluble peptide present in a suspension composition, portions of the swirled suspension were centrifuged and an aliquot of the clear supernatant was analyzed by HPLC as described above.

Method 6

FTIR Analysis

FTIR (Fourier Transform Infrared Raman) spectra were acquired on a Nicolet Nic-Plan FTIR microscope using a Nicolet 760 Spectrometer optical bench (Nicolet Instrument Corporation, Madison, WI, USA) and Omnic version 5.1 software. Microliter portions of the peptide samples were placed on a glass slide and allowed to evaporate to dryness in a desiccator overnight at ambient temperature (20°C to 25°C). The dried peptide was removed from the slide using a micro-tool and placed on a diamond anvil cell (Spectra-Tech, Inc., Shelton, CT, USA) for analysis. Spectra were acquired at 4 cm^{-1} resolution from 128 scans and were baseline adjusted. The background spectra were similarly obtained with half of the diamond anvil cell in the beam. For analysis of secondary structure, second derivatives and deconvolved spectra were obtained using Nicolet software. Second derivatives were inverted (multiplied by -1000) and smoothed (11 points over 10.6 cm^{-1}). Deconvolved spectra were obtained using a 23 cm^{-1} bandwidth and 2.2 enhancement factor.

The following examples are provided merely to further illustrate the invention. The scope of the invention shall not be construed as merely consisting of the following examples.

Example 1

Crystallization of Val^8-GLP-1(7-37)OH

Val^8-GLP-1(7-37)OH was dissolved in about 0.57 mL of 0.015 N NaOH at a concentration of about 17 mg/mL, based on the mass of the peptide. The protein solution was adjusted to about pH 10.5 with dilute NaOH. The solution was held at ambient temperature for about 1 hour.

To a 390 μL aliquot of this peptide solution was added 25 μL of a 1.0 M glycine pH 8 solution, giving a final concentration of about 16 mg/mL of Val^8-GLP-1(7-37)OH and about 60 mM glycine. The pH of the solution (about pH 9.0) was adjusted to about pH 9.9 with dilute HCl and/or dilute NaOH as needed.

The solution was then filtered into a 0.5 mL Eppendorf tube through a sterile 0.22 μm Millex®-GV (Millipore Corporation, Waltham, MA, USA) 4 mm filter unit.

To 300 μL of the filtered peptide solution in a clean test tube was added 66 μL of a 50% ethanol solution in water. To this solution was added, in small increments, a total of 14.1 μL of a 150 mM zinc oxide pH 2.3 solution (prepared with dilute HCl), with mixing by hand performed after each increment was added until the solution became clear. The molar ratio of zinc:peptide was about 1.5:1.

The final solution was adjusted to about pH 8.9 and crystallization proceeded at ambient temperature. The crystallization solution comprised about 12.6 mg/mL Val^8-GLP-1(7-37)OH, 47 mM glycine, 8.7% ethanol by volume, and about 1.5 moles of zinc per mole of Val^8-GLP-1(7-37)OH at pH 8.9.

After about three days at ambient temperature, thin plate crystals of Val^8-GLP-1(7-37)OH were observed under a microscope at 400X magnification.

Example 2

Crystallization of Val^8-GLP-1(7-37)OH at pH 8.2

The operations in this example were conducted at ambient temperature.

To 25 mL of deionized water in a 50 mL beaker was added, with slow stirring, 477.1 mg of solid Val^8-GLP-1(7-37)OH. While stirring, 180 μL of 10% (w/v) NaOH was added

to dissolve the peptide, resulting in a solution pH of 10.53. The solution was then held without stirring for 30 minutes.

With slow stirring, 93.7 mg of solid glycine was added to the solution. After the glycine was dissolved, 140 µL of 10% (w/v) NaOH was added to raise the solution pH from pH 8.9 to pH 9.5. This solution was then passed through a 0.22 µm sterile, low protein-binding filter into a glass beaker.

With slow stirring, 2.5 mL of absolute ethanol was added, followed by 1.147 mL of a 0.15 M zinc solution (which was prepared by dissolving zinc oxide in 10% HCl and diluting appropriately with deionized water). The pH of the solution was then raised from pH 8.8 to pH 9.0 using 10% (w/v) NaOH.

The pH of the solution was then lowered by a stepwise addition of a 10% HCl solution. Aliquots were removed from the crystallization solution at various pH levels and held quiescently at ambient temperature for about 1 day (16 to 24 hours). The aliquot taken when the solution was at pH 8.2 was evaluated for crystallization yield by ultraviolet spectroscopy and was examined under the microscope at 630X magnification. This examination revealed individual needle-like crystals and needle-like clusters at a crystallization yield of greater than 98%.

