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(54) **Title**: GLUCAGON DELIVERY APPARATUSES AND RELATED METHODS

(57) **Abstract**: Disclosed is a glucagon delivery apparatus, and methods for its use, comprising a reservoir containing a composition comprising glucagon, a glucagon analogue, or a salt form of either thereof, a sensor configured to measure a patient's blood glucose level, and an electronic pump configured to intradermally, subcutaneously or intramuscularly deliver at least a portion of the composition to a patient based on the patient's measured blood glucose level.

DESCRIPTION

GLUCAGON DELIVERY APPARATUSES AND RELATED METHODS

CROSS-REFERENCE WITH RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Applications serial numbers 62/170,942 filed June 4, 2015 and 62/233,032 filed September 25, 2015, and U.S. Patent Application serial number 15/136,650 filed April 22, 2016, each of which is incorporated herein by references in its entirety.

BACKGROUND

1. Field of Invention

[0002] The present invention relates generally to glucagon delivery systems. In particular, the invention concerns the use of closed-loop, open-loop, and no-loop glucagon delivery systems that can be used to treat or prevent hypoglycemia from occurring in a patient.

2. Description of Related Art

[0003] Diabetes is a serious health problem in modern society. Insulin is a critical treatment for both type I and type II diabetes. Studies over the past two decades have demonstrated that tight metabolic control of glucose through the use of insulin not only reduces the incidence, but also delays the development of complications in people with type 1 and type 2 diabetes. Unfortunately, the intensive insulin therapy required to achieve tight glucose control is also associated with a significantly increased risk of developing hypoglycemia or “low blood sugar.”

[0004] Symptoms of hypoglycemia vary greatly among patients, but typically include tremor, palpitations, irritability, anxiety, nervousness, hunger, tachycardia, headache and pallor. The symptoms typically subside once plasma glucose is restored to normal levels. If hypoglycemia is not reversed, a further decrease in plasma glucose can lead to depletion of glucose in the central nervous system and associated neuroglycopenic symptoms, such as difficulty in concentration, slurred speech, blurred vision, reduction in body temperature, behavioral changes and, if not treated, unconsciousness, seizure and possibly death.

[0005] In general, hypoglycemia can be defined as minor to moderate hypoglycemia or as severe hypoglycemia as follows:

Minor to moderate hypoglycemia: Episodes that the patient can self-treat, regardless of the severity of symptoms, or any asymptomatic blood glucose measurements in which blood glucose levels are less than 70 mg/dL (3.9 mmol/L) and greater than 50 mg/dL (2.8 mmol/L).

5 **Severe hypoglycemia:** Operationally defined as an episode of hypoglycemia that the patient cannot self-treat so that external help is required. Typically, neuroglycopenic symptoms and cognitive impairment begin at a blood glucose level of about 50 mg/dL (2.8 mmol/L) and less.

[0006] Most episodes of minor to moderate hypoglycemia can be self-treated relatively 10 easily by ingesting fast-acting carbohydrates such as glucose tablets or food (juice, soft drinks or sugary snacks). Severe hypoglycemia, by definition, cannot be self-treated and thus requires external intervention. If the patient can swallow and is cooperative, it is appropriate to use gels or products such as honey or jelly placed inside the cheek. If the patient is unable to swallow, glucagon, which is injected subcutaneously or intramuscularly, is used to treat 15 severe hypoglycemia.

[0007] Glucagon is a naturally occurring peptide hormone that is 29 amino acids in length and is secreted by the α-cells of the pancreas. The principal function of glucagon is to maintain glucose production through both glycogenolysis and gluconeogenesis, mostly mediated via the liver. Glucagon is the primary counter-regulatory hormone to insulin and is 20 used as a first-line treatment of severe hypoglycemia in patients with diabetes.

[0008] Numerous attempts have been made to create a glucagon rescue medication for treating severe hypoglycemia in emergency situations. Currently, there are two glucagon kits currently available in the United States, manufactured by Eli Lilly (Glucagon Emergency Kit) and Novo Nordisk (GlucaGen® HypoKit). Both products combine a vial of freeze-dried 25 glucagon with a pre-filled syringe of aqueous diluent to prepare a 1 mg/mL glucagon solution. The freeze-dried glucagon must be reconstituted using a complex procedure that is difficult to use in an emergency situation. These products also provide a large volume injection because glucagon is poorly soluble in water. Recently, attempts have been made to improve the stability of glucagon in an aqueous solution, to create more stable glucagon analogs and/or to 30 improve delivery of glucagon via powder injection.

[0009] Although some progress has been made with glucagon formulations and deliver methods, there still remains a need for more user-friendly methods of delivering glucagon to

patients to reduce or even prevent the occurrence of situations where the patient develops mild to moderate or severe hypoglycemic conditions.

SUMMARY OF THE INVENTION

[0010] A solution to the current problems associated with delivering glucagon to patients has been discovered. The solution is premised on using stable glucagon containing compositions in combination with pump-based delivery systems rather than the direct injection devices discussed above (i.e., the Eli Lilly and Novo Nordisk products). Such pump-based systems can be closed-loop, open-loop, or no-loop systems. The formulations that can be used with such systems are designed to be carried or stored in a pump container without having to be reconstituted (i.e., they are readily available to be administered to the patient from the pump container). Further, the formulations are stable at non-refrigerated temperatures (20-35 °C) for extended periods (>2 months) (i.e., the formulations can be safely stored in the pump container without risking substantial loss in activity of the glucagon in the formulation).

[0011] The pump-based system can include: (1) a glucose sensor that is or can be inserted in a patient and that is capable of measuring blood glucose levels (e.g., either directly via contact with the patient's blood or indirectly via contact with the patient's interstitial fluid); (2) a transmitter that sends the glucose information from the sensor to a monitor (e.g., via radio frequency transmission); (3) a pump that is designed to store and deliver the glucose formulation to the patient; and/or (4) a monitor (e.g., one that can be built into the pump device or a stand-alone monitor) that displays or records glucose levels. For a closed-loop system, the glucose monitor can be capable of modifying the delivery of the glucagon formulation to the patient via the pump based upon an algorithm. Such a closed-loop system requires little to no input from the patient and instead actively monitors blood glucose levels and administers the needed amount of the glucagon formulation to the patient to maintain an appropriate glucose level and prevent the occurrence of hypoglycemia. For an open-loop system, the patient would actively participate by reading their glucose monitor and adjusting the delivery rate/dose based on information provided by the monitor. For a no-loop system, the pump would deliver the glucagon formulation at a fixed (or basal) dose. The no-loop system can be used without a glucose monitor and without a glucose sensor if so desired.

[0012] In aspect of the present invention there is disclosed a glucagon delivery apparatus comprising a reservoir containing a composition comprising glucagon, a glucagon analogue, or a salt form of either thereof, a sensor configured to measure a patient's blood glucose level,

and an electronic pump configured to intradermally, subcutaneously or intramuscularly deliver at least a portion of the composition to a patient based on the patient's measured blood glucose level. The sensor can be positioned on the patient such that it contacts the patient's blood or contacts the patient's interstitial fluid or both. The sensor can be configured to
5 transmit data (for example, wirelessly, via radio frequency, or via a wired connection) to a processor configured to control operation of the electronic pump. The processor can be configured to control operation of the pump based, at least in part, on the data obtained by the sensor. In one instance, the processor can be configured to control operation of the pump to intradermally, subcutaneously or intramuscularly inject at least a portion of the composition if
10 the data obtained by the sensor indicates a glucose level below a defined threshold or indication that a defined threshold will breached in a particular period of time (e.g., an indication of impending hypoglycemia or an indication that the blood glucose levels will fall to below 70, 60, or 50 mg/dL within a certain period of time (*e.g.*, within 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 minute(s)). Such an indication can be determined by identifying a
15 downward trend of blood glucose levels (*e.g.*, by the blood glucose monitoring device) as well as the speed or trajectory of this downward trend. The glucagon delivery apparatus can also include a monitor configured to communicate information indicative of the patient's glucose level. The monitor can include a speaker or a display device, or both. The monitor can be configured to communicate an alert when a glucose level of the patient is estimated to
20 be at a defined threshold. Still further, the apparatus can be configured to allow manual adjustment of at least one of a delivery rate and a dose of the composition intradermally, subcutaneously or intramuscularly delivered by the pump.

[0013] In certain instances, the composition does not include a drug capable of decreasing the blood glucose level in the patient. Similarly, and in certain instances, the apparatus can be
25 configured such that it is not capable of injecting a composition comprising a drug capable of decreasing the blood glucose level in the patient (e.g., the apparatus does not include such a composition in its reservoir to be administered to a patient). In other instances, the composition can also include a drug capable of decreasing the blood glucose level in the patient. Similarly, and in certain instances, the apparatus can be configured such that it is
30 capable of injecting a composition comprising a drug capable of decreasing the blood glucose level (e.g., the apparatus includes in its reservoir a second composition having such a drug). The reservoir of the apparatus can have a single container or can have multiple containers for multiple compositions. By way of example only, a reservoir having at least two containers

can include one composition in one container that increases blood glucose levels (e.g., glucagon containing composition) and another that decreases blood glucose levels in a second container. This can result in the apparatus of the present invention operating as a fully operational artificial pancreas. Non-limiting examples of drugs that can decrease blood glucose levels in the patient include insulin, an insulin mimetic peptide, incretin, or an incretin mimetic peptide.

[0014] In certain aspects of the present invention, the apparatus is configured to be a closed-loop system. In other instances, it is configured to be an open-loop system. In still further instances, it is configured to be a no-loop system.

[0015] The composition than can be included in the reservoir of the apparatus can be a single-phase solution comprising the glucagon, glucagon analogue, or a salt form of either thereof, dissolved in a non-aqueous solvent. In particular instances, the glucagon, glucagon analogue, or a salt form of either thereof, can be fully solubilized in an aprotic polar solvent. Therapeutic molecules typically require an optimal or beneficial ionization profile in order to exhibit prolonged stability when solubilized in an aprotic polar solvent system. An optimal or beneficial ionization profile of a therapeutic molecule may be obtained by direct dissolution of the therapeutic agent in an aprotic polar solvent system containing a specified concentration of at least one ionization stabilizing excipient. Compositions for use with the present invention are stable formulations containing at least one therapeutic molecule solubilized in an aprotic polar solvent system. In certain aspect the therapeutic molecule does not need to be previously dried from a buffered aqueous solution prior to reconstitution in the aprotic polar solvent system.

[0016] In certain aspects a therapeutic agent is directly dissolved (e.g. a powder as received from a commercial manufacturer or supplier) along with an effective amount of an ionization stabilizing excipient for establishing an appropriate ionization of the therapeutic agent in the aprotic polar solvent system.

[0017] In particular, the ability to circumvent the need for drying the peptide from a buffered aqueous solution, for example via lyophilization, prior to reconstitution in the aprotic polar solvent system is anticipated to save considerable time and cost throughout the various product development stages. It is well-known that the development of a drying method is an expensive and time-intensive processing step that often must be tailored to each therapeutic molecule. Further, during manufacturing the ability to scale-up the drying step is complicated by the use of equipment and/or instruments that differ considerably from those employed at

the lab-scale, where the processing steps were initially studied and optimized. Accordingly, the ability to prepare a stable therapeutic peptide formulation via direct dissolution of the active ingredient in the aprotic polar solvent system, in the absence of such a drying step, will facilitate scale-up and manufacturing by eliminating a costly and time-consuming processing step. Further, during drying the therapeutic agent is exposed to multiple stresses than can degrade the molecule, and stabilizing excipients (e.g., disaccharides such as trehalose and sucrose) are often added to the formulation primarily to protect against degradation of the active agent during the drying process. By eliminating or minimizing the drying step the use of additional stabilizing excipients, particularly those that are often included to provide stability during the drying step, may be minimized, thereby allowing for the overall formulation to be simplified.

[0018] Stable solutions of a therapeutic agent(s) solubilized in non-aqueous aprotic polar solvents (e.g. DMSO), can be prepared by adding a specific predetermined amount of a compound, or combination of compounds, that function as an ionization stabilizing excipient. The amount can be determined by titration studies using the therapeutic agent and the ionization stabilizing excipient. Without wishing to be bound by theory, it is believed that the ionization stabilizing excipient can act as a proton source (e.g., a molecule that can donate a proton to the therapeutic molecule) in the aprotic polar solvent system that may protonate the ionogenic groups on the therapeutic molecule such that the therapeutic molecule possesses an ionization profile having an improved physical and chemical stability in the aprotic polar solvent system.

[0019] Certain embodiments are directed to a formulation of a therapeutic agent comprising a therapeutic agent at a concentration of at least, at most, or about 0.1, 1, 10, 50, or 100 mg/mL to 150, 200, 300, 400, or 500 mg/ml or up to the solubility limit of the therapeutic agent in the aprotic polar solvent system comprising a concentration of at least one ionization stabilizing excipient that provides physical and chemical stability to the therapeutic agent. In certain aspects the therapeutic agent is a peptide. The formulation can comprise an ionization stabilizing excipient at a concentration of at least, at most, or about 0.01, 0.1, 0.5, 1, 10, or 50 mM to 10, 50, 75, 100, 500, 1000 mM, or up to the solubility limit of the ionization stabilizing excipient in the aprotic polar solvent system. In certain aspects the ionization stabilizing excipient concentration is between 0.1 mM to 100 mM. In certain embodiments the ionization stabilizing excipient may be a suitable mineral acid, such as hydrochloric acid. In certain aspects the ionization stabilizing excipient may be an organic

acid, such as an amino acid, amino acid derivative, or the salt of an amino acid or amino acid derivative (examples include glycine, trimethylglycine (betaine), glycine hydrochloride, and trimethylglycine (betaine) hydrochloride). In a further aspect the amino acid can be glycine or the amino acid derivative trimethylglycine. In certain aspects a peptide is less than 150, 100, 75, 50, or 25 amino acids. In further aspects the aprotic solvent system comprises DMSO. The aprotic solvent can be deoxygenated, e.g., deoxygenated DMSO. In certain aspects the therapeutic agent is glucagon or salt thereof.

