

(19) **DANMARK**

(10) **DK/EP 3257524 T3**



(12) **Oversættelse af
europæisk patentskrift**

Patent- og
Varemærkestyrelsen

-
- (51) Int.Cl.: **A 61 K 9/00 (2006.01)** **A 61 K 9/08 (2006.01)** **A 61 K 38/26 (2006.01)**
A 61 K 47/12 (2006.01) **A 61 K 47/18 (2017.01)** **A 61 K 47/26 (2006.01)**
A 61 P 3/04 (2006.01) **A 61 P 3/08 (2006.01)** **A 61 P 3/10 (2006.01)**
C 07 K 14/00 (2006.01) **C 07 K 16/00 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2020-11-23**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2020-08-26**
- (86) Europæisk ansøgning nr.: **16748688.5**
- (86) Europæisk indleveringsdag: **2016-02-03**
- (87) Den europæiske ansøgnings publiceringsdag: **2017-12-20**
- (86) International ansøgning nr.: **CN2016073279**
- (87) Internationalt publikationsnr.: **WO2016127887**
- (30) Prioritet: **2015-02-11 CN 201510071304**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
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- (54) Benævnelse: **STABILISERET OPLØSNINGSPRÆPARAT AF FARMACEUTISK GLP-1R-ANTISTOFFUSIONSPROTEIN**
- (56) Fremdragne publikationer:
EP-A1- 2 238 985
WO-A1-2006/068910
WO-A2-2009/009562
CN-A- 101 128 487
CN-A- 102 665 691
CN-A- 102 961 745
CN-A- 104 371 019
US-A1- 2011 098 443

DESCRIPTION

FIELD

[0001] The present invention relates to the technical field of biomedicines, especially relating to a stable pharmaceutical solution formulation of a GLP-1R antibody fusion protein.

BACKGROUND

[0002] GLP-1 derivatives are used in clinical trials to treat type II diabetes and obesity (Gallwitz B, Eur. Endocrinol., 2015; 11:21-5). GLP-1 induces multiple biological effects, for example, stimulation of insulin secretion, inhibition of glucagon secretion, inhibition of gastric emptying, inhibition of gastric and intestinal movement, as well as induction of loss of body weight (Lund A et al., Eur. J. Intern. Med., 2014; 25:407-14). One prominent characteristic of GLP-1 is its capability of stimulating the secretion of insulin without the risk of inducing hypoglycemia, which is one of the concerns in insulin treatment and some other oral therapies that promote endogenous insulin secretion. Endogenous GLP-1 is degraded very quickly and its extremely short half-life limits the effectiveness of GLP-1 as a therapeutic peptide.

[0003] Currently, there are various ways to prolong the half-life of GLP-1 and its derivatives and maintain their biological activity at the same time (Verspohl EJ, Pharmacol. Rev., 2012; 64:A-AX), including fusion of GLP-1 and its derivatives with an IgG Fc fragment or human serum albumin (HSA). The new method we have chosen is fusing GLP-1 with a full-length anti-GLP-1R antibody (IgG). The IgG can extend the *in vivo* half-life of its fusion partner and its own half-life in human is 21 days. In addition to maintaining the biological activity of GLP-1, a GLP-1R antibody fusion protein has advantageous stability provided by the IgG moiety. At the same time, the IgG moiety provides the GLP-1R antibody fusion protein molecular targeting properties, thus increasing the possibility of the interactions between GLP-1 and GLP-1R. Furthermore, the antibody molecule has lower immunogenicity than most of other commonly used fusion partners. As to a drug for a long-term or even life-long use, low immunogenicity is a necessity.

[0004] Fusion proteins are often produced by mammalian cell lines, for example, CHO, SP2/0 or NSO. It is noted in the present invention that, when CHO cells were used to produce a GLP-1R antibody fusion protein, the fusion protein was subjected to degradation by endogenous proteases or under certain cell culturing physicochemical conditions. The degradation process was faster and more apparent under low pH conditions, and on top of that, the GLP-1R antibody fusion protein was more prone to aggregation when pH was higher than 7. Furthermore, it was discovered in the present invention that under refrigeration conditions (2-8 °C) and in a single buffer solution (for example, a citrate buffer system), the solubility of the GLP-1R antibody fusion protein is too low to meet the requirements for therapeutic dosages. In

the present invention, the problems mentioned above are solved through pH control, using a specific combination of certain excipients and a specific concentration of the GLP-1R antibody fusion protein.

SUMMARY

[0005] The objective of the present invention is to provide a stable pharmaceutical solution formulation of a GLP-1R antibody fusion protein, and the formulation is stable with a long half-life *in vivo* and efficacious, and can be used for the treatment of diabetes, obesity and related diseases.

[0006] To solve the technical problems mentioned above, the present invention provides the following technical solutions.

[0007] A stable pharmaceutical solution formulation of a GLP-1R antibody fusion protein, comprises a therapeutically effective amount of the GLP-1R antibody fusion protein, an amino acid, a surfactant and a buffer system, wherein the final concentration of the amino acid is 100-180 mM, the final concentration of the surfactant is 0.05%-0.15%, and the pH of the stable solution formulation is from 5.0 to 8.0; wherein the amino acid is arginine and the surfactant is TWEEN-80.