The above experiment was repeated two more times under similar conditions and comparable results were obtained each time.

Example 3

Crystallization of Val^8-GLP-1(7-37)OH at a Low Glycine Concentration

About 524 mg of solid Val^8-GLP-1(7-37)OH was dissolved in 50 mL of Sterile Water for Irrigation (Abbott Laboratories). The pH of the solution was raised to 12.0

using 5 N NaOH and held at ambient temperature for five minutes. To this solution was added 0.75 mL of a 1 M glycine stock solution. The pH of the resulting solution was lowered to pH 9.0 using 5 N HCl and the solution was then filtered.

To each of nine aliquots (4.5 mL each) of the filtered Val^8-GLP-1(7-37)OH-glycine pH 9.0 solution was added 0.5 mL of absolute ethanol. To each solution was then added from 17 μL to 130 μL of a 10 mg/mL zinc oxide solution in dilute HCl, followed by up to 113 μL of water such that the total volume of the zinc oxide solution and water added was 130 μL. The pH of each solution was then adjusted to the values indicated in Table 1. Each aliquot had an ethanol concentration of about 9.9% (v/v) and a glycine concentration of about 13 mM. The concentration of Val^8-GLP-1(7-37)OH in the crystallization solutions was about 6.8 mg/mL, as determined by UV absorbance measurements of a later-acidified aliquot. The molar ratio of zinc:Val^8-GLP-1(7-37)OH in the crystallization samples ranged from 0.22:1 to 1.55:1, as shown in Table 1.

The precipitation and crystal morphology of each crystallization sample was evaluated under a microscope at 630-1000X magnification after overnight storage at ambient temperature. The results are reported in Table 1.

Table 1

Morphology of Val^8-GLP-1(7-37)OH Precipitation Obtained at a Low Glycine Concentration

Zinc Ratio	Final pH	Precipitation Observed
1.55	9.25	amorphous
1.10	9.14	amorphous
0.85	8.76	amorphous
0.60	8.46	long, thin plates
0.49	8.91	long, thin plates
0.49	8.56	long, thin plates
0.34	8.40	long, thin plates
0.29	8.34	small, thin plates
0.22	7.90	long, thin plates

Example 4

Large-Scale Crystallization of Val^8-GLP-1(7-37)OH

The procedures described in Examples 4 and 5 herein were conducted two times at about the one liter scale noted herein and three times at a larger scale to successfully prepare thin plate crystals of Val^8-GLP-1(7-37)OH and compositions thereof.

To about 400 gm of water for injection was added about 70 µL to about 100 µL of 10% NaOH to adjust the pH to about 10 to 11. About 6 grams of Val^8-GLP-1(7-37)OH, quantified by main peak area in HPLC analysis, was added to the solution in 3 to 5 portions, with most of the peptide dissolving before the next portion was added. Small

aliquots of 10% NaOH were added after each portion of peptide was added, if needed, to maintain a pH about 10 to about 10.5. About 700 µL of 10% NaOH was added to the solution to reach a final pH of about 10.5. The solution was held quiescently for about 1 hour at ambient temperature, or about 23°C.

While stirring, about 2.25 gm of solid glycine (free base) was added to the solution. The glycine dissolved in the solution in about 5 minutes. The pH of the resulting solution was about 8.8 and was readjusted to about pH 9.5 with the addition of about 3 mL of 10% NaOH. After stirring for 5 minutes the pH of the solution was readjusted, if needed, to about 9.5 and water for injection was added to a total volume of about 500 mL (about 503 gm). An additional volume of 10% NaOH was added, if needed, to readjust the pH to about 9.5.

The solution was then pressure filtered through a 0.22 µm Millex®-GV (Millipore S.A., Molsheim, France) sterilizing filter membrane unit. Approximately the first 100 mL of the filtrate was discarded and the remaining filtrate was collected in a sterile bottle.

To about 393 mL of the stirred, sterile filtrate was added about 86.4 mL of a sterile-filtered 50% (by volume) ethanol solution (about 42.5% by weight) that had been prepared from absolute ethanol and water for injection.