[0020] Compositions to be used in conjunction with the present invention can be made by: (a) calculating or determining the appropriate ionization stabilizing excipient or proton concentration needed to achieve a stabilizing ionization profile of a target therapeutic agent (e.g., a peptide(s) or small molecule(s)) in an aprotic polar solvent system; (b) mixing at least one ionization stabilizing excipient with the aprotic polar solvent system to attain an appropriate ionization environment that provides the ionization profile determined in step (a); and (c) solubilizing the target therapeutic agent(s) in the aprotic solvent having an appropriate environment to physically and chemically stabilize the therapeutic agent. In certain aspects the dissolution of the therapeutic agent and the addition of the ionization stabilizing excipient to the aprotic polar solvent system can be done in any order or concurrently, thus the ionization stabilizing excipient can be mixed first followed by dissolution of the therapeutic agent, or the therapeutic agent can be dissolved followed by addition of the ionization stabilizing excipient to the solution, or the ionization stabilizing excipient and the therapeutic agent can be added or dissolved in an aprotic polar solvent system concurrently. In a further aspect the entire amount of a component (e.g., a therapeutic agent or an ionization stabilizing excipient) need not to be mixed at a particular point; that is, a portion of the one or more components can be mixed first, second, or concurrently, and another portion mixed at another time, first, second, or concurrently. In certain aspects the therapeutic agent can be a peptide, and the ionization stabilizing excipient may be a suitable mineral acid, such as hydrochloric acid. In certain aspects the peptide(s) is less than 150, 100, 75, 50, or 25 amino acids. The concentration of the therapeutic agent and/or ionization stabilizing excipient added to the solution can be between 0.01, 0.1, 1, 10, 100, 1000 mM to its solubility limit, including all values and ranges there between. In certain aspects the aprotic polar solvent system is deoxygenated. In a further aspect the aprotic polar solvent system comprises, consists essentially of, or consists of DMSO or deoxygenated DMSO.

[0021] In other aspects a composition can further include a carbohydrate, an amphoteric molecule, and optionally an acid. In one instance, the aprotic polar solvent can be DMSO, the carbohydrate can be trehalose, the amphoteric molecule can be glycine, and the optional acid can be hydrochloric acid. The composition can include at least 80 wt.% of the aprotic polar solvent, 3 to 7 wt. % of the carbohydrate, 0.001 to 0.1 wt. % of the amphoteric molecule, and 0 wt. % to less than 0.1 wt. % of the acid. The composition can comprise, consists essentially of, or consist of glucagon, the glucagon analogue, or the salt form of either thereof, the aprotic polar solvent, the amphoteric molecule, the carbohydrate, and optionally the acid. The composition can have a water content of 0 to less than 15 wt. %, 0 to less than 3 wt. %, 3 to 10 wt. %, or 5 to 8 wt. %. The glucagon, glucagon analogue, or salt form of either thereof, can have been previously dried from a buffer, wherein the dried glucagon, glucagon analogue, or salt form of either thereof, has a first ionization profile that corresponds to an optimal stability and solubility for the glucagon, glucagon analogue, or salt form thereof, wherein the dried glucagon, glucagon analogue, or salt form of either thereof, is reconstituted into an aprotic polar solvent and has a second ionization profile in the aprotic polar solvent, and wherein the first and second ionization profiles are within 1 pH unit of one another. The first or second or both ionization profiles can correspond to the ionization profile of glucagon when solubilized in an aqueous solution having a pH range of about 1 to 4 or 2 to 3. The composition can be capable of providing a T_{max} for glucagon or the glucagon analogue of 1 minutes to 160 minutes after subcutaneous administration to the subject, and/or is capable of providing a C_{max} for glucagon or the glucagon analogue of 50 pg/dL to 1300 pg/dL after subcutaneous administration to the subject. The composition can be capable of providing a T_{max} for glucagon or the glucagon analogue of 5 minutes to 50 minutes after subcutaneous administration to the subject and/or wherein the composition is capable of providing a C_{max} for glucagon or the glucagon analogue of 150 pg/dL to 550 pg/dL after subcutaneous administration to the subject. The composition can be capable of providing an AUC_{60} for glucagon or the glucagon analogue of 2000 pg min./dL to 40000 pg min./dL.

[0022] In another instance, the composition can be structured as a two-phase mixture of a powder dispersed in a liquid that is a non-solvent to the solid, where the powder comprises the glucagon, glucagon analogue, or a salt form of either thereof, and where the liquid is a pharmaceutically acceptable carrier, where the powder is homogeneously contained within a pharmaceutically acceptable carrier. The composition can be a paste, slurry, or suspension.

The powder can have a mean particle size ranging from 10 nanometers (0.01 microns) to about 100 microns, with no particles being larger than about 500 microns.

[0023] Due to the stability of the glucagon formulations being used with the apparatuses of the present invention, said formulations can be pre-loaded and stored in the reservoir and used over a period of time (e.g., at least 1, 2, 3, 4, 5, 6, 7, 14, 21, 30, 45, or 60 days). This allows the apparatuses to be used as closed-loop, open-loop, or no-loop pump devices for maintaining appropriate blood glucose levels to prevent or treat hypoglycemia in the patient. In particular instances, the composition is capable of remaining stable after being stored for one month or 6 months or 12 months or 18 months at room temperature.

[0024] Also disclosed is a method of treating or preventing hypoglycemia in a patient, the method comprising using any one of the glucagon delivery apparatus of the present invention to intradermally, subcutaneously or intramuscularly deliver at least a portion of a glucagon containing composition to the patient. The composition can be administered with the apparatus such that the sensor measures the patient's blood glucose level, data containing the patient's blood glucose level is transmitted to the processor, and the processor determines how much of the composition to administered to the patient. In a particular instance, the patient's measured blood glucose level can be from 0 mg/dL to less than 50 mg/dL or the data shows an indication of impending hypoglycemia before delivery of the composition, and where the patient has a blood glucose level from 50 mg/dL to 180 mg/dL within 1 to 20 minutes after delivery of the composition. The patient can have been previously diagnosed with Type 1, Type II, or gestational diabetes or with a hyperinsulinemic hypoglycemia disorder or both. Non-limiting examples of hyperinsulinemic hypoglycemia disorders include congenital hyperinsulinism, insulinoma, reactive hypoglycemia, or drug-induced hypoglycemia.

[0025] "Coupled" is defined as connected, although not necessarily directly, and not necessarily mechanically; two items that are "coupled" may be unitary with each other. The terms "a" and "an" are defined as one or more unless this disclosure explicitly requires otherwise. The term "substantially" is defined as largely but not necessarily wholly what is specified (and includes what is specified; e.g., substantially 90 degrees includes 90 degrees and substantially parallel includes parallel), as understood by a person of ordinary skill in the art.

[0026] Further, a device or system that is configured in a certain way is configured in at least that way, but it can also be configured in other ways than those specifically described.

[0027] A peptide's "optimal stability and solubility" refers to the pH environment wherein solubility of the peptide is high (at or near the maximum on a solubility versus pH profile, or suitable for the requirements of the product) and its degradation minimized relative to other pH environments. Notably, a peptide may have more than one pH of optimal stability and solubility. A person having ordinary skill in the art can easily ascertain a given peptide's optimal stability and solubility by referencing literature or by performing assays.

[0028] The term "dissolution" as used herein refers to a process by which a material(s) in a gas, solid, or liquid state becomes a solute(s), a dissolved component(s), of a solvent, forming a solution of the gas, liquid, or solid in the solvent. In certain aspects a therapeutic agent or an excipient, e.g., an ionization stabilizing excipient, is present in an amount up to its solubility limited or is fully solubilized. The term "dissolve" refers to a gas, liquid, or solid becoming incorporated into a solvent to form a solution.

[0029] The term "excipient" as used herein refers to a natural or synthetic substance formulated alongside the active or therapeutic ingredient (an ingredient that is not the active ingredient) of a medication, included for the purpose of stabilization, bulking, or to confer a therapeutic enhancement on the active ingredient in the final dosage form, such as facilitating drug absorption, reducing viscosity, enhancing solubility, adjusting tonicity, mitigating injection site discomfort, depressing the freezing point, or enhancing stability. Excipients can also be useful in the manufacturing process, to aid in the handling of the active substance concerned such as by facilitating powder flowability or non-stick properties, in addition to aiding in vitro stability such as prevention of denaturation or aggregation over the expected shelf life.

[0030] As used herein an "ionization stabilizing excipient" is an excipient that establishes and/or maintains a particular ionization state for a therapeutic agent. In certain aspects the ionization stabilizing excipient can be, or includes, a molecule that donates at least one proton under appropriate conditions or is a proton source. According to the Bronsted-Lowry definition, an acid is a molecule that can donate a proton to another molecule, which by accepting the donated proton may thus be classified as a base. As used in this application, and as will be understood by the skilled technician, the term "proton" refers to the hydrogen ion, hydrogen cation, or H^+. The hydrogen ion has no electrons and is composed of a nucleus that typically consists solely of a proton (for the most common hydrogen isotope, protium). Specifically, a molecule that can donate a proton to the therapeutic agent is considered an acid

or proton source, regardless of whether it is completely ionized, mostly ionized, partially ionized, mostly unionized, or completely unionized in the aprotic polar solvent.

[0031] As used herein a "mineral acid" is an acid that is derived from one or more inorganic compounds. Accordingly, mineral acids may also be referred to as "inorganic acids." Mineral acids may be monoprotic or polyprotic (e.g. diprotic, triprotic, etc.). Examples of mineral acids include hydrochloric acid (HCl), sulfuric acid (H_2SO_4), and phosphoric acid (H_3PO_4).

[0032] As used herein an "organic acid" is an organic compound with acidic properties (i.e. can function as a proton source). Carboxylic acids are one example of organic acids. Other known examples of organic acids include, but are not limited to, alcohols, thiols, enols, phenols, and sulfonic acids. Organic acids may be monoprotic or polyprotic (e.g. diprotic, triprotic, etc.)

[0033] "Charge profile," "charge state," "ionization state," and "ionization profile" may be used interchangeably and refer to the ionization state (i.e. due to protonation and/or deprotonation) of the peptide's ionogenic groups.

[0034] "Therapeutic agent" encompasses peptide compounds together with pharmaceutically acceptable salts thereof. Useful salts are known to those skilled in the art and include salts with inorganic acids, organic acids, inorganic bases, or organic bases. Therapeutic agents useful in the present invention are those peptide compounds that affects a desired, beneficial, and often pharmacological, effect upon administration to a human or an animal, whether alone or in combination with other pharmaceutical excipients or inert ingredients.

[0035] "Peptide," "polypeptide" and "peptide compound" refer to polymers of up to about 100 or more preferably up to about 80 amino acid residues bound together by amide (CONH) linkages. Analogs, derivatives, agonists, antagonists and pharmaceutically acceptable salts of any of the peptide compounds disclosed here are included in these terms. The terms also include peptides and peptide compounds that have D-amino acids, modified, derivatized or naturally occurring amino acids in the D- or L-configuration and/or peptomimetic units as part of their structure.

[0036] The term "glucagon" refers to the glucagon peptide, analogues thereof, and salt forms of either thereof.

[0037] As used herein, a "co-formulation" is a formulation that contains two or more therapeutic agents dissolved in an aprotic polar solvent system. The therapeutic agents may belong to the same class (for example, a co-formulation comprising two or more therapeutic peptides, such as insulin and pramlintide), or the therapeutic agents may belong to different classes (for example a co-formulation comprising one or more therapeutic small molecules and one or more therapeutic peptide molecules, such as GLP-1 and lisofylline).

[0038] "Patient," "subject," or "individual" refers to a mammal (*e.g.*, human, primate, dog, cat, bovine, ovine, porcine, equine, mouse, rate, hamster, rabbit, or guinea pig). In particular aspects, the patient is a human.

[0039] "Inhibiting" or "reducing" or any variation of these terms includes any measurable decrease or complete inhibition to achieve a desired result.

[0040] "Effective" or "treating" or "preventing" or any variation of these terms means adequate to accomplish a desired, expected, or intended result.

[0041] As used herein, the term "aprotic polar solvent" refers to a polar solvent which does not contain acidic hydrogen and thus does not act as a hydrogen bond donor. Polar aprotic solvents include, but are not limited to dimethylsulfoxide (DMSO), dimethylformamide (DMF), ethyl acetate, n-methyl pyrrolidone (NMP), dimethylacetamide (DMA), and propylene carbonate. An "aprotic polar solvent system" refers to a solution wherein the solvent is a single aprotic polar solvent (for example, neat DMSO), or a mixture of two or more aprotic polar solvents (for example, a mixture of DMSO and NMP).