[0008] To overcome the problems that a solution formulation of the GLP-1R antibody fusion protein is unstable at low pH and easily aggregated at high pH, and the GLP-1R antibody fusion protein does not have a sufficient solubility in a single buffer solution, the present invention provides a physiochemically stable formulation comprising a GLP-1R antibody fusion protein, and further comprising a buffering system, an amino acid as a stabilizer and an osmoregulator, and a surfactant. The stable solution formulation is stable for at least 6 months at 25 °C. Preferably, the stable solution formulation of the present invention comprises the GLP-1R antibody fusion protein at the final concentration of about 0.1-100 mg/mL, a citrate buffer at the concentration of 5-30 mM, TWEEN-80 at the final concentration of 0.05%-0.15%, and L-arginine at the final concentration of 100-180 mM, wherein the pH is from 5 to 8. The stable solution formulation enhances the solubility of the GLP-1R antibody fusion protein and its stability under special circumstances, especially at high temperatures. The present invention also includes a stable solution formulation for use in a method of treating diabetes, obesity and related diseases, which comprises administering the GLP-1R antibody fusion protein formulation of the present invention.

[0009] Preferably, the final concentration of the amino acid is 100-180 mM, the final concentration of the surfactant is 0.05%-0.15%, and the buffer system is a citrate buffer, and the pH of the stable solution formulation is from 5.5 to 7.0.

[0010] Preferably, the concentration of the citrate buffer is 5-30 mM.

[0011] Preferably, the amino acid is L-arginine, the final concentration of L-arginine is 100-180 mM, the surfactant is TWEEN-80, and the final concentration of TWEEN-80 is 0.05%-0.15%.

[0012] Preferably, the final concentration of the therapeutically effective amount of the GLP-1R antibody fusion protein is 0.1 mg/mL-100 mg/mL.

[0013] Preferably, the final concentration of the therapeutically effective amount of GLP-1R antibody fusion protein is 5 mg/mL-40 mg/mL.

[0014] Preferably, the amino acid sequence of the light chain variable domain of the GLP-1R antibody fusion protein is one selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, and the amino acid sequence of the heavy chain variable domain is one selected from SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9.

[0015] Preferably, the amino acid sequence of the light chain constant domain of the GLP-1R antibody fusion protein is SEQ ID NO: 10 or SEQ ID NO: 11, and the amino acid sequence of the heavy chain constant domain is SEQ ID NO: 12.

[0016] The GLP-1R antibody fusion protein of the present invention comprises GLP-1 or an analog thereof, which is fused at its C-terminus to the N-terminus of the light chain of a GLP-1R antibody by a peptide linker. The antibody fusion protein has a similar or improved biological activity and an extended half-life compared to GLP-1 and its analogs. The sequence of the light chain variable domain of the preferred GLP-1R antibody fusion protein comprises all or part of SEQ ID NO: 1, 2, 3, 4, 5 or 6, the sequence of the light chain constant domain comprises all or part of SEQ ID NO: 10 or 11, the sequence of the heavy chain variable domain comprises all or part of SEQ ID NO: 7, 8 or 9, and the sequence of the heavy chain constant domain comprises all or part of SEQ ID NO: 12. The sequence of the light chain variable domain of the more preferred GLP-1R antibody fusion protein is substantially SEQ ID NO: 1, 2, 3, 4, 5 or 6, the sequence of the light chain constant domain is substantially SEQ ID NO: 10 or 11, the sequence of the heavy chain variable domain is substantially SEQ ID NO: 7, 8 or 9, and the sequence of the heavy chain constant domain is substantially SEQ ID NO: 12. The sequence of the light chain variable domain of the most preferred GLP-1R antibody fusion protein is SEQ ID NO: 1, 2, 3, 4, 5 or 6, the sequence of the light chain constant domain is SEQ ID NO: 10 or 11, the sequence of heavy chain variable domain is SEQ ID NO: 7, 8 or 9, and the sequence of the heavy chain constant domain is SEQ ID NO: 12.

[0017] The stable solution formulation of the present invention can be used to treat diabetes or obesity.

[0018] The stable solution formulation of the present invention can be used to treat irritable bowel syndrome, and other diseases that benefit from decreasing plasma glucose, inhibition of gastric and/or bowel movement, inhibition of gastric and/or intestinal emptying, or inhibition of food intake.

[0019] The beneficial effects of the present invention are: stability, an *in vivo* long half-life, good efficacy, and suitability for the treatment of diabetes, obesity, irritable bowel syndrome and related diseases.

DETAILED DESCRIPTION

[0020] Through the following specific embodiments, the technical solutions of the present invention are further illustrated.

[0021] In this invention, unless referred specifically, the raw materials and instruments and the like employed are all commercially available or are commonly used in the art. The methods of the following embodiments, if not indicated specifically, are all conventional methods in the art.

[0022] The preparation of the GLP-1R antibody fusion protein of the present invention is described in a prior patent applicant: CN104371019A, WO 2015/021871.

[0023] Biological activity refers to the ability of a GLP-1R antibody fusion protein to bind and activate GLP-1R *in vivo* to elicit a response. Responses include, but are not limited to, promotion of insulin secretion, inhibition of glucagon secretion, inhibition of appetite, induction of weight loss, induction of satiety, inhibition of pancreatic β -cell apoptosis and induction of pancreatic β -cell proliferation.