To this stirred solution was added, very slowly, 20 mL of a sterile-filtered zinc oxide solution that had been prepared by combining about 1.22 gm zinc oxide and about 12 mL of 10% HCl and bringing the volume up to about 100 mL by adding water for injection. The addition of the zinc oxide solution to the peptide solution proceeded slowly and in small aliquots to allow any transient haze or precipitation to redissolve prior to the addition of the successive aliquots.

The pH of the resulting solution was adjusted to about 9.1 by carefully adding aliquots of 10% NaOH that totaled about 1000 µL. After gently mixing for about 5 minutes, the crystallization solution was covered tightly and held quiescently at ambient temperature.

After about 18 hours the suspension was stirred for about 5 minutes to allow removal of a 1-2 mL aliquot for analysis of particle size (by Coulter Model LS230, see Method 3), crystal morphology (by microscopy) and yield (for example, by HPLC analysis as described in Method 5). At this point the analyses generally showed a yield of about 92-96% of well-defined thin plate crystals that were about 1 µm thick, about 4-6 µm wide and about 20-60 µm long, and had a median volume percent distribution of about 6-14 µm.

After another few hours the suspension was restirred and the pH was adjusted to about 8 by adding about 1.5 mL to about 2.5 mL of 10% HCl. After holding the composition quiescently another 2 hours or less at ambient temperature, the crystallization process was complete. Analyses at this point typically showed mostly thin plate crystals in a yield greater than 98% and a median volume percent distribution of about 6-14 µm.

Example 5

Preparing a Stable Pharmaceutical Composition of Thin Plate Crystals of Val^8-GLP-1(7-37)OH

To the completed crystallization suspension described in Example 4 was added, with stirring, an equal volume, that is, about 495 mL, of a sterile-filtered solution comprising about 30 mM TRIS, 220 mM NaCl, and 6 mg/mL m-cresol at about pH 7.5. The pH was readjusted to about pH 7.5 by the addition of small aliquots of 10% HCl and/or 10% NaOH as needed.

The addition of the sodium chloride-preservative-buffer solution appeared to diminish somewhat the sharpness of the edges of the thin plate crystals, but the chemical and physical stability of the resulting crystalline composition was acceptable. The stable pharmaceutical suspension composition prepared as described above comprised about 6 mg/mL of thin plate crystals of Val^8-GLP-1(7-37)OH, about 23 mM glycine, about 3 mg/mL m-cresol, about 110 mM sodium chloride, about 4% ethanol by volume, about 0.2 mg/mL zinc, about 15 mM TRIS and has a pH of about 7.5.

Example 6

Preparation of Compositions for Stability Testing

Seven different stock solutions were prepared as described below. Stock solutions 3 through 6 comprise buffers that have approximately equal buffering capacities at the designated pH values.

Stock solutions

1. A suspension of crystals of the peptide Val^8-GLP-1(7-37)OH prepared in a manner similar to that described in Example 4. This particular composition of crystals had a Val^8-GLP-1(7-37)OH concentration of about 7.5 mg/mL, a glycine concentration of about 37 mM, a zinc concentration of about 4.3 mM, and an ethanol concentration of about 7.4% by volume.
2. 1 M sodium chloride in water.
3. 60 mM TRIS solution adjusted to about pH 7.5.
4. 52 mM sodium phosphate solution adjusted to about pH 7.5.
5. 36 mM maleic acid solution adjusted to about pH 6.5.
6. 28 mM succinic acid solution adjusted to about pH 5.5.
7. 15 mg/mL m-cresol in water.

Multiple test cartridges comprising 14 different suspension compositions designated A through L were prepared from the 7 stock solutions described above. The stock solutions were combined according to the volumes indicated in Table 2 and order of addition was from left to right. The compositions were then brought up to a final volume of 50.0 mL with water and adjusted to the pH values noted in the last column of Table 2.

Table 2

Volume (mL) of Stock Solutions 1 through 7
Combined to Form Test Compositions A through L
at the Indicated Final pH Values

	1	2	3	4	5	6	7	pH
A	19.9	0	0	0	0	0	10.0	8.5
B	19.9	7.5	0	0	0	0	10.0	8.5
C	19.9	0	0	12.5	0	0	10.0	7.5
D	19.9	7.5	0	12.5	0	0	10.0	7.5
E	19.9*	0	12.5	0	0	0	10.0	7.5
F	19.9	7.5	12.5	0	0	0	10.0	7.5
G	19.9	0	0	12.5	0	0	10.0	6.5
H	19.9	7.5	0	12.5	0	0	10.0	6.5
I	19.9	0	0	0	12.5	0	10.0	6.5
J	19.9	7.5	0	0	12.5	0	10.0	6.5
K	19.9	0	0	0	0	12.5	10.0	5.5
L	19.9	7.5	0	0	0	12.5	10.0	5.5

*Quantitation of the peptide in composition E by HPLC suggested about 15% less of stock solution 1 was added than indicated.