[0042] "Single-phase solution" refers to a solution prepared from a powder dissolved in a solvent, or solvent system (e.g., mixture of two or more solvents), wherein the particulate matter is completely dissolved in the solvent and there is no longer particulate matter visible, such that the solution can be described as optically clear.

[0043] A "paste" is a two-phase mixture of a solid (e.g., a powder containing a medicament and stabilizing excipients, if necessary) dispersed in a liquid (e.g., a biocompatible diluent), which is a non-solvent to the solid (e.g., and thus, the diluent is typically, but not always, lipophilic in nature). Pastes are substances that behave as solids until a sufficiently large load or stress is applied, at which point the pastes flow like a liquid (e.g., pastes are semi-solids). The term pastes may include a concentrate of the therapeutic agent dispersed in a pharmaceutically acceptable carrier having a thick consistency to form a viscous semisolid.

[0044] "Slurry" means a thin paste.

[0045] "Buffer" refers to a weak acid or base that prevents rapid or significant changes in the pH of a solution following the addition of other acids and/or bases. When buffering agent are added to water, a buffered solution is formed. For example, a buffer solution may contain both a weak acid and its conjugate base, or a weak base and its conjugate acid. In common chemical usage, a pH buffer is a substance or a mixture of substances, which permits solutions to resist large changes in pH upon addition of small amounts of H^+ and OH^- ions. A common buffer mixture contains two substances, a conjugate acid (proton donor) and a conjugate base (proton acceptor). Together, the two species (the conjugate acid-base pair of a conjugate acid and conjugate base) resist large changes in pH of the solution by partially absorbing additions of H^+ and OH^- ions to the solution.

[0046] "Non-volatile buffer" refers to a buffer where the buffer components are not sufficiently volatile that they may be removed from the composition during drying (e.g., during lyophilization). Glycine, citrate, or phosphate buffers, or mixtures thereof are a few non-limiting examples of non-volatile buffers. In preferred instances, glycine buffers can be used as the non-volatile buffer.

[0047] "Partially-volatile buffer" refers to a buffer wherein one of the buffer components is sufficiently volatile that it may be removed from the composition during drying (e.g., during lyophilization). Ammonium phosphate is one non-limiting example of a partially-volatile buffer.

[0048] "Volatile buffer" refers to both of the buffer components being sufficiently volatile such that they may be removed from the composition during drying (e.g., during lyophilization). Ammonium formate is one non-limiting example of a volatile buffer.

[0049] "Organic phase buffer" refers to sufficiently hydrophobic weak acids/bases and their conjugate bases/acids such that even the ionized form of the buffer is soluble into the organic phase (e.g., hexane, heptane), and not be able to partition into an adjacent aqueous phase.

[0050] "Isoelectric point" (pI) of a peptide corresponds to the pH value where the overall net charge of the peptide is zero. Due to their varying composition with respect to their primary structures, peptides may have varying isoelectric points. In peptides there may be many charged groups (e.g., ionogenic groups that have been protonated or deprotonated) and at the isoelectric point the net sum of all these charges is zero, i.e. the number of negative

charges balances the number of positive charges. At a pH above the isoelectric point the overall net charge of the peptide will be negative, and at pH values below the isoelectric point the overall net charge of the peptide will be positive. There are multiple methods known in the art for determining the isoelectric point of a peptide, including experimental methods such 5 as isoelectric focusing, and theoretical methods where the isoelectric point may be estimated from the amino acid sequence of the peptide by computational algorithms.

[0051] "Reconstituted," when referring to a pharmaceutical composition, refers to a composition which has been formed by the addition of an appropriate non-aqueous solvent to a solid material comprising the active pharmaceutical ingredient. Pharmaceutical 10 compositions for reconstitution are typically applied where a liquid composition with acceptable shelf-life cannot be produced. An example of a reconstituted pharmaceutical composition is the solution which results when adding a biocompatible aprotic polar solvent (e.g., DMSO) to a freeze dried composition.

[0052] "Primary structure" refers to the linear sequence of amino acid residues that 15 comprise a peptide/polypeptide chain.

[0053] "Analogue" and "analog," when referring to a peptide, refers to a modified peptide wherein one or more amino acid residues of the peptide have been substituted by other amino acid residues, or wherein one or more amino acid residues have been deleted from the peptide, or wherein one or more amino acid residues have been added to the peptide, or any 20 combination of such modifications. Such addition, deletion or substitution of amino acid residues can take place at any point, or multiple points, along the primary structure comprising the peptide, including at the N-terminal of the peptide and/or at the C-terminal of the peptide.

[0054] "Derivative," in relation to a parent peptide, refers to a chemically modified parent 25 peptide or an analogue thereof, wherein at least one substituent is not present in the parent peptide or an analogue thereof. One such non-limiting example is a parent peptide which has been covalently modified. Typical modifications are amides, carbohydrates, alkyl groups, acyl groups, esters, pegylations and the like.

[0055] An "amphoteric species" is a molecule or ion that can react as an acid as well as a 30 base. These species can either donate or accept a proton. Examples include amino acids, which possess both amine and carboxylic acid functional groups. Amphoteric species further

include amphiprotic molecules, which contain at least one hydrogen atom, and have the ability to donate or accept a proton.

[0056] “Non-aqueous solvent” refers to a solvent that is not water.

[0057] A “therapeutically equivalent” drug is one that has essentially the same effect in the
5 treatment of a disease or condition as one or more other drugs. A drug that is therapeutically equivalent may or may not be chemically equivalent, bioequivalent, or generically equivalent.

[0058] “Water” or “moisture” content of the formulations of the present invention refers to the total amount of water present in a given formulation. Water can be “residual moisture” or “residual water”, either of which refers to the amount of water that a given formulation takes
10 up and retains from its environment. By way of example, the formulations of the present invention can be hygroscopic in that the formulation after initially being prepared may have 0 wt. % water but after a period of time (e.g., storage for one month), its water content increases to 2 wt. %. Therefore, the residual moisture or residual water content in such a formulation is 2 wt. %. Additionally, residual moisture may be present in the powder following drying (e.g.
15 via lyophilization) of the initial aqueous solution containing the peptide. The amount of residual moisture remaining due to incomplete removal during drying varies according to, among other factors, the instrument, batch size, processing parameters, but is typically less than 10 wt. %.

[0059] Alternatively, water may be used as a co-solvent in the context of the present
20 formulations, where the water can be used to depress the freezing point of the formulation. For example, a formulation could include 10 wt. % water as a co-solvent such that the formulation after its initial preparation has 10 wt. % water but after a period of time (e.g., storage for one month), its water content increases to 11 wt. %. Therefore, the residual moisture or residual water content in such a formulation is 1 wt. %, but the total water or
25 moisture content is 11 wt. %. In either instance, the formulations of the present invention can have a water or moisture content of less than 15 wt. %, less than 10 wt. %, less than 5 wt. %, less than 4 wt. %, less than 3 wt. %, less than 2 wt. %, or less than 1 wt. % after the formulations have been prepared. In specific embodiments, the composition can have a water content of 0 to less than 15 wt. %, 0 to less than 3 wt. %, 3 to 10 wt. %, or 5 to 8 wt. %.

30 **[0060]** “Bioavailability” refers to the extent to which the therapeutic agent, such as a peptide compound, is absorbed from the formulation.

[0061] "Systemic," with respect to delivery or administration of a therapeutic agent, such as a peptide compound, to a subject, that therapeutic agent is detectable at a biologically significant level in the blood plasma of the subject.

[0062] "Controlled release" refers to the release of the therapeutic agent at such a rate that blood (e.g., plasma) concentrations are maintained within the therapeutic range, but below toxic concentrations over a period of time of about one hour or longer, preferably 12 hours or longer.

[0063] "Pharmaceutically acceptable carrier" refers to a pharmaceutically acceptable solvent, suspending agent or vehicle for delivering a drug compound of the present invention to a mammal such as an animal or human.

[0064] "Pharmaceutically acceptable" ingredient, excipient or component is one that is suitable for use with humans and/or animals without undue adverse side effects (such as toxicity, irritation and allergic response) commensurate with a reasonable benefit/risk ratio.

[0065] "Chemical stability," when referring to a therapeutic agent, such as a peptide or salt thereof, refers to an acceptable percentage of degradation products produced by chemical pathways such as oxidation or hydrolysis is formed. In particular, a formulation is considered chemically stable if no more than about 20% breakdown products are formed after one year of storage at the intended storage temperature of the product (e.g., room temperature); or storage of the product at 30° C/ 60% relative humidity for one year; or storage of the product at 40° C /75% relative humidity for one month, and preferably three months. In some embodiments, a chemically stable formulation has less than 20%, less than 15%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% breakdown products formed after an extended period of storage at the intended storage temperature of the product.

[0066] "Physical stability," when referring to a therapeutic agent, such as a peptide or salt thereof, refers to an acceptable percentage of aggregates (e.g., dimers, trimers and larger forms) being formed. In particular, a formulation is considered physically stable if no more that about 15% aggregates are formed after one year of storage at the intended storage temperature of the product (e.g., room temperature); or storage of the product at 30° C / 60% relative humidity for one year; or storage of the product at 40° C / 75% relative humidity for one month, and preferably three months. In some embodiments, a physically stable formulation has less than less than 15%, less than 10%, less than 5%, less than 4%, less than

3%, less than 2%, or less than 1% aggregates formed after an extended period of storage at the intended storage temperature of the product.

[0067] "Stable formulation" refers to at least about 65% chemically and physically stable therapeutic agents, such as peptides or salts thereof, remain after two months of storage at room temperature. Particularly preferred formulations are those in which at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% chemically and physically stable therapeutic agent remains under these storage conditions. Especially preferred stable formulations are those which do not exhibit degradation after sterilizing irradiation (e.g., gamma, beta or electron beam).

[0068] "Mammal" or "mammalian" includes murine (e.g., rats, mice) mammals, rabbits, cats, dogs, pigs, and primates (e.g., monkey, apes, humans). In particular aspects in the context of the present invention, the mammal can be murine or human. The patient can be a mammal or a mammalian patient.

[0069] "Parenteral injection" refers to the administration of therapeutic agents, such as peptide compounds, via injection under or through one or more layers of skin or mucus membranes of an animal, such as a human. Standard parenteral injections are given into the intradermal, subcutaneous, or intramuscular region of an animal, e.g., a human patient. In some embodiments, a deep location is targeted for injection of a therapeutic agent as described herein.

[0070] The term "about" or "approximately" or "substantially unchanged" are defined as being close to as understood by one of ordinary skill in the art, and in one non-limiting embodiment the terms are defined to be within 10%, preferably within 5%, more preferably within 1%, and most preferably within 0.5%. Further, "substantially non-aqueous" refers to less than 5%, 4%, 3%, 2%, 1%, or less by weight or volume of water.

[0071] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[0072] The words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0073] The apparatuses, compositions, and methods of the present invention can "comprise," "consist essentially of," or "consist of" any of the claimed elements or steps disclosed throughout the specification. With respect to the transitional phase "consisting essentially of," in one non-limiting aspect, a basic and novel characteristic of the apparatuses of the present invention are their ability to deliver stable glucagon formulations to patients via closed-loop, open-loop, or no-loop pump-based devices.

[0074] The feature or features of one embodiment may be applied to other embodiments, even though not described or illustrated, unless expressly prohibited by this disclosure or the nature of the embodiments.

[0075] Some details associated with the embodiments described above and others are described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0076] The following drawings illustrate by way of example and not limitation. For the sake of brevity and clarity, every feature of a given structure is not always labeled in every figure in which that structure appears. Identical reference numbers do not necessarily indicate an identical structure. Rather, the same reference number may be used to indicate a similar feature or a feature with similar functionality, as may non-identical reference numbers. The figures are drawn to scale (unless otherwise noted), meaning the sizes of the depicted elements are accurate relative to each other for at least the embodiment depicted in the figures.

[0077] **FIG. 1** is a perspective view of a first embodiment of the present glucagon delivery apparatuses.

[0078] **FIG. 2** is a cross-sectional side view of various components of the glucagon delivery apparatus of FIG. 1 shown coupled to a patient.

[0079] **FIG. 3** is a schematic depicting various components of the glucagon delivery apparatus of FIG. 1.

[0080] **FIGS. 4A-4C** are side views of reservoirs containing various compositions of the present disclosure that are suitable for use in some embodiments of the present glucagon delivery apparatuses.

[0081] **FIG. 4D** is a top view of a reservoir suitable for use in some embodiments of the present glucagon delivery apparatuses.

[0082] **FIG. 5** depicts an illustrative flow chart of one example of closed-loop control of one embodiment of the present glucagon delivery apparatuses.

[0083] **FIG. 6** shows blood glucose data obtained from a human subject in an open-loop clinical trial. When the patient consumed a mixed-meal beverage (labeled 'MMTT') they experienced an increase in blood glucose followed by a rapid decline. When the 150 µg dose of glucagon was administered to the patient (labeled '150 mcg') hypoglycemia was arrested and blood glucose levels rose above 70 mg/dL.

DETAILED DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0084] Prior to the present invention, the typical process of treating hypoglycemia was with formulations such as those offered by Eli Lilly (Glucagon Emergency Kit) and Novo Nordisk (GlucaGen® HypoKit). These products are not made for nor used with pump-based devices, thereby limiting their use to effectively manage hypoglycemia on a consistent basis.