[0024] The stable pharmaceutical solution formulation of a GLP-1R antibody fusion protein comprises the GLP-1R antibody fusion protein in a buffer, which further comprises an amino acid as a stabilizer and an osmoregulator, and a surfactant. At 25 °C, the stable solution formulation is stable for at least 6 months, and at 25 °C, more precisely, the stable solution formulation is stable for 6 months to 8 months, 6 months to 12 months, 6 months to 18 months, 6 months to 24 months, 8 months to 12 months, 8 months to 18 months, 8 months to 24 months, 12 months to 18 months, 12 months to 24 months, 18 months to 24 months.

[0025] The buffer system used in the present invention includes, but is not limited to, one or several of the following organic or inorganic compounds: citric acid, salts of citric acid, ascorbic acid, salts of ascorbic acid, gluconic acid, salts of gluconic acid, carbonic acid, salts of carbonic acid, tartaric acid, salts of tartaric acid, succinic acid, salts of succinic acid, acetic acid, salts of acetic acid, phthalic acid, salts of phthalic acid, phosphoric acid, phosphate, hydrochloric acid, Tris, thomethamine, and an amino acid, including, but not limited to, histidine, arginine, glycine.

[0026] The osmoregulator as defined in the present invention is a substance capable of increasing the osmotic pressure of a formulation upon addition. The osmoregulators are free amino acids, including, but not limited to, arginine, histidine, methionine, lysine, ornithine, leucine, isoleucine, alanine, glycine, glutamic acid and aspartic acid. The basic amino acid is preferably arginine, histidine, lysine or a combination thereof. The amino acid used in the present invention is arginine. The amino acid can be added in the form of a salt of an amino

acid where the added amino acid can be a D-amino acid such as D-arginine or an L-amino acid such as L-arginine.

[0027] The added amino acid is not limited to functioning just as an osmoregulator, but also as a stabilizer at the same time, including, but not limited to, arginine, histidine, methionine, lysine, ornithine, leucine, isoleucine, alanine, glycine, glutamine, glutamic acid, asparagine, aspartic acid, phenylalanine, tyrosine, serine, proline and tryptophan. Preferably, the concentration of the amino acid as a stabilizer and osmoregulator is from 1 to 500 mM.

[0028] Surfactants as defined in the present invention are amphiphilic organic compounds, that is, compounds contain groups having different soluble properties. Typically, these compounds contain both lipophilic hydrocarbon groups and hydrophilic ionic groups. The surfactants include, but are not limited to, sorbitan fatty acid esters, e.g., sorbitan monocaprylate, sorbitan monolaurate, sorbitan monopalmitate, sorbitan trioleate; glycerine fatty acid esters, e.g., glycerine monocaprylate, glycerine monomyristate, glycerine monostearate; polyglycerine fatty acid esters, e.g., decaglyceryl monostearate, decaglyceryl distearate, decaglyceryl monolinoleate; polyoxyethylene sorbitan fatty acid esters, e.g., polyoxyethylene sorbitan monolaurate, wherein polyoxyethylene (20) sorbitan monolaurate is TWEEN-20 and polyoxyethylene sorbitan monopalmitate is TWEEN-40, polyoxyethylene sorbitan monooleate, wherein polyoxyethylene (80) sorbitan monooleate is TWEEN-80, polyoxyethylene sorbitan monostearate, wherein polyoxyethylene (60) sorbitan monostearate is TWEEN-60, polyoxyethylene sorbitan trioleate is TWEEN-85, and polyoxyethylene sorbitan tristearate is TWEEN-65; polyoxyethylene sorbitol fatty acid esters, e.g., polyoxyethylene sorbitol tetrastearate, polyoxyethylene sorbitol tetraoleate; polyoxyethylene glycerine fatty acid esters, e.g., polyoxyethylene glyceryl monostearate; polyethylene glycol fatty acid esters, e.g., polyethylene glycol distearate; polyoxyethylene alkyl ethers, e.g., polyoxyethylene lauryl ether; polyoxyethylene polyoxypropylene alkyl ethers, e.g., polyoxyethylene polyoxypropylene glycol, polyoxyethylene polyoxypropylene propyl ether, polyoxyethylene polyoxypropylene cetyl ether; polyoxyethylene alkylphenyl ethers, e.g., polyoxyethylene nonylphenyl ether; polyoxyethylene hydrogenated castor oils, e.g., polyoxyethylene castor oil, polyoxyethylene hydrogenated castor oil; polyoxyethylene beeswax derivatives, e.g., polyoxyethylene sorbitol beeswax; polyoxyethylene lanolin derivatives, e.g., polyoxyethylene lanolin; and polyoxyethylene fatty acid amides, e.g., polyoxyethylene stearic acid amide; C10-C18 alkyl sulfates, e.g., sodium cetyl sulfate, sodium lauryl sulfate, sodium oleyl sulfate; polyoxyethylene C10-C16 alkyl ether sulfate with an average of 2 to 4 moles of ethylene oxide units added, e.g., sodium polyoxyethylene lauryl sulfate; and C1-C18 alkyl sulfosuccinate ester salts, e.g., sodium lauryl sulfosuccinate ester; and natural surfactants such as lecithin, glycerophospholipid, sphingophospholipids, e.g., sphingomyelin, and sucrose esters of C12-C18 fatty acids. The surfactant can include one or more of the surfactants described hereinabove. More suitable surfactants are polyoxyethylene sorbitan fatty acid esters, e.g., TWEEN-20, TWEEN-40, TWEEN-60 and TWEEN-80.