Based on the compositions of the stock solutions and the data shown in Table 2, the test compositions should have a Val^8-GLP-1(7-37)OH concentration of about 3 mg/mL, a glycine concentration of about 14.7 mM, a zinc concentration of about 1.7 mM, an ethanol concentration of about 3%, a m-cresol concentration of 3 mg/mL, a sodium chloride concentration of 0 or 150 mM, a buffer concentration of 15

mM TRIS, 13 mM phosphate, 9 mM maleate or 7 mM succinate, and a pH of 5.5, 6.5, 7.5 or 8.5.

Example 7

Sedimentation Rate Analysis of Peptide Compositions

One set of cartridges for each test composition A through L of Example 6 was evaluated visually for crystal settling rate and volume. After thoroughly suspending the insoluble material in the cartridges, each composition was allowed to settle quiescently at ambient temperature (about 20°C to about 25°C). The resulting sedimentation volume of the suspended material in each of the compositions after 1 hour was about 87% to 100% of total volume. After sitting quiescently for 24 hours at ambient temperature, the sedimentation volume for each cartridge sample was about 53% to about 72% of total volume.

Example 8

Visual Evaluation of Peptide Compositions in the TCR Test

Sets of five cartridges containing each of compositions A through L as described in Example 6 were subjected to the TCR Test (25°C to 37°C temperature cycling) as described in Method 1.

At various times during the 28-day test, the pH values of the suspensions were measured. All samples showed pH values within 0.16 pH units of the originally targeted pH values as shown in the last column of Table 2.

At various times during the 28-day test, cartridges were also evaluated by the visual examination criteria described in Method 4. Table 3 discloses the results of these examinations and reports the last day visual examination established that the cartridge compositions passed the test and the first day in which the test compositions failed the test.

Table 3

Peptide Compositions

in the 28-Day TCR Test

Sample	Day of Last PASS	Day of First FAIL
A	0	7
B	0	6
C	7	14
D	0	6
E	21	28
F	21	28
G	7	8
H	7	8
I	14	21
J	14	21
K	7	14
L	7	14

Example 9

Visual Examination of Peptide

Compositions in the Modified TCR Test

Sets of five cartridges containing each of compositions A through L as described in Example 6 were subjected to the Modified TCR Test (5°C to 25°C temperature cycling) as described in Method 2.

At various times during the 28-day test, the pH values of the suspensions were measured. All samples showed pH

values within 0.23 pH units of the originally targeted pH values as reported in Table 2.

At various times during the 28-day test, cartridges were evaluated by the visual examination criteria described in Method 4. Table 4 displays the results of these examinations, showing the last day visual examination established that the test compositions passed the test and the first day in which the test compositions failed the test.

Table 4

Peptide Compositions in the 28-Day Modified TCR Test

Sample	Day of Last PASS	Day of First FAIL
A	0	1
B	All PASS	All PASS
C	All PASS	All PASS
D	14	21
E	All PASS	All PASS
F	All PASS	All PASS
G	14	21
H	14	21
I	All PASS*	All PASS*
J	All PASS	All PASS
K	14	21
L	14	21

*Clumpy material at day 21 in 1 of 5 cartridges.

Example 10

Evaluation of Particle Sizes of Crystals in Peptide Compositions

Suspensions A through L prepared as described in Example 6 and evaluated by the TCR Test (Example 8) and Modified TCR Test (Example 9) were examined at the 14-day and 28-day time points for particle size measurements as described in Method 3. The starting mean volume percent distribution of particle sizes in each of the compositions was about 5 μm to about 6 μm. An increasing mean particle size observed during the TCR or Modified TCR Tests reflects the presence of agglomerated or clumped material. After 28 days in the TCR test, the mean particle size for virtually all of the test compositions that could be measured were greater than 30 μm, so only the results for the TCR Test after 14 days and the results for the Modified TCR Test after 14 days and 28 days are reported in Table 5.