[0085] The present invention offers a solution to the current glucagon products and methods for managing or treating hypoglycemia. The solution is premised, in part, on a discovery that pump-based delivery systems that have been traditionally used to administered insulin to diabetic patients can be used to also treat hypoglycemia. Such systems, when combined with storage stable glucagon formulations, allow for a more consistent process for monitoring, treating, and preventing hypoglycemia in patients and avoiding the deleterious effects caused by hypoglycemia.

[0086] These and other aspects of the present invention are provided in non-limiting detail in the following subsections.

A. Glucagon Delivery Apparatuses and Related Methods

[0087] Referring now to FIGS. 1-4, shown therein and designated by the reference numeral 100 is a first embodiment of the present glucagon delivery apparatuses. In the depicted embodiment, apparatus 100 comprises a housing 104, which generally functions to locate and/or secure components of apparatus 100 relative to one another. In the embodiment shown, glucagon delivery apparatus 100 is configured to intradermally, subcutaneously or intramuscularly deliver a composition comprising glucagon to a patient.

[0088] In the depicted embodiment, apparatus 100 comprises a reservoir 108a, which in this embodiment, may be disposed and/or disposable within housing 104. For example, in this embodiment, housing 104 defines and/or is configured to allow access to a receptacle

112, which may be dimensioned to receive and/or allow removal and/or replacement of reservoir 108a within housing 104.

[0089] In this embodiment, reservoir 108a may comprise a composition (e.g., 116a, 116b, 116c, and/or the like) (sometimes referred to collectively as "composition 116" or "compositions 116"). The present glucagon delivery apparatuses can be used with any suitable storage stable composition, such as, for example, the glucagon containing formulations described throughout the present application.

[0090] In the embodiment shown, reservoir 108a comprises a cap 120. In this embodiment, cap 120 includes a puncturable seal 124 (e.g., which may be punctured by a needle or other sharp object external to or within apparatus 100, for example, when reservoir 108a is inserted into receptacle 112, to allow for communication of composition 116 from reservoir 108a to pump 128). In this way, compositions 116 can be stored prior to use, which may be facilitated by the stability of the compositions.

[0091] While some embodiments of the present glucagon delivery apparatuses do not comprise a composition having a protein or peptide capable of decreasing the blood glucose level of a patient, other embodiments may comprise a composition including a glucose-reducing formulation (e.g., insulin, an insulin memetic peptide, incretin, an incretin mimetic peptide, and/or the like, as described above). For example, some embodiments may comprise a (e.g., additional to reservoir 108a) reservoir 108b containing the glucose-reducing formulation, and, in such embodiments, pump 128 (described in more detail below) may be configured to intracutaneously delivery at least a portion of the glucose-reducing formulation (an example of such a configuration is depicted in FIG. 3, which may include a valve to selectively place either reservoir 108a or reservoir 108b in communication with pump 128). In these and similar embodiments, housing 104 may comprise a receptacle (e.g., 112) dimensioned to receive and/or allow removal and/or replacement of reservoir 108b within housing 104.

[0092] In the embodiment shown, apparatus 100 comprises an electronic pump 128 configured to intracutaneously delivery at least a portion of the composition to a patient. Pumps of the present disclosure can comprise any suitable pump, such as, for example, positive displacement pumps (e.g., gear pumps, screw pumps, peristaltic pumps, piston pumps, plunger pumps, and/or the like), centrifugal pumps, and/or the like. In this embodiment, pump 128 is electronic (e.g., is configured to be actuated electrically, for example, by an electric motor with power supplied from a battery 132); however, in other

embodiments, the pump may be actuated manually (e.g., via application of force by a user, for example, to a plunger, lever, crank, and/or the like). In this embodiment, pump 128 is in communication with a needle 136 via an (e.g., flexible) conduit 140 such that actuation of pump 128 may cause communication of composition 116 from reservoir 108a, through conduit 140, and into the patient via needle 136 (e.g., which, in some embodiments, may be configured to be received within an implanted port of the patient). An example of such composition communication is depicted in FIG. 3, in which composition communication is indicated by dashed lines 144, and electrical communication is indicated by dotted lines 148.

[0093] In the embodiment shown, apparatus 100 comprises a sensor 152 configured to obtain data indicative of a glucose level within interstitial fluid of the patient (e.g., by measuring a current generated as glucose oxidase (GOx) catalyzes the reaction of glucose in the interstitial fluid with oxygen). The level can then be used to determine the blood glucose level of the patient or can be used to determine how much of the glucagon formulation to administer to the patient. For example, and referring particularly to FIG. 2, in this embodiment, a portion of sensor 152 (e.g., which may include a needle, electrodes, and/or the like) is inserted into a patient's skin 156 and is in communication with the interstitial fluid.

[0094] In the embodiment shown, sensor 152 is configured to transmit data wirelessly. For example, in this embodiment, sensor 152 is configured to transmit data via radio frequency (e.g., whether in response to a signal generated by a reader 160 and/or facilitated by a battery in electrical communication with the sensor). However, in other embodiments, sensor 152 can be configured to transmit data via a wired connection.

[0095] In the embodiment shown, apparatus 100 comprises a monitor 164 configured to communicate information indicative of the glucose level within the interstitial fluid of the patient. Monitors 164 of the present disclosure can comprise any suitable monitor, and can be configured to communicate information audibly (e.g., via a speaker 164a), tactilely (e.g., via a vibratory motor), visually (e.g., via a display device 164b), and/or the like. For example, in this embodiment, monitor 164 comprises a speaker 164a and a display device 164b. While monitor 164 is depicted as attached to housing 104 of apparatus 100, in other embodiments, monitors (or components thereof, such as, for example, speaker 164a or display device 164b) may be physically separate from housing 104 (e.g., and in wireless and/or wired communication with other components of apparatus 100). In this way, by receiving information communicated by monitors 164, a patient using apparatus 100 may gain insight

into how food intake, physical activity, medication, illness, and/or the like impact blood glucose levels.

[0096] In the embodiment shown, monitor 164 can be configured to communicate alerts under any suitable circumstance (e.g., triggers for which may be stored within a memory in electrical communication with processor 172). To illustrate, in this embodiment, apparatus 100 is configured such that monitor 164 communicates an alert when a glucose level within interstitial fluid of the patient is estimated to be at least one of: above a threshold (e.g., indicating an existing or impending hypoglycemic condition) and below a threshold (e.g., indicating an existing or impending hyperglycemic condition). Processor 172 may detect impending conditions by analyzing data received from sensor 152 over a time period to anticipate a patient's blood glucose level at a future time period (e.g., by determining trends within the patient's blood glucose level over time).

[0097] In this embodiment, apparatus 100 is configured to allow manual adjustment of at least one of a delivery rate and a dose of the composition intracutaneously delivered by pump 128. For example, in the embodiment shown, apparatus 100 comprises one or more user input devices (e.g., buttons) 168. User input devices 168 can be configured to allow a user to activate and/or deactivate apparatus 100 and/or pump 128, set a time and/or time period for activation and/or deactivation of apparatus 100 and/or pump 128, set a desired blood glucose level, set a desired composition delivery rate and/or dose (e.g., basal and/or bolus doses), and/or the like. User input devices 168 may work in conjunction with monitor 164 (or a display device 164b thereof) (e.g., to provide information to assist a user in interacting with apparatus 100, to provide for menu navigation, to display current parameters (e.g., target blood glucose level, composition delivery rate and/or dose, and/or the like), and/or the like). While in the depicted embodiment, user input devices 168 comprise buttons, in other embodiments, user input devices 168 can comprise any suitable structure, such as, for example, touch sensitive surface(s) of a display device 164b.

[0098] In the embodiment shown, apparatus 100 comprises a processor 172 configured to control operation of pump 128. In the embodiment shown, processor 172 control can be open-loop or closed-loop (e.g., based, at least in part, on data obtained by sensor 152). To illustrate, in this embodiment, processor 172 is configured to control operation of pump 128 to intracutaneously inject at least a portion of composition 116 if the data obtained by the sensor indicates a blood glucose level within interstitial fluid of the patient below a threshold (e.g., indicating an existing or impending hyperglycemic condition). FIG. 5 provides an

illustrative flow chart of such closed-loop processor-based control. For example, at step 176, processor may receive data from sensor 152 indicative of the glucose level within interstitial fluid of the patient (e.g., through communication with reader 160). At step 180, in this embodiment, processor 172 may compare the received data to a targeted or threshold value. In the depicted embodiment, at step 184, if the data indicates a blood glucose level within interstitial fluid of the patient is below the targeted or threshold value, processor 172 may command pump 128 to actuate to cause intracutaneous delivery of composition 116 to the patient. Embodiments configured for such closed-loop control may require no input from a patient, and may be suited for treating patients having, for example, type II insulin dependent diabetes, post-bariatric surgery reactive hypoglycemia, hypoglycemia associated autonomic failure, insulinoma, and/or the like.

[0099] In some embodiments (e.g., 100), the present apparatuses can be configured to communicate (e.g., via a display 164b) data indicative of current blood glucose level to a patient, whereby the patient may adjust the delivery rate, dose, and/or the like of composition 116 (e.g., controlling apparatus 100 in an open-loop fashion). Embodiments configured for such open-loop control may be suited for treating patients having, for example, type I insulin dependent diabetes, type II insulin dependent diabetes, and/or the like.

[00100] Some embodiments may be configured to provide intradermal, subcutaneous or intramuscular delivery of composition 116 in a no-loop fashion. For example, some embodiments may be configured such that pump 128 actuates to deliver a fixed (e.g., basal) dose of composition 116. In these and similar embodiments, sensor 152, reader 160, monitor 164, user input devices 168, processor 172, and/or the like may be omitted. Such embodiments may be suitable for treating patients having, for example, congenital hyperinsulinism, post-bariatric surgery reactive hypoglycemia, and/or the like.

[00101] Some embodiments of the present methods for treating hypoglycemia in a patient comprise using a glucagon delivery apparatus (e.g., 100) to intradermally, subcutaneously or intramuscularly deliver at least a portion of a composition (e.g., 116) to the patient. In some embodiments, the patient has been diagnosed as having a blood glucose level from 0 mg/dL to less than 50 mg/dL or has an indication of impending hypoglycemia before delivery of the composition, and the patient has a blood glucose level from 50 mg/dL to 180 mg/dL within 1 to 20 minutes after delivery of the composition. In some embodiments, the patient has been diagnosed as having a blood glucose level between from 10 mg/dL to less than 40 mg/dL. In some embodiments, the patient has a blood glucose level from 50 mg/dL to 180 mg/dL within

1 to 10 minutes after delivery of the composition. In some embodiments, the patient has a blood glucose level from 50 mg/dL to 180 mg/dL within 1 to 5 minutes after delivery of the composition. In some embodiments, the patient has been diagnosed with type I, type II, or gestational diabetes. Some embodiments comprise measuring, with a sensor (e.g., 152), the
5 blood glucose level of the patient.

B. Glucagon Formulations

1. Non-Aqueous Ionization Stabilized Compositions

[00102] To address the physical and/or chemical instability that many therapeutic molecules exhibit in water, formulations may be prepared wherein the therapeutic agent is dissolved in a
10 biocompatible non-aqueous liquid, such as an aprotic polar solvent. The use of aprotic polar solvents to prepare non-aqueous therapeutic formulations to inhibit many common degradation pathways, particularly those involving water, can significantly improve the stability of the solubilized or dissolved therapeutic molecule(s). However, problems still remain with the compositions and methods disclosed in the prior art. In particular, direct
15 dissolution of a therapeutic molecule in an aprotic polar solvent is not a suitable approach for preparing stable compositions of most therapeutic molecules. For example, when solubilized directly in DMSO at a concentration of 5 mg/mL the peptide hormone glucagon will form insoluble aggregates within one day of storage at room temperature. For a composition comprising only glucagon and DMSO, 5 mg/mL corresponds to approximately 0.45% (w/w)
20 of the peptide compound, indicating that at even relatively low concentrations, direct dissolution in an aprotic polar solvent system is by itself incapable of preventing physical aggregation and/or gelation of a therapeutic molecule. Moreover, therapeutic molecules that may not form insoluble aggregates in an aprotic polar solvent system may nonetheless be prone to chemical degradation when solubilized directly in an aprotic polar solvent system.
25 **[00103]** The drying process is well known to impose several stresses on the therapeutic molecule, and additional excipients (e.g., lyoprotectants such as trehalose and sucrose, and/or surfactants such as polysorbate 80) must be included in the aqueous solution in sufficient amounts to protect the therapeutic molecule, thereby increasing the cost and complexity of the formulation. Further, the drying process (e.g., spray drying, freeze drying) must often be
30 optimized for a given therapeutic molecule, both at the lab-scale during initial research and development where the process is initially developed, and then during the manufacturing-scale as the process is scaled-up and transferred to instruments and facilities capable of producing commercial-scale batches. Consequently, the combination of initially developing

and optimizing a drying process for a given therapeutic molecule, coupled with the time and costs associated with both transferring the method and incorporating an additional step in the manufacturing process can be very expensive. Thus, there is a need for a method of providing the therapeutic molecule(s) with an appropriate ionization profile in an aprotic polar solvent system without the requirement of drying the molecule from a buffered aqueous solution where the pH of the aqueous solution is set to provide an appropriate ionization profile for the molecule.