[0029] The stable pharmaceutically solution formulation of a GLP-1R fusion protein comprises the GLP-1R antibody fusion protein at a final concentration from about 0.1 to about 100

mg/mL. The preferred concentration (mg/mL) of the GLP-1R antibody fusion protein ranges from about 0.1 to 1, 1 to 5, 5 to 10, 5 to 20, 10 to 20, 20 to 30, 20 to 40, 30 to 40, 40 to 50, 50 to 60, 60 to 70, 70 to 80, 80 to 90, 90 to 100 mg/mL. More preferably, the concentration (mg/mL) of the GLP-1R antibody fusion protein is about 0.1, about 0.25, about 0.5, about 1, about 2, about 3, about 4, about 5, about 6.5, about 8, about 10, about 12.5, about 15, about 17.5, about 20, about 22.5, about 25, about 27.5, about 30, about 32.5, about 35, about 37.5, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100 mg/mL.

[0030] The preferred buffer system of the stable solution formulation is a citrate salt at it's a concentration ranging from about 5 to 30 mM. More preferably, the citrate concentration (mM) is ranging from about 5 to 25, 5 to 20, 5 to 15, 5 to 12.5, 5 to 10, 7.5 to 30, 7.5 to 25, 7.5 to 20, 7.5 to 15, 7.5 To 12.5, 7.5 to 10, 8 to 30, 8 to 25, 8 to 20, 8 to 15, 8 to 12.5, 8 to 11, 8 to 10, 9 to 30, 9 to 25, 9 to 20, 9 to 15, 9 to 12.5, 10 to 30, 10 to 25, 10 to 20, 10 to 17.5, 10 to 15, 10 to 12.5, 12.5 to 30, 12.5 to 25, 12.5 to 20, 12.5 to 15, 15 to 30, 15 to 25, 15 to 20, 17.5 to 30, 17.5 to 25, 17.5 to 22.5, 17.5 to 20, 20 to 30, 20 to 27.5, 20 to 25, 20 to 22.5, 22.5 to 30, 22.5 to 27.5, 22.5 to 25 , 25 to 30, 25 to 27.5, 27.5 to 30. A further preferred citrate concentration is about 5 to 20 mM. A particularly preferred citrate concentration is about 10, 10.0, 20 or 20.0 mM.

[0031] The pH of the stable pharmaceutical solution formulation of the GLP-1R fusion protein of the present invention ranges from about 5 to 8. The pH range provides acceptable stability for the formulation to maintain the solubility of the GLP-1R antibody fusion protein and to promote the activity of stimulating insulin secretion, and suitable for parenteral administration. The pH can be adjusted to the desired pH by adding an acid, e.g., HCl, or by adding a base, e.g., NaOH or by a combination of a citrate buffer and citric acid to achieve the desired buffer concentration and pH value. The preferred pH ranges from 5 to 7.75, 5 to 7.5, 5 to 7.25, 5 to 7.0, 5 to 6.75, 5 to 6.5, 5 to 6.25, 5 to 6.0, 5 to 5.75, 5 to 5.5, 5 to 5.25, 5.25 to 8.0, 5.25 to 7.75, 5.25 to 7.5, 5.25 to 7.25, 5.25 to 7.0, 5.25 to 6.75, 5.25 to 6.5, 5.25 to 6.25, 5.25 to 6.0, 5.25 to 5.75, 5.25 to 5.5, 5.5 to 8.0, 5.5 to 7.75, 5.5 to 7.5, 5.5 to 7.25, 5.5 to 7.0, 5.5 to 6.75, 5.5 to 6.5, 5.5 to 6.25, 5.5 to 6.0, 5.5 to 5.75, 5.75 to 8.0, 5.75 to 7.75, 5.75 to 7.5, 5.75 to 7.25, 5.75 to 7.0, 5.75 to 6.75, 5.75 to 6.5, 5.75 to 6.25, 5.75 to 6.0, 6.0 to 8.0, 6.0 to 7.75, 6.0 to 7.5, 6.0 to 7.25, 6.0 to 7.0, 6.0 to 6.75, 6.0 to 6.5, 6.0 to 6.25, 6.25 to 8.0, 6.25 to 7.75, 6.25 to 7.5, 6.25 to 7.25, 6.25 to 7.0, 6.25 to 6.75, 6.25 to 6.5, 6.5 to 8.0, 6.5 to 7.75, 6.5 to 7.5, 6.5 to 7.25, 6.5 to 7.0, 6.5 to 6.75, 6.75 to 8.0, 6.75 to 7.75, 6.75 to 7.5, 6.75 to 7.25, 6.75 to 7.0, 7.0 to 8.0, 7.0 to 7.75, 7.0 to 7.5, 7.0 to 7.25, 7.25 to 8.0, 7.25 to 7.5, 7.5 to 8.0, 7.5 to 7.75, 7.75 to 8.0. The further preferred pH ranges from 6 to 7, and particularly preferred pH is about 6.5 or 6.50.