Table 5

Mean Particle Size (μm) in Peptide Compositions in the TCR Test and the Modified TCR Test

Sample	14 Days Modified TCR	28 Days Modified TCR	14 Days TCR
A	*	*	*
B	5.95	18.76	*
C	5.93	5.42	*
D	8.06	150.20	*
E	5.51	5.88	5.13
F	5.85	5.87	5.35
G	34.56	107.20	*
H	29.99	148.00	*
I	5.96	5.82	22.90
J	5.82	9.14	32.31
K	7.67	78.87	69.03
L	20.94	121.80	88.20

*Excessive clumping and aggregation prevented quantitation.

Example 11

Chemical Stability of Tested Peptide Compositions

Selected suspensions from compositions A through L prepared as described in Example 6 were evaluated by HPLC analysis for peptide purity by Method 5 when they were initially prepared, after 14 days in the TCR Test and after 28 days in the Modified TCR Test. The main peak purity of the peptide Val^8-GLP-1(7-37)OH in the test compositions are shown in Table 6.

Table 6

Percent Peptide Purity in Compositions in the TCR Test and the Modified TCR Test

Sample	Initial	14 Days TCR Test	28 Days Modified TCR
A	97.26	NT	NT
B	97.08	NT	96.70
C	97.07	NT	96.73
D	97.16	NT	96.38
E	97.06	96.43	96.77
F	97.14	96.40	96.65
G	97.04	NT	96.36
H	96.88	NT	96.10
I	97.13	95.85	96.47
J	97.12	95.85	96.51
K	97.04	95.79	95.83
L	96.94	95.98	96.00

NT = not tested

Example 12

Soluble Peptide Concentrations in Tested Compositions

Selected suspensions from compositions A through L prepared as described in Example 6 were evaluated by HPLC analysis for soluble Val^8-GLP-1(7-37)OH by Method 5 soon after they were prepared, after 14 days in the TCR Test and after 28 days in the Modified TCR Test. The concentrations of soluble peptide measured by these analyses are shown in Table 7. The total concentration of Val^8-GLP-1(7-37)OH in

these suspension compositions was about 3 mg/mL, except for composition E which had a concentration of about 2.54 mg/mL (see Example 6 and Table 2).

Table 7

Soluble Peptide (μg/mL) in Compositions in the TCR Test and the Modified TCR Test

Sample	Initial	14 Days TCR Test	28 Days Modified TCR
A	NT	NT	NT
B	6	360	10
C	7	110	30
D	4	60	10
E	3	2	6
F	3	1	6
G	6	2	170
H	5	19	40
I	2	3	7
J	NT	1	8
K	2	2	4
L	2	2	3

NT = not tested

Example 13

FTIR Analysis of Tested Peptide Compositions

Compositions A through L prepared as described in Example 6 were evaluated after storage at 5°C for 28 days by FTIR analysis as described in Method 6 (spectra not shown).

Selected compositions were also examined after 14 days in the TCR Test (spectra not shown).

FTIR analysis of the compositions prepared and stored for 28 days at 5°C showed that the crystals in compositions B through L had a conformation consistent with a predominantly α-helix secondary structure. Duplicate analyses of the same samples resulted in similar spectra. The spectra for the sample prepared for composition A suggested an acid-like contaminant was present, so a definitive conclusion regarding the conformation of the crystals in composition A could not be made.

After 14 days in the TCR Test, compositions E, F, I, J, K and L were evaluated by FTIR analysis. For the spectra of samples E, F and I, the deconvolved and second derivative were consistent with a secondary structure that is mainly α-helix. Samples J and K appeared to have a slightly broader OH stretch region (3100-3500 cm^{-1}) with a peak at 3200 cm^{-1}, which could be due to normal spectral variation or the presence of an OH contributing species. Compositions K and L showed significant levels of β-sheet structure.

Example 14

Subcutaneous Injection of a Suspension Composition of Peptide Crystals in Dogs

A composition of crystals of Val^{8}-GLP-1(7-37)OH was prepared essentially as described in Examples 4 and 5. Each milliliter of this aqueous composition at about pH 7.4 contained approximately:

5.9 mg Val^8-GLP-1(7-37)OH;
6.43 mg sodium chloride;
1.82 mg TRIS;
1.78 mg glycine;
4.35% ethanol (by volume);
3 mg m-cresol; and
0.216 mg zinc.

Portions of the swirled suspension composition were injected subcutaneously into the dorsal region of beagle dogs of about 7 to 9 months of age. Each test dose was injected into 3 female and 3 male dogs each weighing about 5.8 kg to about 8.5 kg. The peptide composition was injected at three different doses based on the weights of the dogs; 0.2 mg/kg, 0.8 mg/kg and 3.0 mg/kg. A solution comprising only the liquid vehicle represented the control sample and was injected at a volume equivalent to the 3.0 mg/kg peptide dose.