[00104] The solution resides in dissolving an ionization stabilizing excipient(s) directly in the aprotic polar solvent, coupled with dissolution of the peptide molecule or small molecule directly in the aprotic polar solvent solution. Without wishing to be bound by theory, it is believed that by providing a sufficient quantity of ionization stabilizing excipient to achieve an appropriate or optimal ionization profile of the therapeutic molecule, electrostatic repulsion between therapeutic molecules possessing the same charge polarity (i.e. negatively or positively charged) may be sufficient in magnitude to prevent physical degradation (e.g., via short-range hydrophobic interaction between molecules that lead to aggregation). This is especially important for molecules that exhibit a tendency to aggregate in solution, particularly as the concentration of the molecule in solution is increased. Further, by controlling and optimizing the extent of the ionization (i.e., protonation or deprotonation) of the therapeutic agent, chemical degradation can be minimized, as, for example, an excess of protonation may promote chemical instability via degradative reactions such as oxidation (for example, oxidation of methionine residues) and fragmentation (for example, cleavage of the peptide backbone). Accordingly, for some therapeutic molecules there may be an optimal or beneficial ionization profile achieved via protonation such that physical and/or chemical degradation reactions are minimized. For a therapeutic peptide, the extent of protonation required for stability, and thus the amount of the ionization stabilizing excipient required in the solution, will depend on, among other things, the primary structure (i.e., amino acid sequence) and the peptide concentration in the solution.

[00105] Without wishing to be bound by theory, it is thought that in order to exhibit enhanced or optimal stability and solubility when formulated in an aprotic polar solvent system, a therapeutic molecule may require a specific ionization profile. The ionization profile is the charge state acquired via protonation and/or deprotonation of the therapeutic molecule's ionogenic groups. For example, protonation of the ionogenic amino acid residues (e.g. arginine, lysine) comprising a therapeutic peptide will confer an overall positive charge

on the molecules in solution. The relatively long-range electrostatic repulsions between positively charged peptide molecules may inhibit the short-range hydrophobic interactions that can result in physical aggregation and/or gelation. Thus, in the absence of sufficient protonation (i.e., an optimal or beneficial ionization profile), therapeutic molecules dissolved in an aprotic polar solvent system may be physically unstable and lead to the formation of soluble and/or insoluble aggregates.

[00106] Accordingly, it may be necessary to include at least one excipient in a sufficient concentration to function as an ionization stabilizing agent that is capable of imparting the ionization profile for improved physical and/or chemical stability to the active agent in the aprotic polar solvent system. An appropriate concentration of the ionization stabilizing excipient(s) to be added to the solution depends on several factors including, but not limited to, the chemical structure of the ionization stabilizing excipient, the chemical structure of the active agent(s), the concentration of the active(s), the solvent system used, the presence of co-solvents, and the presence of additional excipients or formulation components and their respective concentrations.

[00107] In certain aspects a composition may be prepared by first adding the ionization stabilizing excipient to the aprotic polar solvent system, followed by addition of the therapeutic molecule. Alternatively, the therapeutic molecule may initially be solubilized in the aprotic polar solvent system followed by addition of the ionization stabilizing excipient. In a further aspect, the ionization stabilizing excipient and the therapeutic molecule may be solubilized simultaneously in the aprotic polar solvent system.

[00108] Each molecule that functions as an ionization stabilizing excipient will exhibit a certain tendency to donate protons to the therapeutic molecule(s) in a given solvent system; this tendency to donate protons may be referred to as the relative acidic strength of the molecule. For a fixed concentration of a proton-donating molecule, (and for simplicity it is assumed only monoprotic molecules in this example) molecules that have a greater acidic strength will protonate the therapeutic molecule to a greater extent than a weaker acid. Accordingly, the concentration of a given proton-donating molecule (ionization stabilizing excipient) required to achieve an appropriate or optimal ionization profile for the therapeutic molecules will be inversely proportional to its acidic strength. These and other non-limiting aspects of the present invention are discussed herein.

[00109] In certain aspects the aprotic polar solvent can be deoxygenated prior to preparation of the formulation. Many different techniques can be used in the context of the present

invention to deoxygenate or remove oxygen from aprotic polar solvents (degasification or deoxygenation). For instance, it is contemplated that deoxygenation can, but is not limited to, remove oxygen that is dissolved in a liquid aprotic polar solvent either by the liquid alone, by the liquid and other solute molecules (e.g. micelles, cyclodextrins, etc.), or by other solute molecules alone. Non-limiting examples of deoxygenation techniques include placing the aprotic polar solvent under reduced pressure and/or heating the liquid to decrease the solubility of dissolved gas, fractional distillation, membrane degasification, substitution by inert gas, using a reducing agent, freeze-pump-thaw cycling, or long time storage in a container with air-locks.

[00110] Once treated or deoxygenated, the aprotic polar solvents may have less than 0.1 mM of dissolved oxygen, preferably less than 0.05 mM of dissolved oxygen. Methods known to those of skill in the art can be used to determine the amount of dissolved oxygen in any given aprotic polar solvent (e.g., a dissolved oxygen meter or probe device can be used such as the Dissolved Oxygen Probe commercially available by Vernier (Beaverton, Oregon, USA)).

2. Obtaining the Dried Glucagon Peptide

[00111] Glucagon is a naturally occurring peptide hormone that is 29 amino acids in length and is secreted by the α-cells of the pancreas. The principal function of glucagon is to maintain glucose production through both glycogenolysis and gluconeogenesis, mostly mediated via the liver. Glucagon is the primary counter-regulatory hormone to insulin and is used as a first-line treatment of severe hypoglycemia in patients with diabetes. Its amino acid sequence is His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr. A commercial source of human, rat, and porcine glucagon is Bachem AG (Switzerland) and Bachem Americas, Inc. (Torrance, CA). In addition to native glucacon, glucagon analogues can also be used in the context of the present invention. Non-limiting examples of such analogues include ([$^{13}C_6$]Leu^{14})-Glucagon (1-29) (human, rat, porcine), Biotinyl-Glucagon (1-29) (human, rat, porcine), (Des-His^{1},Glu^{9})-Glucagon (1-29) (human, rat, porcine), (Des-Thr^{5})-Glucagon (human, rat, porcine), (Des-Thr^{7})-Glucagon (human, rat, porcine), and (Met(O)^{27})-Glucagon (1-29) (human, rat, porcine), each of which is also commercially available from Bachem AG and Bachem Americas, Inc. Another commercial source for native human, rat, or porcine glucagon and analogues thereof is American Peptide Company (Sunnyvale, CA). The data obtained in Examples 1-10 is human glucagon (1-29) obtained from American Peptide Company under

the name Glucagon, human. The data obtained in the Examples 11-12 is human glucagon (1-29) obtained from Bachem Americas, Inc. under the name Glucagon H-6790.

[00112] Glucagon can degrade via a number of different mechanisms, including deamidation, oxidation, hydrolysis, disulfide interchange and racemization. Further, water acts as a plasticizer, which facilitates unfolding of protein molecules and irreversible molecular aggregation. Therefore, in order to provide a peptide formulation that is stable over time at ambient or physiological temperatures, a non-aqueous or substantially non-aqueous glucagon peptide formulation is generally required.

[00113] Reduction of aqueous glucagon peptide formulations to dry powdered formulations is one way to increase the stability of pharmaceutical glucagon peptide formulations. For example, glucagon peptide formulations can be dried using various techniques, including spray-drying, lyophilization or freeze-drying, desiccation, spray freeze drying, or any combination thereof. The dry glucagon powder peptide formulations achieved by such techniques exhibit significantly increased stability over time at ambient or even physiological temperatures.

[00114] The present invention is based, in part, on the surprising discovery that a stable glucagon peptide formulation (e.g., a stable glucagon rescue formulation) can be readily prepared by first freeze-drying glucagon from an aqueous solution containing the peptide to a dry peptide powder. The dried glucagon peptide molecules will have a defined ionization profile conferred to them during the drying process. This ionization state may be about equal to the ionization state of the glucagon peptide in the aqueous solution from which it was dried (i.e., "about equal" refers to when the ionization profile of the peptide dried from an aqueous solution of pH X corresponds to the ionization profile of the peptide in an aqueous solution wherein the difference between the pH of said aqueous solution and pH X is within (or equal to) 1 pH unit). Whether the ionization state of the glucagon peptide in the powder is different (i.e., when the ionization profile of the peptide dried from an aqueous solution of pH X corresponds to the ionization profile of the peptide in an aqueous solution where the difference between the pH of said aqueous solution and pH X is greater than 1 pH unit) from the ionization state of the glucagon peptide in the aqueous solution in which it was dried may be dependent on the glucagon peptide itself, the components of the aqueous solution (e.g., excipients) and/or the drying method used to prepare the glucagon peptide powder.

[00115] As a non-limiting example, if the glucagon peptide is dried from an aqueous solution containing a non-volatile buffer, the ionization state of the peptide in the powder may

be about equal to that of the peptide in the aqueous solution from which it was dried. This ability of a dried glucagon peptide molecule to retain (or "remember") the ionization profile it possessed in the aqueous solution from which it was dried is termed "pH memory," as the peptide in the solid state remembers the pH of the aqueous solution from which it was dried to
5 a powder. Accordingly, discussing the pH memory of a molecule may serve as an alternative method for describing the ability of a peptide molecule in the solid state to retain the ionization profile it possessed in the aqueous solution from which it was dried to a powder. As an example, a glucagon peptide molecule dried from an aqueous solution of pH X that maintains the ionization profile it possessed in said aqueous solution upon reconstitution in an
10 aprotic polar solvent (e.g. DMSO) may be said to have a pH memory of X.

[00116] In the foregoing process, drying of the peptide compound from the aqueous solution (optionally containing excipients) is carried out using spray-drying techniques, freeze-drying techniques or lyophilization techniques. Spray-drying techniques are well known to those skilled in the art. Spray-drying includes the steps of atomization of a solution
15 containing one or more solutes (e.g., therapeutic agent, excipients) via a nozzle spinning disk, or other device, followed by evaporation of the solvent from the droplets. The nature of the powder that results is the function of several variables processing parameters, including the initial solute concentration, size distribution of droplets produced and the rate of solute removal. The particles produced may comprise aggregates of primary particles which consist
20 of crystals and/or amorphous solids depending on the rate and conditions of solvent removal. A spray-drying process for preparing ultra-fine powders of biological macromolecules such as proteins, oligo-peptides, high molecular weight polysaccharides, and nucleic acids is described in, for example, U.S. Pat. No. 6,051,256. Freeze-drying procedures are well-known in the art, and are described, for example, in U.S. Pat. No. 4,608,764 and U.S. Pat. No.
25 4,848,094. Spray-freeze-drying processes are described, e.g., in U.S. Pat. No. 5,208,998. Other spray-drying techniques are described, for example, in U.S. Pat. Nos. 6,253,463; 6,001,336; 5,260,306; and PCT International Publication Nos. WO 91/16882 and WO 96/09814.

[00117] Lyophilization techniques are well-known to those skilled in the art.
30 Lyophilization is a dehydration technique that takes place while a product is in a frozen state (ice sublimation under a vacuum) and under a vacuum (drying by gentle heating). These conditions stabilize the product, and minimize oxidation and other degradative processes. The conditions of freeze drying permit running the process at low temperatures, therefore

thermally labile products can be preserved. Steps in freeze drying include pre-treatment, freezing, primary drying and secondary drying. Pre-treatment includes any method of treating the product prior to freezing. This may include concentrating the product, formulation revision (i.e., addition of components to increase stability and/or improve processing), decreasing a high vapor pressure solvent or increasing the surface area. Methods of pre-treatment include: freeze concentration, solution phase concentration, and formulating specifically to preserve product appearance or to provide lyoprotection for reactive products, and are described, e.g., in U.S. Pat. No. 6,199,297. “Standard” lyophilization conditions, are described, e.g., in U.S. Pat. No. 5,031,336, and in “Freeze Drying of Pharmaceuticals” (DeLuca, Patrick P., J. Vac. Sci. Technol., Vol. 14, No. 1, January/February 1977); and “The Lyophilization of Pharmaceuticals: A Literature Review” (Williams, N. A., and G. P. Polli, Journal of Parenteral Science and Technology, Vol. 38, No. 2, March/April 1984).

[00118] In certain preferred embodiments, the lyophilization cycle is partially performed above the glass transition temperature (T_g) of the therapeutic agent formulation to induce a collapse of the mass to form a dense cake containing residue moisture. In other embodiments, the lyophilization cycle is carried out below the glass transition temperature in order to avoid a collapse in order to achieve a complete drying of the particles.

[00119] Methods for measuring the ionization state of the dry peptide, or of a peptide in an aprotic polar solvent, are known in the field. In one well-known and accepted method, the ionization state of a peptide can be obtained by reconstituting the dried peptide into un-buffered water and measuring the pH of the reconstituted peptide with a pH indicator such as pH paper or a calibrated pH electrode. Alternatively, the ionization state of a peptide can be determined for a peptide that has been reconstituted in the aprotic polar solvent (e.g., DMSO) by adding at least 20% water to the aprotic polar solvent (e.g., DMSO) and measuring the pH with a pH indicator. See, e.g., Baughman and Kreevoy, “Determination of Acidity in 80% Dimethyl Sulfoxide-20% Water,” Journal of Physical Chemistry, 78(4):421-23 (1974). Measurement of pH in an aprotic polar solvent-water solution may require a small correction (e.g., 0.2 pH unit as per Baughman and Kreevoy, supra). Alternatively, spectroscopic techniques can be used to measure the ionization state of a given peptide.