[0032] The present description preferably utilizes L-arginine as an osmoregulator and stabilizer for the stable pharmaceutical solution formulation of the GLP-1R fusion protein at a concentration ranging from 1 to 500 mM to stabilize the GLP-1R antibody fusion protein and to increase the solubility, and to adjust the osmotic pressure of the formulation so that it is suitable for parenteral administration and the like. The preferred L-arginine concentration

ranges from 80 to 200 mM. Further preferred concentration ranges from 80 to 190, 80 to 180, 80 to 170, 80 to 160, 80 to 150, 80 to 140, 80 to 130, 80 to 120, 80 to 110, 80 to 100, 80 to 90, 90 to 200, 90 to 190, 90 to 180, 90 to 170, 90 to 160, 90 to 150, 90 to 140, 90 to 130, 90 to 120, 90 to 110, 90 to 100, 100 to 200, 100 to 190, 100 to 180, 100 to 170, 100 to 160, 100 to 150, 100 to 140, 100 to 130, 100 to 120, 100 to 110, 110 to 200, 110 to 190, 110 to 180, 110 to 170, 110 to 160, 110 to 150, 110 to 140, 110 to 130, 110 to 120, 120 to 200, 120 to 190, 120 to 180, 120 to 170, 120 to 160, 120 to 150, 120 to 140, 120 to 130, 130 to 200, 130 to 190, 130 to 180, 130 to 170, 130 to 160, 130 to 150, 130 to 140, 140 to 200, 140 to 190, 140 to 180, 140 to 170, 140 to 160, 140 to 150, 150 to 200, 150 to 190, 150 to 180, 150 to 170, 150 to 160, 160 to 200, 160 to 190, 160 to 180, 160 to 170, 170 to 200, 170 to 190, 170 to 180, 180 to 190, 190 to 200. Further preferred L-arginine concentration is 100 to 180 mM. Particularly preferred concentration is about 138 mM or about 138.0 mM.

[0033] The present description preferably utilizes TWEEN-80 as a surfactant for the stable pharmaceutical solution formulation of a GLP-1R fusion protein at a concentration ranging from 0.01% to 0.5%. The preferred concentration of TWEEN-80 ranges from about 0.01% to about 0.2%, and the preferred concentration range is determined by the combination of GLP-1R antibody fusion protein and arginine so that the formation of soluble aggregates and insoluble particles are minimized. The further preferred concentration of TWEEN-80 ranges from about 0.01% to 0.2%, 0.01% to 0.15%, 0.01% to 0.1%, 0.01% to 0.05%, 0.01% to 0.025%, 0.025% to 0.2%, 0.025% to 0.15%, 0.025% to 0.1%, 0.025% to 0.05%, 0.05 to 0.2%, 0.05 to 0.15%, 0.05 to 0.1%, 0.05 to 0.075%, 0.075% to 0.2%, 0.075% to 0.15%, 0.075% to 0.1%, 0.1% to 0.2%, 0.1% to 0.15%, 0.15% to 0.2%. The further preferred TWEEN-80 concentration ranges from about 0.05% to about 0.15%. Particularly preferred TWEEN-80 concentration is about 0.1%.

[0034] A particularly preferred stable pharmaceutical solution formulation of a GLP-1R fusion protein comprises a GLP-1R antibody fusion protein at the concentration ranging from about 5 to about 20 mg/mL, a citrate buffer at the concentration of about 20 mM, TWEEN-80 at the concentration of about 0.1%, L-arginine at the concentration of about 138 mM, with a pH of about 6.5. Another particularly preferred stable pharmaceutical solution formulation of the GLP-1R fusion protein comprises a GLP-1R antibody fusion protein at the concentration ranging from about 20 to about 40 mg/mL, a citrate buffer at the concentration of about 20 mM, TWEEN-80 at the concentration of about 0.1%, L-arginine at the concentration of about 138 mM, with a pH of about 6.5. Another particularly preferred stable solution formulation comprises a GLP-1R antibody fusion protein at the concentration ranging from about 5 to about 20 mg/mL, a citrate buffer at the concentration ranging from about 5 to 20 mM, TWEEN-80 at the concentration ranging from about 0.05 to about 0.15%, L-arginine at the concentration ranging from about 100 to 180 mM, with a pH ranging from about 6.0 to 7. Another particularly preferred stable solution formulation comprises a GLP-1R antibody fusion protein at the concentration ranging from about 20 to about 40 mg/mL, a citrate buffer at the concentration ranging from about 5 to 20 mM, TWEEN-80 at the concentration ranging from about 0.05 to 0.15%, L-arginine at the concentration ranging from about 100 to 180 mM, with a pH ranging from about 6.0 to 7.

[0035] The administration of the stable solution formulation can be carried out by any effective route known to physicians skilled in the art. Parenteral administration is one of the methods. Parenteral administration is generally understood in the medical literature to inject a dosage form into an individual *via* a sterile syringe or some other mechanical devices such as an infusion pump. Parenteral routes can include intravenous, intramuscular, subcutaneous and intraperitoneal routes. Subcutaneous administration is the preferred route.

[0036] The stable solution formulation of the present invention can be used to treat an individual with non-insulin dependent diabetes mellitus or in progression to non-insulin dependent diabetes mellitus, insulin-dependent diabetes mellitus or obesity. The effective amount of the GLP-1R antibody fusion protein in the stable solution formulation described herein is an amount that causes the desired therapeutic and/or prophylactic effect when administered to an individual in need of GLP-1R stimulation without causing undesirable side effects.

[0037] It is preferable that the fusion proteins are administered either once every two weeks or once a week. Depending on the disease being treated, it may be necessary to administer the fusion protein more frequently such as two to three times per week.