Blood samples were collected from the jugular vein of each dog at 0.5, 1, 2, 4, 6, 8, 12, and 24 hours post-dose. Potassium EDTA was used as an anticoagulant. The plasma concentration of Val^8-GLP-1(7-37)OH at each time point was quantified by an RIA (radioimmunoassay) directed to the peptide. The mean plasma concentrations (n=6) and SEM (standard error of the mean) obtained from this experiment are described in Table 8.

Table 8

Plasma Concentrations (pg/mL ± SEM) of Val^8-GLP-1(7-37)OH from a Suspension Composition Injected Subcutaneously at 3 Doses into Dogs (n=6)

Time (hr) Post-dose	0.2 mg/kg	0.8 mg/kg	3.0 mg/kg
0	100*	100*	100*
0.5	2444 ± 616	5748 ± 926	8395 ± 1968
1	3043 ± 661	6530 ± 597	7855 ± 1435
2	3216 ± 667	7727 ± 777	8608 ± 1350
4	3685 ± 527	8837 ± 857	10663 ± 1198
6	2329 ± 242	6769 ± 754	11913 ± 1639
8	2019 ± 204	7217 ± 842	11505 ± 1664
12	1921 ± 275	6794 ± 1127	10649 ± 1090
24	1779 ± 373	5188 ± 775	8919 ± 501

*Pre-dose measurements typically showed peptide levels at or below the RIA limit of detection, which is 100 pg/mL.

Example 15

A Pharmaceutical Composition of Val^8-GLP-1(7-37)OH

A suspension of thin plate crystals of Val^8-GLP-1(7-37)OH further comprising glycine, ethanol and about 1.5 moles of zinc per mole of Val^8-GLP-1(7-37)OH is diluted with a solution comprising TRIS, NaCl and m-cresol at about pH 7.5 to provide a composition at a pH of about 7.5 and containing approximately:

6 mg/mL Val^8-GLP-1(7-37)OH;
110 mM sodium chloride;
15 mM TRIS;
23.7 mM glycine;
4.3% ethanol (by volume);
3 mg/mL m-cresol; and
1.5 moles of zinc per mole of Val^8-GLP-1(7-37)OH.

Example 16

A Pharmaceutical Composition of Val^8-GLP-1(7-37)OH

A suspension of thin plate crystals of Val^8-GLP-1(7-37)OH further comprising glycine, ethanol and about 1.5 moles of zinc per mole of Val^8-GLP-1(7-37)OH is diluted with a solution comprising TRIS, NaCl and m-cresol at about pH 7.5 to provide a composition at a pH of about 7.5 and containing approximately:

5 mg/mL Val^8-GLP-1(7-37)OH;
110 mM sodium chloride;
15 mM TRIS;
19.7 mM glycine;
3.6% ethanol (by volume);
3 mg/mL m-cresol; and
1.5 moles of zinc per mole of Val^8-GLP-1(7-37)OH.

Example 17

A Pharmaceutical Composition of Val^8-GLP-1(7-37)OH

A suspension of thin plate crystals of Val^8-GLP-1(7-37)OH further comprising glycine, ethanol and about 1.5 moles of zinc per mole of Val^8-GLP-1(7-37)OH is diluted with a solution comprising TRIS, NaCl and m-cresol at about pH 7.5 to provide a composition at a pH of about 7.5 and containing approximately:

4 mg/mL Val^8-GLP-1(7-37)OH;
110 mM sodium chloride;
15 mM TRIS;
15.8 mM glycine;
2.9% ethanol (by volume);
3 mg/mL m-cresol; and
1.5 moles of zinc per mole of Val^8-GLP-1(7-37)OH.

Example 18

A Pharmaceutical Composition of Val^8-GLP-1(7-37)OH

A suspension of thin plate crystals of Val^8-GLP-1(7-37)OH further comprising glycine, ethanol and about 1.5 moles of zinc per mole of Val^8-GLP-1(7-37)OH is diluted with a solution comprising TRIS, NaCl and m-cresol at about pH 7.5 to provide a composition at a pH of about 7.5 and containing approximately:

3 mg/mL Val^8-GLP-1(7-37)OH;
110 mM sodium chloride;
15 mM TRIS;
11.9 mM glycine;
2.2% ethanol (by volume);
3 mg/mL m-cresol; and
1.5 moles of zinc per mole of Val^8-GLP-1(7-37)OH.