[00120] In some embodiments, a dried glucagon peptide has an ionization profile that is about equal to the ionization profile of the peptide in the aqueous solution from which it is dried, when the ionization profile of the peptide when it is reconstituted in an aprotic polar solvent is within one pH unit of the pH of the peptide in the aqueous solution from which it is

dried (thus, for example, a glucagon peptide dried from an aqueous solution of pH 3.0 is said to have an ionization profile about equal to the ionization profile of the peptide in the aqueous solution from which it was dried if the ionization profile of the glucagon peptide, when measured in the powder following drying or following reconstitution of said powder in the aprotic polar solvent corresponds to an ionization profile of the glucagon peptide in an aqueous solution with pH between 2.0 to 4.0).

[00121] In some embodiments, a dried glucagon peptide has an ionization profile that is about equal to the ionization profile of the peptide in the aqueous solution from which it is dried, when the ionization profile of the glucagon peptide when it is reconstituted in an aprotic polar solvent is within half of a pH unit of the pH of the peptide in the aqueous solution from which it is dried (thus, for example, a glucagon peptide dried from an aqueous solution of pH 3.0 is said to have an ionization profile about equal to the ionization profile of the peptide in the aqueous solution from which it was dried if the ionization profile of the peptide, when measured in the powder following drying or following reconstitution of said powder in the aprotic polar solvent corresponds to an ionization profile of the glucagon peptide in an aqueous solution with pH between 2.5 to 3.5).

[00122] Once dried, the resulting glucagon peptide powder, e.g., the freeze-dried glucagon, is dissolved in an aprotic polar solvent, thereby forming a stable formulation, wherein the moisture or water content of the formulation is less than 15%, 10%, 5%, 1%, 0.5%, 0.25%, 0.15%, or less than 0.1%. The dried glucagon peptide maintains its defined ionization profile when reconstituted in the aprotic polar solvent, i.e., the ionization profile of the peptide when reconstituted in the aprotic polar solvent is about equal to the ionization profile of the dried peptide. Advantageously, once prepared, the glucagon formulation is stable for extended periods of time, is ready for use without the need for reconstitution, and is functional over a range of temperatures.

[00123] In certain preferred embodiments, the stable formulations described herein preserve the glucagon peptide drug in a stable form for a prolonged period of time, e.g., for a period of time sufficient to provide a desired shelf life of the formulation without unacceptable levels of physical and chemical degradation of the therapeutic agent prior to use. A desired property of the injectable formulations is that they be non-aqueous and non-reactive with respect to the peptide. In such embodiments, it is possible to store the injectable formulations directly in the injection device itself.

[00124] The stable injectable formulations of the present invention contain the desired delivered dose of glucagon (e.g., the dose required for drug therapy) and are preferably low volume. For example, in some embodiments an injectable formulation comprising a therapeutic dose of a glucagon peptide has a volume of at least about 1.0 microliters (the
5 lower limit being a function of the filling equipment), more preferably from about 10 milliliters to about 250 microliters. The delivery of a therapeutic dose of glucagon peptide at a low volume is accomplished in certain preferred embodiments by concentrating the dose of the therapeutic glucagon peptide in a stable form in a suitable aprotic polar solvent for injection in accordance with the invention.

10 **[00125]** Furthermore, the stable formulations of the present invention are suitable for administration without requiring dilution prior to injection. By comparison, the currently commercially available glucagon formulations are produced in a solid particulate form to promote stability while on the shelf. These formulations are diluted prior to injection in sterile water, phosphate buffer solution, or isotonic saline. In particular, there are two
15 glucagon kits currently available in the United States, manufactured by Eli Lilly (Glucagon Emergency Kit) and Novo Nordisk (GlucaGen® HypoKit). Both products combine a vial of freeze-dried glucagon with a pre-filled syringe of aqueous diluent. The freeze-dried glucagon must be reconstituted using a complex procedure that is difficult to use in an emergency situation.

20 **[00126]** By comparison, in certain preferred embodiments of the present invention, the therapeutic glucagon peptide is concentrated using the particle preparation processing techniques (e.g., spray drying, lyophilization, etc.) routinely employed by the pharmaceutical industry to prepare formulations for injection. In preferred embodiments, therapeutic dosages of glucagon are achieved by dissolving the peptide, which have first been freeze-dried (and
25 optionally additional components such as a stabilizing excipient) to a dried powder having very little residual moisture content. Once prepared, the dried glucagon peptide powder is dissolved in an aprotic polar solvent, such as DMSO, NMP, ethyl acetate, or blends of these solvents. Thus, in accordance with the goals of the present invention, the low volume, stable formulations of the present invention are injected, infused, or otherwise administered into an
30 animal (e.g., human patient), without first diluting the formulation prior to injection as required by most reconstitution products. As such, in preferred embodiments, the low volume formulations of the present invention are administrable without being first being diluted, or reconstituted, or refrigerated.

3. Reconstitution of Dried Peptides in Aprotic Polar Solvents

[00127] In the stable formulations of the present invention, once the glucagon peptide (and optional components) are dried to a powder, the dried peptide powder is dissolved or reconstituted in an aprotic polar solvent. In some embodiments, the aprotic polar solvent is selected from dimethylsulfoxide (DMSO), dimethylformamide (DMF), ethyl acetate, n-methyl pyrrolidone (NMP), dimethylacetamide (DMA), propylene carbonate, and mixtures thereof. In some embodiments, the aprotic polar solvent is a mixture of two or more of dimethylsulfoxide (DMSO), dimethylformamide (DMF), ethyl acetate, n-methyl pyrrolidone (NMP), dimethylacetamide (DMA), and propylene carbonate. Dimethylsulfoxide (DMSO), ethyl acetate, and n-methyl pyrrolidone (NMP) are particularly preferred aprotic polar solvents, each of which is a biocompatible solvent. In some embodiments, the aprotic polar solvent is dimethylsulfoxide (DMSO). In other embodiments, the aprotic polar solvent is n-methyl pyrrolidone (NMP). In other embodiments, the aprotic polar solvent is a mixture of dimethylsulfoxide (DMSO) and n-methyl pyrrolidone (NMP). In still other embodiments, the aprotic polar solvent is a mixture of dimethylsulfoxide (DMSO) and ethyl acetate. In some embodiments, the dried glucagon peptide powder is reconstituted in an aprotic polar solvent that is "neat," i.e., that does not contain a co-solvent. In some embodiments, the dried peptide powder is reconstituted in a solution that comprises an aprotic polar solvent and that does not contain water as a co-solvent.

[00128] In some embodiments, the formulations of the present invention further comprise at least one co-solvent that depresses the freezing point of the formulation. The co-solvent can be a polar protic solvent. In some embodiments, the co-solvent is selected from ethanol, propylene glycol (PG), glycerol, water, and mixtures thereof. In some embodiments, the co-solvent is ethanol or propylene glycol (PG). In other embodiments, the co-solvent is water. The co-solvent may be present in the formulation in an amount ranging from about 10% (w/v) to about 50% (w/v), e.g., about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50% (w/v). In some embodiments, the co-solvent is present in the formulation in an amount ranging from about 10% (w/v) to about 50% (w/v), from about 10% (w/v) to about 40% (w/v), from about 10% (w/v) to about 30% (w/v), from about 10% (w/v) to about 25% (w/v), from about 15% (w/v) to about 50% (w/v), from about 15% (w/v) to about 40% (w/v), from about 15% (w/v) to about 30% (w/v), or from about 15% (w/v) to about 25% (w/v). In some embodiments, the at least one co-solvent depresses the freezing point of the formulation by at least 5 °C., at least 10 °C, at least 15 °C, at least 20 °C

or more as compared to an otherwise identical formulation that does not comprise the co-solvent. In some embodiments, the at least one co-solvent depresses the freezing point of the formulation to about 3 °C, to about 2 °C, to about 1 °C, or to about 0 °C or below.

4. Partially Volatile Buffer

5 **[00129]** In one aspect of the present invention a composition is a stable formulation that includes: (a) a peptide or a salt thereof that has been previously dried from an aqueous composition comprising a partially volatile buffer, a volatile buffer, a strong acid, or a strong base, or any combination thereof, wherein the dried peptide or salt thereof has a first ionization profile that corresponds to the peptide's optimal stability and solubility; and an 10 aprotic polar solvent, wherein the dried peptide or salt thereof is reconstituted into an aprotic polar solvent and has a second ionization profile in the aprotic polar solvent, wherein the first and second ionization profiles are substantially the same, such as within 1 pH unit of one another. For a more detailed description see PCT application PCT/US2015/014756, which is incorporated herein by reference in its entirety. One non-limiting method for measuring the 15 ionization state of the dry peptide includes reconstituting the dried peptide into un-buffered water and measuring the pH of the reconstituted peptide with a pH indicator such as pH paper or a calibrated pH electrode. One non-limiting method for measuring the ionization state of the peptide that has been reconstituted in the aprotic polar solvent includes adding at least 20% water to the aprotic polar solvent and measuring the pH with a pH indicator.

20 **[00130]** The peptide or salt thereof can have a third ionization profile when the peptide is in the aqueous composition prior to the aforementioned drying step. The third ionization profile can be different from the first or second ionization profiles by at least 1 pH unit (e.g., the aqueous composition can be formulated such that the pH of the aqueous composition compensates for the loss of counter-ions or buffer components or both during drying of said 25 aqueous composition). Alternatively, the third ionization profile can be substantially the same as the first or second ionization profiles, such as within 1 pH unit of one another. One non-limiting method for measuring the ionization state of the peptide in the aqueous composition prior to said drying step is to measure the pH of the aqueous solution with a pH indicator. In some particular aspects, the aqueous composition is formulated such that the third ionization 30 profile shifts to the first ionization profile during drying of said aqueous composition.

[00131] The dried peptide can be partially or fully solubilized within the aprotic polar solvent. Full solubilization can be obtained by adding the dried peptide to the aprotic polar

solvent up to the solubility limit of said peptide. For partial solubilization, suspensions and pastes can be formed such that a percentage of the peptide is solubilized in the aprotic polar solvent and a percentage is suspended or dispersed within said aprotic polar solvent. The aqueous composition can include a partially volatile buffer, non-limiting examples of which include sodium acetate or ammonium phosphate or any combination thereof. The aqueous composition can include a volatile buffer, non-limiting examples of which include ammonium acetate, ammonium formate, ammonium carbonate, ammonium bicarbonate, pyridine acetate, pyridine formate, or triethylammonium acetate, or any combination thereof. The aqueous composition can include a strong acid, a non-limiting example of which includes hydrochloric acid. The aqueous composition can include a strong base, non-limiting examples of which include sodium hydroxide, potassium hydroxide, lithium hydroxide, or calcium hydroxide, or any combination thereof.

[00132] In certain aspects, the aqueous composition does not include any buffer or does not include a non-volatile buffer. Alternatively, the aqueous composition can include a mixture of different buffers. In one non-liming aspect, the mixture can include a mixture of non-volatile buffers, a mixture of partially volatile buffers, a mixture of volatile buffers, a mixture of non-volatile and partially volatile buffers, a mixture of non-volatile and volatile buffers, a mixture of partially volatile and volatile buffers, or a mixture of non-volatile, partially volatile, and volatile buffers. The drying step can be performed by lyophilization, spray drying, desiccation, thin-film freezing, spray freeze drying, or any combination thereof. The moisture or water content of the formulation can be less than 15%, 10%, 5%, 1%, or less.

[00133] Non-limiting examples of aprotic polar solvents includes dimethylsulfoxide (DMSO), n-methyl pyrrolidone (NMP), ethyl acetate, dimethylformamide (DMF), propylene carbonate, or mixtures thereof. The formulation can further include a co-solvent that depresses the freezing point of the formulation (e.g., ethanol, propylene glycol, glycerol, and mixtures thereof). The formulation can further include a stabilizing excipient (e.g., a sugar, a starch, or mixtures thereof). In preferred aspects, the peptide in the formulation is glucagon or a salt thereof. In instances where the peptide is glucagon or a salt thereof, the first or second ionization profiles can correspond to the ionization profile of glucagon when solubilized in an aqueous solution having a pH range of about 2 to 3. The third ionization profile can correspond to the ionization profile of glucagon when solubilized in an aqueous solution having a pH range of about 2 to 3 or can correspond to the ionization profile of glucagon when solubilized in an aqueous solution having a pH range of greater than 3, or

greater than 3 to 14, or greater than 3 to 10, or greater than 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 or any range therein. Alternatively, the third ionization profile can correspond to the ionization profile of glucagon when solubilized in an aqueous solution having a pH range of less than 2, or less than 2 to 0 or less than 2 to 1 or 1 or 0 or any range therein. Also, the first ionization profile can be maintained by reconstituting the dried peptide or salt thereof in an organic solvent system comprising an organic solvent and an organic phase buffer prior to reconstituting said dried peptide or salt thereof into polar aprotic solvent.