[0038] The present invention will now be described by the following non-limiting examples.

SPECIFIC EMBODIMENTS OF THE INVENTION

[0039] A reporter gene assay was used to determine the *in vitro* activation of GLP-1R by a GLP-1 fusion protein.

[0040] The CHO-DHFR- cells co-expressing hGLPIR-CRE-luciferase were seeded into a 96-well cell culture plate with 20000 cells per well, and the plate was cultured at 37 °C overnight. The culture supernatant was removed the next day. The cells were washed twice with serum free medium and the residual liquid was removed by suction. After adding 100 µL of a GLP-1R antibody fusion protein sample that was pre-diluted with a serum free medium or a control, the cells were incubated at 37 °C for 4 h. After the incubation, 100 µL of BRIGHT-GLO™ chemiluminescence substrate (Promega) were added. Finally, the cell lysates were transferred into a white 96-well plate, and the relative luminescence intensities were recorded in a SPECTRAMAX® L (Molecular Devices) microplate reader. The dose-response curves (response intensities versus logarithmic concentrations) were analyzed using GraphPad to determine EC50. SEC-HPLC was used to analyze the purity of a GLP-1R antibody fusion protein.

[0041] Size-exclusion chromatography (SEC-HPLC) was used to determine the formation of aggregates (soluble aggregates) of a GLP-1R antibody fusion protein and the loss of its monomeric form. In AGILENT 1100 HPLC at 25 °C, a TSK-G3000SWxl high performance SEC column was flushed with 200 mM phosphate buffer (pH 6.8) as a mobile phase till the baseline

of the UV absorbance was constant and stable. A stable pharmaceutical solution formulation of a GLP-1R antibody fusion protein at the concentration of 1-3 mg/mL (pre-diluted with the mobile phase) was injected in the amount of 50 μ L. The sample was eluted with the mobile phase at a flow rate of 0.5 mL/min and the absorbance at UV 280 nm was recorded. After each run, the AUCs of absorbance peaks of the monomer (the main peak), dimers and multimers were calculated, the percentage of the AUC for the main peak versus the total AUC was calculated and reported as the purity of the sample.

Example 1: Effect of pH on the stability of a GLP-1R antibody fusion protein:

[0042] pH can affect the solubility and stability of a GLP-1R antibody fusion protein, and is one of the most critical parameters in formulation. We determined the effect of pH on the stability of the GLP-1R antibody fusion protein in Example 1 by measuring the amount of soluble aggregates (dimers and multimers) formed, by measuring the ratio of the soluble aggregates formed (aggregate %) in each sample after 1 week or 3 weeks at 45 °C to determine the effect of each specific pH on the stability of the GLP-1R antibody fusion protein.

[0043] Stable pharmaceutical solution formulations of the GLP-1R fusion protein were prepared according to Table 1:

Table 1. GLP-1R antibody fusion protein formulation at different pH conditions

GLP-1R antibody fusion protein 4.5 mg/mL	pH	Buffer
1	6.5	0.1 M PBS
2	7	0.1 M PBS
3	7.5	0.1 M PBS
4	8	0.1 M PBS

[0044] A pharmaceutical stable solution formulation of a GLP-1R fusion protein was sterilized by filtration through 0.22 μ m polyvinylidene difluoride (PVDF) membrane. The solution was stored at 45 °C in 2 mL borosilicate glass vials until analysis or up to 3 weeks. The ratio of soluble aggregates in each sample was determined by SEC-HPLC. As shown in Table 2, the ratio of soluble aggregates formed was the lowest at the particularly preferred pH of 6.5 and the stability of the formulation was the best; and the stability tends to increase as the pH decreases.

Table 2. Ratios of soluble aggregates formed in a GLP-1R antibody fusion protein formulation at different pH conditions

GLP-1R antibody fusion protein 4.5 mg/mL	pH	Unit	Time point (week)	
			1	3
1	6.5	%	0.52	4.51
2	7.0	%	0.65	4.80

GLP-1R antibody fusion protein 4.5 mg/mL	pH	Unit	Time point (week)	
			1	3
3	7.5	%	1.12	8.16
4	8.0	%	1.39	14.79

Example 2: The robustness of the preferred formulation

[0045] We conducted a robustness study on GLP-1R antibody fusion protein formulations at a concentration of 10 mg/mL to examine if errors ($\pm 15\%$) in the three most important excipients or factors (L-arginine, pH and TWEEN-80) which would have a significant effect on the quality and stability of the drug in the actual production process. Based on general formulation rules and the properties of the GLP-1R antibody fusion protein, the changes in the monomeric purity (that is, the percentage of the main peak) were chosen as the criteria.

[0046] Formulations of a GLP-1R antibody fusion protein were prepared according to Table 3 for DOE experiments:

Table 3. GLP-1R antibody fusion protein formulations for DOE experiments

Formulation	L-arginine(mM)	TWEEN-80 (%)	pH
DOE-1	110	0.05	7
DOE-2	110	0.15	6
DOE-3	166	0.05	6
DOE-4	166	0.15	7
Preferred formulation (repeat 1)	138	0.1	6.5
Preferred formulation (repeat 2)	138	0.1	6.5
DOE-5	110	0.15	7
DOE-6	110	0.05	6
DOE-7	166	0.15	6
DOE-8	166	0.05	7

[0047] Formulations were prepared and sterilized by filtration through 0.22 μm polyvinylidene difluoride (PVDF) membrane. The formulations were stored in a 2 mL glass vial at 40 °C until analysis or up to 1 month. The purity of each formulation was determined by SEC-HPLC. As shown in Table 4, in the range of pH 6 to 7, L-arginine 110 to 166 mM and TWEEN-80 concentration of 0.05% to 0.15%, the purity for each formulation appeared to be similar and there was no significant difference, indicating that the preferred formulation was of good robustness, the deviation in production should not significantly affect the stability of the actual formulation.