We claim:

1. A pharmaceutical composition comprising:
crystals of a peptide of Formula I (SEQ ID NO: 1):

7 8 9 10 11 12 13 14 15 16 17
His-Xaa-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Val-Ser-
18 19 20 21 22 23 24 25 26 27 28
Ser-Tyr-Leu-Glu-Xaa-Gln-Ala-Ala-Lys-Xaa-Phe-
29 30 31 32 33 34 35 36 37
Ile-Xaa-Trp-Leu-Val-Lys-Gly-Arg-R

Formula I (SEQ ID NO: 1)

wherein:

Xaa at position 8 is Val;

Xaa at position 22 is Gly;

Xaa at position 27 is Glu;

Xaa at position 30 is Ala;

R is Gly;

glycine at a concentration of about 5 mM to about 100 mM;

an alcohol selected from the group consisting of ethanol and isopropanol, at a concentration by volume of about 1% to about 10%;

zinc at a concentration of about 0.5 moles to about 2.5 moles per mole of the peptide;

a buffer selected from the group consisting of TRIS, maleate and succinate; and

a pharmaceutically acceptable preservative;

wherein the composition has a pH of about 6.0 to about 8.5.

2. The composition according to claim 1, further comprising a tonicity agent.

3. The composition according to claim 2, wherein the tonicity agent is glycerin.

4. The composition according to claim 2, wherein the tonicity agent is sodium chloride.

5. The composition according to claim 4, wherein the sodium chloride is at a concentration of about 50 mM to about 150 mM.

6. The composition according to any one of claims 1 to 5, wherein the glycine concentration is about 10 mM to about 50 mM.

7. The composition according to claim 6, wherein the glycine concentration is about 20 mM to about 30 mM.

8. The composition according to any one of claims 1 to 7, wherein the alcohol concentration is about 2% to about 6% by volume.

9. The composition according to any one of claims 1 to 8, wherein the alcohol is ethanol.

10. The composition according to any one of claims 1 to 9, wherein the zinc concentration is about 1.1 to about 2.0 moles per mole of the peptide.

11. The composition according to any one of claims 1 to 10, wherein the buffer is selected from the group consisting of TRIS and maleate.

12. The composition according to claim 11, wherein the buffer is TRIS.

13. The composition according to any one of claims 1 to 12, wherein the preservative is a phenolic preservative.

14. The composition according to claim 13, wherein the phenolic preservative is m-cresol.

15. The composition according to any one of claims 1 to 14, wherein the preservative concentration is about 2.0 mg/mL to about 8.0 mg/mL.

16. The composition according to any one of claims 1 to 15, wherein the peptide concentration is about 2.0 mg/mL to about 30.0 mg/mL.

17. The composition according to claim 16, wherein the peptide concentration is about 2.0 mg/mL to about 8.0 mg/mL.

18. The composition according to any one of claims 1 to 17, wherein the pH of the composition is about 7.0 to about 8.0.

19. A composition comprising; crystals of Val^{8}-GLP-1(7-37)OH at a concentration of about 6 mg/mL, glycine at a concentration of about 23 mM, ethanol at a concentration of about 4% by volume, zinc at a concentration of about 0.2 mg/mL, sodium chloride at a concentration of about 110 mM, TRIS at a concentration of about 15 mM, m-cresol at a concentration of about 3 mg/mL and a pH of about 7.5.

20. The composition according to any one of claims 1 to 19, wherein the crystals are thin plate crystals.

21. The composition according to any one of claims 1 to 20, for the treatment of a human or animal by therapy.

22. The use of the composition according to any one of claims 1 to 20, in preparing a medicament for treating diabetes, hyperglycemia or obesity in a mammal.

23. A method of treating diabetes, hyperglycemia or obesity in a mammal, comprising administering to the mammal the composition according to any one of claims 1 to 20.

24. A process for preparing crystals of a peptide of Formula I (SEQ ID NO: 1):

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

18 19 20 21 22 23 24 25 26 27 28
Ser-Tyr-Leu-Glu-Xaa-Gln-Ala-Ala-Lys-Xaa-Phe-

29 30 31 32 33 34 35 36 37
Ile-Xaa-Trp-Leu-Val-Lys-Gly-Arg-R

Formula I (SEQ ID NO: 1)

wherein:

Xaa at position 8 is Val;

Xaa at position 22 is Gly;

Xaa at position 27 is Glu;

Xaa at position 30 is Ala;

R is Gly;

comprising the steps;

(a) preparing a glycine-free solution of the peptide at a pH of about 9.0 to about 12.0,

(b) adding glycine to a concentration between about 5 mM and about 250 mM,

(c) adding about 2% to about 20% by volume of an alcohol selected from the group consisting of ethanol and isopropanol, and about 0.2 to about 2.5 moles of zinc per mole of the peptide,

(d) adjusting the pH of the solution to between about pH 7.5 and about pH 10.5, and

(e) allowing crystals of the peptide to form.