[00134] The dried peptide or salt thereof can be reconstituted into the polar aprotic solvent with mixing the organic solvent system with the polar aprotic solvent. The organic solvent system can be separated from the polar aprotic solvent via separation methods known in the art. The organic solvent system can be substantially anhydrous (e.g., less than 1 wt. %, less than 0.5 wt. %, or less than 0.1 wt. % water) or anhydrous.

5. Stabilizing Excipients

[00135] In certain preferred embodiments, the formulations described herein may be further stabilized to ensure the stability of the glucagon peptide incorporated therein. In some embodiments, the stability of the injectable formulation is enhanced by the inclusion of one or more stabilizing agents or stabilizing excipients into the formulation prior to the drying of the glucagon peptide. In other embodiments, the stability of the injectable formulation is enhanced by reconstituting the dried glucagon peptide with a stabilizing agent or stabilizing excipient (e.g., trehalose) in the aprotic polar solvent. Alternatively, the stabilizing agent or stabilizing excipient (e.g., trehalose) can be added to the buffered aqueous solution prior to lyophilization, such that when the dried glucagon powder is reconstituted in the aprotic polar solvent, the stabilizing agent or excipient is present.

[00136] In some embodiments, the stabilizing excipient is a cryoprotectant. The addition of a cryoprotectant, such as trehalose, protects the peptide formulations of the present invention against instability associated with freeze-thaw cycles. The addition of the cryoprotectant trehalose also promotes enhanced thawing of a frozen glucagon peptide formulation. This property of enhanced thawing is surprisingly advantageous, particularly in emergency medical situations, such as a severe hypoglycemia episode, wherein a peptide formulation of the present invention is frozen and needs to be administered quickly. Thus, in another aspect of the present invention, the stable formulation has an improved freeze-thaw stability, an enhanced thawing rate, and/or an enhanced thawing profile.

[00137] In some embodiments, the stabilizing excipient is selected from sugars, starches, sugar alcohols, and mixtures thereof. Examples of suitable sugars for stabilizing excipients include, but are not limited to, trehalose, glucose, sucrose, etc. Examples of suitable starches for stabilizing excipients include, but are not limited to, hydroxyethyl starch (HES). Examples of suitable sugar alcohols for stabilizing excipients include, but are not limited to, mannitol and sorbitol. In some embodiments, the at least one stabilizing excipient (e.g., a sugar, a starch, a sugar alcohol, or a mixture thereof) is capable of enhancing the stability of the peptide during a freeze-thawing process, enhancing the thawing rate of the formulation, or enhancing the thawing profile of the formulation.

[00138] In some embodiments, the stabilizing excipient is present in the formulation in an amount ranging from about 1% (w/v) to about 60% (w/v), from about 1% (w/v) to about 50% (w/v), from about 1% (w/v) to about 40% (w/v), from about 1% (w/v) to about 30% (w/v), from about 1% (w/v) to about 20% (w/v), from about 5% (w/v) to about 60% (w/v), from about 5% (w/v) to about 50% (w/v), from about 5% (w/v) to about 40% (w/v), from about 5% (w/v) to about 30% (w/v), from about 5% (w/v) to about 20% (w/v), from about 10% (w/v) to about 60% (w/v), from about 10% (w/v) to about 50% (w/v), from about 10% (w/v) to about 40% (w/v), from about 10% (w/v) to about 30% (w/v), or from about 10% (w/v) to about 20% (w/v). In some embodiments, the stabilizing excipient is present in the formulation in an amount that is about 1%, about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, or about 60% (w/v).

[00139] In some embodiments, the formulation further comprises additional stabilizing agents including, for example, antioxidants, chelators and preservatives. Examples of suitable antioxidants include, but are not limited to, ascorbic acid, cysteine, methionine, monothioglycerol, sodium thiosulphate, sulfites, BHT, BHA, ascorbyl palmitate, propyl gallate, N-acetyl-L-cysteine (NAC), and Vitamin E. Examples of suitable chelators include, but are not limited to, EDTA, tartaric acid and salts thereof, glycerin, and citric acid and salts thereof. Examples of suitable preservatives include, but are not limited to, benzyl alcohols, methyl parabens, propyl parabens, and mixtures thereof.

[00140] In some embodiments, the formulation further comprises a stabilizing polyol. Such formulations and materials are described, for example, in U.S. Pat. Nos. 6,290,991 and 6,331,310, the contents of each of which are incorporated by reference herein.

C. Therapeutic Methods

[00141] In some embodiments, a therapeutic method of the present invention comprises treating hypoglycemia by administering to a subject having hypoglycemia a stable formulation as described herein in an amount effective to treat the hypoglycemia. Administration is performed via the pump-based delivery systems described throughout this application. The hypoglycemia can be caused by diabetes or non-diabetes related diseases, conditions, and disorders.

[00142] As described by the Workgroup of the American Diabetes Association and the Endocrine Society, (Seaquist, et al, (2013), *Diabetes Care,* Vol 36, pages 1384 – 1395) with respect to hypoglycemia, a single threshold value for plasma glucose concentration that defines hypoglycemia in diabetes is not typically assigned because glycemic thresholds for symptoms of hypoglycemia (among other responses) shift to lower plasma glucose concentrations after recent antecedent hypoglycemia and to higher plasma glucose concentrations in patients with poorly controlled diabetes and infrequent hypoglycemia.

[00143] Nonetheless, an alert value can be defined that draws the attention of both patients and caregivers to the potential harm associated with hypoglycemia. Patients at risk for hypoglycemia (i.e., those treated with a sulfonylurea, glinide, or insulin) should be alert to the possibility of developing hypoglycemia at a self-monitored plasma glucose - or continuous glucose monitoring subcutaneous glucose - concentration of ≤ 70 mg/dL (≤ 3.9 mmol/L).

[00144] Because it is higher than the glycemic threshold for symptoms in both nondiabetic individuals and those with well-controlled diabetes, it generally allows time to prevent a clinical hypoglycemic episode and provides some margin for the limited accuracy of monitoring device at low-glucose levels.

[00145] Severe hypoglycemia is an event requiring assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions. Plasma glucose concentrations may not be available during an event, but neurological recovery following the return of plasma glucose to normal is considered sufficient evidence that the event was induced by a low plasma glucose concentration. Typically, these events begin occurring at plasma glucose concentrations of ≤50 mg/dL (2.8 mmol/L).

[00146] Documented symptomatic hypoglycemia is an event during which typical symptoms of hypoglycemia are accompanied by a measured plasma glucose concentration ≤ 70 mg/dL (≤ 3.9 mmol/L).

[00147] Asymptomatic hypoglycemia is an event not accompanied by typical symptoms of hypoglycemia but with a measured plasma glucose concentration ≤ 70 mg/dL (≤ 3.9 mmol/L).

[00148] Probable symptomatic hypoglycemia is an event during which symptoms typical of hypoglycemia are not accompanied by a plasma glucose determination but that was 5 presumably caused by a plasma glucose concentration ≤ 70 mg/dL (≤ 3.9mmol/L).

[00149] Pseudo-hypoglycemia is an event during which the person with diabetes reports any of the typical symptoms of hypoglycemia with a measured plasma glucose concentration > 70 mg/dL (> 3.9mmol/L) but approaching that level.

[00150] Iatrogenic hypoglycemia in patients with diabetes is defined as all episodes of an 10 abnormally low plasma glucose concentration that exposes the patient to harm. (see: Alsahli, et al. (2014), Hypoglycemia, chronic kidney disease, and diabetes mellitus," Mayo Clinic Proceedings, 89 (11), 1564 – 1571).

[00151] Further included in the indications which may be treated by the disclosed invention are hypoglycemia-associated autonomic failure (HAAF). As described by Philip E. Cryer, 15 Perspectives in Diabetes, Mechanisms of Hypoglycemia-Associated Autonomic Failure and Its Component Syndromes in Diabetes, Diabetes, Vol. 54, pp. 3592-3601 (2005), "recent antecedent iatrogenic hypoglycemia causes both defective glucose counter-regulation (by reducing epinephrine responses to a given level of subsequent hypoglycemia in the setting of absent decrements in insulin and absent increments in glucagon) and hypoglycemia 20 unawareness (by reducing sympathoadrenal and the resulting neurogenic symptom responses to a given level of subsequent hypoglycemia) and thus a vicious cycle of hypoglycemia." HAAF affects those with type 1 and advanced type 2 diabetes. Additionally, the invention of the present disclosure may also treat hypoglycemia in patients who have had islet cell transplantation.

25 **[00152]** The formulations of the present invention can also be used for the treatment of hyperinsulinemic hypoglycemia, which broadly refers to the condition and effects of low blood glucose levels that are caused by excessive insulin. The most common type of severe, but typically transient, hyperinsulinemic hypoglycemia arises from the administration of exogenous insulin in patients with Type 1 diabetes. This type of hypoglycemia can be 30 defined as iatrogenic hypoglycemia, and is a limiting factor in the glycemic management of type 1 and type 2 diabetes. Nocturnal hypoglycemia (night-time hypo) is a common type of hypoglycemia arising in patients taking exogenous insulin. However, hyperinsulinemic

hypoglycemia can also arise due to endogenous insulin, for example in congenital hyperinsulinism, insulinomas (insulin-secreting tumors), or reactive hypoglycemia. Reactive hypoglycemia is a non-diabetic hypoglycemia, and is due to low blood sugar that occurs following a meal – typically within four hours after eating. Reactive hypoglycemia may also be referred to as postprandial hypoglycemia. Symptoms and signs of reactive hypoglycemia can include hunger, weakness, shakiness, sleepiness, sweating, confusion and anxiety. Stomach surgery (e.g. bariatric surgery) is one possible cause, as following surgery food may pass too quickly into the small intestine. In particular, post-bariatric hypoglycemia syndrome can be caused by bariatric surgery. Additional causes include enzyme deficiencies that make it difficult for the body to breakdown food, or increased sensitivity to the hormone ephinephrine. Further, hyperinsulinemic hypoglycemia can also be drug induced, such as occurs in drug induced hyperinsulinism due to administration of, for example, sulfonylurea, pentamidine, quinine, and aspirin, among other therapeutic compounds.

[00153] The formulations of the present invention can also treat hypoglycemia arising due to glucose depletion, as for example, in exercise-induced hypoglycemia. Further, nocturnal hypoglycemia arising in non-diabetic patients may also be treated by the disclosed formulations.

[00154] Determination of an effective amount or dose is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. Generally, the formulations to deliver these doses may contain a glucagon peptide present at a concentration from about 0.1 mg/mL up to the solubility limit of the peptide in the formulation to produce a solution, wherein the glucagon peptide is fully or completely solubilized in the aprotic polar solvent. This concentration is preferably from about 1 mg/mL to about 100 mg/mL, e.g., about 1 mg/mL, about 5 mg/mL, about 10 mg/mL, about 15 mg/mL, about 20 mg/mL, about 25 mg/mL, about 30 mg/mL, about 35 mg/mL, about 40 mg/mL, about 45 mg/mL, about 50 mg/mL, about 55 mg/mL, about 60 mg/mL, about 65 mg/mL, about 70 mg/mL, about 75 mg/mL, about 80 mg/mL, about 85 mg/mL, about 90 mg/mL, about 95 mg/mL, or about 100 mg/mL.

[00155] The formulations of the present invention may be for subcutaneous, intradermal, or intramuscular administration (e.g., by injection or by infusion). In some embodiments, the formulation is administered subcutaneously.

EXAMPLES

[00156] Some embodiments of the present disclosure will be described in greater detail by way of specific examples. The following examples are offered for illustrative purposes, and are not intended to limit any present invention in any manner. For example, those of skill in 5 the art will readily recognize a variety of noncritical parameters which can be changed or modified to yield essentially the same results.

Example 1

Ionization Stabilized Glucagon Composition

[00157] In this example, glucagon solutions were prepared by dissolving glycine 10 hydrochloride (CAS No. 6000-43-7) directly in DMSO (CAS No. 67-68-5) at 5 mM, 10 mM, and 20 mM concentrations, followed by dissolution of glucagon powder (MW = 3483 g/mol; Bachem AG) to a peptide concentration of 5 mg/mL. The prepared sample solutions are shown in Table 1:

15 Table 1: Glucagon sample solutions prepared by dissolving both glycine hydrochloride and glucagon powder directly in DMSO.

Glucagon Concentration	Solvent	Added Excipient
5 mg/mL	DMSO	5 mM Glycine Hydrochloride
5 mg/mL	DMSO	10 mM Glycine Hydrochloride
5 mg/mL	DMSO	20 mM Glycine Hydrochloride

[00158] The reversed-phase high performance liquid chromatography (RP-HPLC) method used to assess chemical stability was a gradient method with mobile phases A and B 20 respectively consisting of 0.1% (v/v) TFA (trifluoroacetic acid) in water and 0.1% (v/v) TFA in acetonitrile. A C8 column (BioBasic™-8; ThermoScientific) (4.6 mm I.D. x 250 mm length, 5 micron particle size) was used with a column temperature of 37 °C, a 1.0 mL/min flow rate, 6-µL sample injection volume and 280-nm detection wavelength.

[00159] Visual observation indicated that following six weeks (42 days) of storage at 40 °C, 25 the sample solutions containing glycine hydrochloride as a formulation excipient remained clear and colorless, and did not exhibit any precipitation and/or gelation. The stability of 5 mg/mL glucagon formulations were assessed via RP-HPLC as described above.