Table 4. Purities of GLP-1R antibody fusion protein formulations in DOE experiments

Formulation	Purity (%)	Purity (%)	Purity (%)
	0 Day	15 Days	1 Month
DOE-1	99.76	96.18	95.94
DOE-2	99.77	95.59	95.34
DOE-3	99.75	96.42	96.20
DOE-4	99.75	94.43	94.10
Preferred formulation (repeat 1)	99.75	95.35	94.58
Preferred formulation (repeat 2)	99.75	95.84	94.88
DOE-5	99.74	96.38	95.91
DOE-6	99.74	95.60	95.23
DOE-7	99.75	96.44	96.33
DOE-8	99.75	94.43	93.77

Example 3: Effect of the concentrations of a GLP-1R antibody fusion protein on the formulation stability

[0048] We compared the performance of the preferred formulation at two different concentrations of the GLP-1R antibody fusion protein to determine whether the preferred formulation could provide acceptable stability at higher concentrations of GLP-1R antibody fusion protein to facilitate the high dosage use of GLP-1R antibody fusion protein in practice. We selected 10 and 20 mg/mL of the GLP-1R antibody fusion protein for comparison.

[0049] Formulations of the GLP-1R antibody fusion protein were prepared according to Table 5:

Table 5. Formulations of the GLP-1R antibody fusion protein at different protein concentrations

No.	GLP-1R antibody fusion protein concentration (mg/mL)	Citrate concentration (mM)	L-arginine (mM)	TWEEN-80 (%)	pH
5	10	20	138	0.1	6.5
6	20	20	138	0.1	6.5

[0050] The formulations were prepared and sterilized by filtration through 0.22 μ m polyvinylidene difluoride (PVDF) membrane. Each formulation was stored at 37 °C in a 2 mL glass vial until analysis or up to 1 month. The purity of each formulation was determined by

SEC-HPLC and its biological activity was determined by the reporter gene assay. As shown in Table 6, the preferred formulation provided a high stability for the GLP-1R antibody fusion protein at a concentration of 20 mg/mL, the change in its purity at 37 °C was similar to that at 10 mg/mL, only with non-significant changes, and at the same time, there was no significant difference as to their biological activity.

Table 6. Purities and biological activity of GLP-1R antibody fusion protein formulations at different protein concentrations

No.	Purity (%)	Purity (%)	Purity (%)	Purity (%)
	0 Day	7 Days	15 Days	1 Month
5	98.3	98.2	97.6	96.7
6	96.6	96.85	96.5	96.05
No.	Activity (EC50)	Activity (EC50)	Activity (EC50)	Activity (EC50)
	0 Day	7 Day	15 Day	1 Month
5	0.1 nM	0.08 nM	0.09 nM	0.11 nM
6	0.06 nM	0.07 nM	0.08 nM	0.08 nM

Example 4: Stability of the preferred formulation under vibration

[0051] We conducted a study on the stability of the preferred formulation under vibration conditions to investigate whether the formulation could provide stability for the GLP-1R antibody fusion protein in vibratory environments, to assess the effect of sample transportation and daily carrying.

[0052] The formulation of GLP-1R antibody fusion protein was prepared according to Table 7: Table 7. GLP-1R antibody fusion protein formulation for stability under vibration

No.	GLP-1R antibody fusion protein concentration (mg/mL)	Citrate concentration (mM)	L-arginine (mM)	TWEEN-80 (%)	pH
7	10	20	138	0.1	6.5

[0053] A formulation was prepared and sterilized by filtration through 0.22 µm polyvinylidene difluoride (PVDF) membrane. The formulation was stored in a 2 mL glass vial at 37 °C and placed on a shaker at a speed of 70 rpm until analysis or up to 15 days. The purity of each formulation was determined by SEC-HPLC, and the biological activity was determined by the reporter gene assay. As shown in Table 8, the stability and biological activity of the GLP-1R antibody fusion protein were maintained under the vibratory conditions.

Table 8. Purities and biological activity of GLP-1R Antibody Fusion Protein Formulation under vibration

No.	Purity (%)				
	0 Day	1 Day	5 Days	10 Days	15 Days
7	98.8	99.5	99.5	99.5	99.6
No.	Activity (EC50)				
	0 Day	1 Day	5 Days	10 Days	15 Days
7	0.13 nM	0.13 nM	0.13 nM	0.14 nM	0.16 nM

Example 5: Accelerated study on stability of the preferred formulation

[0054] We performed an accelerated stability study on the preferred formulation to rapidly examine the trend of the changes in the stability of the GLP-1R antibody fusion protein in the preferred formulation over time.