25. The process according to claim 24, wherein the glycine-free solution of step (a) is at a pH of about 9.5 to about 11.5.

26. The process according to claim 25, wherein the glycine-free solution of step (a) is at a pH of about 10.0 to about 11.0.

27. The process according to any one of claims 24 to 26, wherein the glycine added in step (b) is in solid form.

28. The process according to any one of claims 24 to 27, wherein the glycine of step (b) is added to a concentration between about 40 mM and about 80 mM.

29. The process according to claim 28, wherein the glycine of step (b) is added to a concentration between about 55 mM and about 65 mM.

30. The process according to any one of claims 24 to 29, wherein the solution after completing step (b) is filtered prior to conducting step (c).

31. The process according to claim 30, wherein the filtration is conducted aseptically.

32. The process according to any one of claims 24 to 31, wherein at step (c) the alcohol is added prior to addition of the zinc.

33. The process according to any one of claims 24 to 32, wherein the addition of zinc in step (c) utilizes a solution comprising zinc and glycine.

34. The process according to any one of claims 24 to 33, wherein the additions of step (c) comprise about 1.0 to about 2.25 moles of zinc per mole of the peptide and result in a composition of about 5% to about 15% ethanol by volume.

35. The process according to any one of claims 24 to 34, wherein the pH of crystallization of step (d) is about 8.0 to about 10.0.

36. The process according to any one of claims 24 to 35, wherein the crystals formed in step (e) are thin plate crystals.

37. A process for preparing a pharmaceutical composition of crystals of a peptide of Formula I (SEQ ID NO: 1):

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

18 19 20 21 22 23 24 25 26 27 28
Ser-Tyr-Leu-Glu-Xaa-Gln-Ala-Ala-Lys-Xaa-Phe-

29 30 31 32 33 34 35 36 37
Ile-Xaa-Trp-Leu-Val-Lys-Gly-Arg-R

Formula I (SEQ ID NO: 1)

wherein:

Xaa at position 8 is Val;

Xaa at position 22 is Gly;

Xaa at position 27 is Glu;

Xaa at position 30 is Ala;

R is Gly;

comprising the steps;

(i) preparing crystals of a peptide of Formula I (SEQ ID NO: 1) by the process according to any one of claims 24 to 36,

(ii) lowering the pH of the crystal suspension formed in step (i) to a pH at which 97% or more of the peptide becomes insoluble,

(iii) adding a pharmaceutically acceptable preservative and a buffer selected from the group consisting of TRIS, maleate and succinate, and

(iv) adjusting the pH of the suspension resulting from step (iii) to between about 6.0 and about 8.5.

38. The process according to claim 37, wherein at step (ii) the pH of the crystal suspension is lowered to a pH at which 98% or more of the peptide becomes insoluble.

39. The process according to any one of claims 37 to 38, wherein the preservative of step (iii) is a phenolic preservative.

40. The process according to claim 39, wherein the phenolic preservative is m-cresol.

41. The process according to any one of claims 37 to 40, wherein the buffer of step (iii) is selected from the group consisting of TRIS and maleate.

42. The process according to claim 41, wherein the buffer of step (iii) is TRIS.

43. The process according to any one of claims 37 to 42, wherein step (iii) further comprises addition of a tonicity agent.

44. The process according to claim 43, wherein the tonicity agent added at step (iii) is glycerin.

45. The process according to claim 43, wherein the tonicity agent added at step (iii) is sodium chloride.

46. The process according to any one of claims 37 to 45, wherein the adjusted pH of step (iv) is about 6.5 to about 8.0.

47. The process according to claim 46, wherein the adjusted pH of step (iv) is about 7.0 to about 8.0.

48. A pharmaceutical composition prepared by the process according to any one of claims 37 to 47.

SEQUENCE LISTING

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Gln Ala Ala Lys Xaa Phe Ile Xaa Trp Leu Val Lys Gly Arg Xaa
            20                  25                  30
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1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
20 25 30