[0055] The formulation of the GLP-1R antibody fusion protein was prepared according to Table 9:

Table 9. GLP-1R antibody fusion protein formulation for accelerated stability study

No.	GLP-1R antibody fusion protein concentration (mg/mL)	Citrate concentration (mM)	L-arginine (mM)	TWEEN-80 (%)	pH
8	10	20	138	0.1	6.5

[0056] The formulation was prepared and sterilized by filtration through 0.22 μ m polyvinylidene difluoride (PVDF) membrane. The formulation was stored at 25 °C in a 2 mL glass vial until analysis or up to 6 months. The purity of each formulation was determined by SEC-HPLC and its biological activity was determined by the reporter gene assay. As shown in Table 10, the GLP-1R antibody fusion protein formulation withstood a storage temperature of 25 °C for at least 6 months with no significant changes in the purity and biological activity, showing superior stability.

Table 10. Purities and biological activity GLP-1R antibody fusion protein formulation under accelerated conditions

No.	Purity (%)					
	0 Month	0.5 Month	1 Month	2 Months	4 Months	6 Months
8	98.4%	98.6%	98.8%	99.4%	97.6%	96.9%

No.	Activity (EC50)					
	0 Month	0.5 Month	1 Month	2 Months	4 Months	6 Months
8	0.17 nM	N/A	0.09 nM	0.08 nM	0.1 nM	0.05 nM

N/A = not tested.

SEQUENCE LISTING

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REFERENCES CITED IN THE DESCRIPTION

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- VERSPOHL EJPharmacol. Rev., 2012, vol. 64, [0003]

Patentkrav

1. Stabil farmaceutisk opløsningsformulering af et GLP-1R-antistoffusionsprotein, omfattende en terapeutisk effektiv mængde af GLP-1R-antistoffusionsproteinet, en aminosyre, et overfladeaktivt stof, og et buffersystem, hvor den endelige
5 koncentration af aminosyren er 100-180 mM, den endelige koncentration af det overfladeaktive stof er 0,05-0,15 %, og pH'en af den stabile opløsningsformulering er fra 5,0 til 8,0; hvor aminosyren er arginin og det overfladeaktive stof er TWEEN-80.
- 10 2. Den stabile opløsningsformulering ifølge krav 1, hvor den stabile opløsningsformulering er stabil i mindst 6 måneder ved 25 °C.
3. Den stabile opløsningsformulering ifølge krav 1, hvor buffersystemet er en citratbuffer og pH'en af den stabile opløsningsformulering er fra 5,5 til 7,0.
15
4. Den stabile opløsningsformulering ifølge krav 3, hvor koncentrationen af citratbufferen er 5-30 mM, fortrinsvis 20 mM.
5. Den stabile opløsningsformulering ifølge krav 3, hvor aminosyren er L-arginin
20 og den endelige koncentration af L-arginin er 120-150 mM, fortrinsvis 138 mM.
6. Den stabile opløsningsformulering ifølge krav 3, hvor den endelige koncentration af TWEEN-80 er 0,1 %.
- 25 7. Den stabile opløsningsformulering ifølge krav 1, hvor den endelige koncentration af GLP-1R-antistoffusionsproteinet er 0,1 mg/mL-100 mg/mL, fortrinsvis 5 mg/mL-40 mg/mL.
- 30 8. Den stabile opløsningsformulering ifølge et hvilket som helst af kravene 1-7, hvor aminosyresekvensen af det letkæde variable domæne af GLP-1R-antistoffusionsproteinet er et valgt fra: SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, og SEQ ID NO: 6; og aminosyresekvensen af det

tungkæde variable domæne af GLP-1R-antistoffusionsproteinet er et valgt fra:
SEQ ID NO: 7, SEQ ID NO: 8, og SEQ ID NO: 9.

9. Den stabile opløsningsformulering ifølge krav 8, hvor aminosyresekvensen af
5 det letkæde konstante domæne af GLP-1R-antistoffusionsproteinet er SEQ ID NO:
10 eller SEQ ID NO: 11, og aminosyresekvensen af det tungkæde konstante
domæne af GLP-1R-antistoffusionsproteinet er SEQ ID NO: 12.

10. Den stabile opløsningsformulering ifølge et hvilket som helst af kravene 1-9,
10 hvor koncentrationen af GLP-1R-antistoffusionsproteinet er 5 mg/mL-40 mg/mL,
koncentrationen af L-arginin er 138 mM, koncentrationen af TWEEN-80 er 0,1 %, og
pH'en er mellem 6 og 7.

11. Den stabile opløsningsformulering ifølge krav 10, hvor pH'en er omkring 6,5.
15

12. Den stabile opløsningsformulering ifølge krav 10, hvor koncentrationen af
GLP-1R-antistoffusionsproteinet er: 5 mg/mL, 10 mg/mL, 20 mg/mL, 30 mg/mL,
eller 40 mg/mL.

20 **13.** Den stabile opløsningsformulering ifølge et hvilket som helst af kravene 1-12,
hvor den stabile opløsningsformulering er lagret i en steril sprøjte.

14. Stabil farmaceutisk opløsningsformulering som defineret i et hvilket som helst
af kravene 1-13 til anvendelse i en fremgangsmåde til behandlingen af diabetes,
25 fedme, eller irritabel tyktarm.

15. Stabil farmaceutisk opløsningsformulering som defineret i et hvilket som helst
af kravene 1-13 til anvendelse i en fremgangsmåde til behandlingen af non-insulin
afhængig diabetes mellitus eller insulin-afhængig diabetes mellitus.