[00160] The sample formulations prepared with varying concentrations of glycine hydrochloride were sealed in 2-mL CZ vials (Crystal-Zenith, West Pharmaceuticals, PA, USA) with 13-mm FluroTec® stoppers (rubber stoppers coated with a fluorocarbon film, produced by West Pharmaceuticals) and stored at 40 °C for up to 6 weeks. The solutions were compared with 5 mg/mL glucagon formulations prepared via drying from a non-volatile buffer and reconstituting in DMSO (the pH memory formulations as described in Prestrelski '644), and direct dissolution of glucagon in DMSO (the method as described in Stevenson '547). The stability of the formulations are presented as glucagon purity and shown in Table 2 below.

Table 2: Stability (provided as peptide purity) of 5 mg/mL glucagon solutions stored at 40 °C.

	Glycine HCl Concentration			pH Memory	Direct Dissolution
Time Point	5 mM	10 mM	20 mM	Formulation	In DMSO
Day 1	100%	100%	100%	100%	Formed Gel
Day 14	99.7%	99.5%	99.3%	99.4%	----
Day 42	97.8%	97.0%	97.0%	96.8%	---

[00161] Within 24 hours at room temperature, the 5 mg/mL glucagon solutions (approximately 0.45% w/w) prepared by direct dissolution of glucagon powder in DMSO exhibited physical aggregation, as noted by the formation of insoluble material (FIG. 1). By contrast, solutions prepared with 5 mg/mL glucagon powder dissolved in DMSO in the presence of 5.0 mM glycine HCl remained clear (i.e. free of precipitation) and colorless throughout the examined incubation period (6 weeks at 40 °C). Glucagon formulations that had previously been lyophilized from a buffered aqueous solution containing 1 mg/mL glucagon, 2 mM glycine and 1% (w/v) trehalose prior to reconstitution to 5-fold the initial concentration with DMSO (i.e., the composition in the aprotic polar solvent system following reconstitution was 5 mg/mL glucagon, 10 mM glycine, and 5% (w/v) trehalose) also exhibited a glucagon purity of approximately 97% following six weeks of storage at 40 °C.

[00162] Accordingly, the compositions prepared by the method of the present invention provide enhanced stability compared to the prior art methods of direct dissolution of the peptide powder in an aprotic polar solvent. Further, the formulations of the present invention may provide an alternative pathway for preparing highly-concentrated, stable glucagon

formulations in aprotic polar solvent systems without the need for drying the peptide from a buffered aqueous solution prior to dissolution in the aprotic polar solvent system.

Example 2
Ionization Stabilized Glucagon Compositions at 5 mg/ml

[00163] In this example glucagon solutions were prepared at a concentration of 5 mg/mL by dissolving glucagon powder in DMSO that included different concentrations of added hydrochloric acid, ranging from 0.001 M (1 mM) to 0.01 M (10 mM). To minimize the amount of water added to the formulation, 5 N HCl was utilized to prepare 10 mM and 5.6 mM HCl in DMSO solutions, while 1 N HCl was used to prepare the 3.2 mM, 1.8 mM, and 1.0 mM solutions. As an example, the 10 mM HCl in DMSO solution was prepared by adding 20 μL of 5 N HCl to 9.98 mL of DMSO (neat), while the 1.0 mM HCl in DMSO solution was prepared by adding 10 μL of 1 N HCl to 9.99 mL of DMSO (neat). Samples of each formulation were stored in CZ vials and incubated at 40 °C.

[00164] Following both 28 and 58 days of storage the chemical stability of the peptide was assessed by RP-HPLC and the purity reported in Table 3. The addition of 1.0 mM HCl was insufficient to prevent the formation of insoluble aggregates in the 5 mg/mL glucagon solutions, and accordingly the chemical stability of these samples were not measured. Conversely, the glucagon molecule exhibited relatively rapid chemical degradation when 10 mM HCl was added to the solution. Decreasing the added HCl concentration in the solution increased the overall stability of the glucagon molecule, with the 3.2 mM and 1.8 mM HCl solutions exhibiting the highest stability over the examined time period.

Table 3: Stability (provided as peptide purity) of 5 mg/mL Glucagon-DMSO Solutions Stored at 40 °C.

Glucagon	Added [HCl]	Day 28	Day 58
5 mg/mL	10.0 mM	36.9%	0%
5 mg/mL	5.6 mM	90.8%	85.3%
5 mg/mL	3.2 mM	98.0%	96.8%
5 mg/mL	1.8 mM	98.3%	97.4%
5 mg/mL	1.0 mM	Insoluble Aggregates	Insoluble Aggregates

Example 3
Ionization Stabilized Glucagon Compositions at 5 mg/ml

[00165] Sample solutions were prepared by dissolving glucagon powder to a concentration of 5 mg/mL in DMSO which contained various added concentrations of glycine hydrochloride (CAS No. 6000-43-7), betaine hydrochloride (CAS No. 590-46-5), or hydrochloric acid (1 N; CAS No. 7647-01-0). The various concentrations of each ionization stabilizing excipient used to prepare the sample formulations are listed in Table 4. Samples of each formulation were stored in CZ vials and incubated at 40 °C. Following 28 days of storage the chemical stability of the glucagon peptide was assessed by RP-HPLC and the purity reported in Table 4. This example demonstrates that the proton-donating ability of the added ionization stabilizing excipient (i.e. its ‘strength’) may influence the concentration required to stabilize the therapeutic molecule. Glucagon was selected as a model peptide due to its tendency to gel (i.e. form insoluble aggregates) when the molecule is insufficiently protonated. A concentration of up to 2 mM glycine hydrochloride was insufficient to prevent the formation of insoluble aggregates in the solution, though this concentration of both betaine hydrochloride and hydrochloric acid was sufficient to prevent the formation of insoluble aggregates following 28 days of storage at 40 °C.

Table 4: Stability (provided as % peptide purity) of 5 mg/mL Glucagon-DMSO Solutions Stored at 40 °C for 28 days.

Glucagon Powder	Ionization Stabilizing Excipient	Added Concentration	% Peptide Purity
5 mg/mL	Glycine HCl	0.5 mM	Insoluble Aggregates
5 mg/mL	Glycine HCl	1.0 mM	Insoluble Aggregates
5 mg/mL	Glycine HCl	2.0 mM	Insoluble Aggregates
5 mg/mL	Glycine HCl	3.0 mM	98.5%
5 mg/mL	Glycine HCl	4.0 mM	98.6%
5 mg/mL	Glycine HCl	5.0 mM	99.1%
5 mg/mL	Betaine HCl	0.5 mM	Insoluble Aggregates
5 mg/mL	Betaine HCl	2.0 mM	98.6%
5 mg/mL	Betaine HCl	5.0 mM	98.4%
5 mg/mL	HCl	1.0 mM	Insoluble Aggregates
5 mg/mL	HCl	1.8 mM	98.3%
5 mg/mL	HCl	3.2 mM	98.0%

Example 4

5 **Ionization Stabilized Glucagon Compositions at 5 mg/ml**

[00166] The following example demonstrates the stability of a glucagon solution prepared according to the method of the present invention in the presence of added formulation components (e.g. inactive agents, excipients). Sample solutions were prepared by dissolving 10 glucagon powder to a concentration of 5 mg/mL in DMSO which contained about 3.2 mM of added HCl (from a stock solution of 1 N HCl). To these solutions were added varying concentrations of moisture, as well as 5.5% (w/v) mannitol (CAS No. 69-65-8), and 1% (v/v) benzyl alcohol (CAS No. 100-51-6). The experimental samples examined are listed in Table 5.

15 **[00167]** Samples of each formulation were stored in CZ vials and incubated at room temperature (22 – 23 °C). Following 180 days (6 months) of storage the chemical stability of the glucagon peptide was assessed by RP-HPLC (according to the method described in Example 1) and the glucagon purity is reported in Table 5. This example demonstrates that

additional formulation components (e.g. inactive agents, excipients) may be included in the formulation and still yield a stable formulation following approximately 6 months of storage at room temperature.

5 Table 5: Stability of 5 mg/mL Glucagon-DMSO Solutions stored at room temperature for 180 days. Stability is provided as glucagon purity as assessed by RP-HPLC

Glucagon	Added [HCl]	Moisture (% v/v)	Mannitol (% w/v)	Benzyl Alcohol (%v/v)	%Glucagon Purity
5 mg/mL	3.2 mM	0%	0%	0%	98.2
5 mg/mL	3.2 mM	1%	0%	0%	98.3
5 mg/mL	3.2 mM	3%	0%	0%	98.1
5 mg/mL	3.2 mM	5%	0%	0%	98.4
5 mg/mL	3.2 mM	1%	5.5%	0%	98.6
5 mg/mL	3.2 mM	3%	5.5%	0%	97.7
5 mg/mL	3.2 mM	5%	5.5%	0%	98.9
5 mg/mL	3.2 mM	1%	5.5%	1%	95.3
5 mg/mL	3.2 mM	3%	5.5%	1%	96.9
5 mg/mL	3.2 mM	5%	5.5%	1%	97.1

Example 5
An Example of One Embodiment

10 **[00168]** This example demonstrates an embodiment of the claimed invention ("Xeris Glucagon") and Lilly Glucagon (Glucagon for Injection) formulation used in other Examples. The Xeris Glucagon formulation prepared is characterized below in Table 6. Human glucagon (1-29) obtained from Bachem Americas, Inc. (Glucagon H-6790) was used.

Xeris Glucagon

15 **[00169]** Pre-lyophilized bulk drug product is prepared in an aqueous solution of 1.0 mg/mL glucagon, 2 mM glycine, and 1% w/w trehalose, at pH 3.0 as follows:

1. Combine glycine, trehalose, and water to prepare a 2.0 mM glycine, 1% (w/v) trehalose solution.
2. Measure pH and adjust to 3.0 with 1.0 N HCl if needed. If HCl added, check pH a
20 second time.

3. Weigh glucagon powder (adjusted for the peptide content per glucagon CofA as peptide content of synthetic glucagon varies, but is approximately 94%) and dissolve glucagon powder in the buffer to a final concentration of 1.0 mg/mL glucagon. Verify concentration with UV-Vis$_{A280}$ (AM500-54).
4. Filter solution with a Millipak 200 0.45 μm PVDF polishing filter to remove particulates.
5. Utilizing a bio-safety cabinet (BSC) fill 10.0 g aliquots into 20 mL glass vials, and stopper with single-vent lyo stoppers.
6. Place vials on a lyophilization tray, load into the BOC Edwards lyophilizer and initiate cycle.

[00170] The intermediate bulk drug product includes glucagon lyophiles reconstituted in DMSO that are pooled and aseptically filtered, creating a formulation of 5.0 mg/mL glucagon, 10 mM glycine, 5.0% w/w trehalose, and 94.5% w/w DMSO, with "pH memory" 3.0. All materials used in intermediate drug product formulation steps are DMSO compatible.

1. Obtain 500 mL DMSO to create the non-aqueous reconstitution solution.
2. Dissolve individual glucagon lyophiles by adding reconstitution solution to each vial, with a target concentration of 5.0 mg/mL glucagon (and a final trehalose concentration of 5.00%).
3. Pool reconstituted vials into a glass filling vessel and mix.
4. Assay for UV-Vis$_{A280}$ (AM500-54) to determine if further dilution with the solvent mixture is required to achieve the target concentration.
5. If dilution with the solvent mixture is required, again assay for UV-Vis$_{A280.}$
6. Place batch in BSC and sterile filter using a Meissner 0.22 μm capsule filter with DMSO-compatible PTFE membrane.

TABLE 6

Component	**Concentration (% w/w)**
Glucagon	0.45
Glycine	$\leq$ 0.1
Trehalose	5.00
Dimethyl sulfoxide (dmso)	94.54
Hydrochloric acid[1]	
Total:	100.00

[1]variable depending on ph adjustment required: usually ~ 5 ml ($\leq$ 0.1 % w/w) per batch

Novo Nordisk A/S Glucagon

[00171] The Novo Nordisk GlucaGen® (glucagon [rDNA origin] for injection) formulation is characterized as including the following ingredients: Glucagon [rDNA origin] for injection is produced by expression of recombinant DNA in a Saccharomyces cerevisiae vector with subsequent purification. The chemical structure of the glucagon in GlucaGen® is identical to human glucagon and to glucagon extracted from beef and pork pancreas. Glucagon induces liver glycogen breakdown, releasing glucose from the liver and increases blood glucose and relaxes smooth muscle of the gastrointestinal tract. Glucagon is available for use intravenously, intramuscularly, or subcutaneously in a kit that contains a vial of powder (containing glucagon) and a syringe of sterile diluent (the diluent syringe contains 1 mL Sterile Water for Reconstitution). The reconstituted solution contains glucagon as hydrochloride 1 mg/mL (1 unit/mL) and lactose monohydrate (107 mg). GlucaGen® is supplied at pH 2.5-3.5 and is soluble in water. (Source GlucaGen® Product Label, Revised December 29, 2011).

EXAMPLE 6

Comparative PD, PK, Safety and Tolerability Study in Human Test Subjects with Type 1 Diabetes Using Doses of Stable Glucagon and GlucaGen® Administered From a Pump

[00172] This example concerns data obtained from a study which examined the pharmacodynamics (PD), pharmacokinetics (PK), safety and tolerability, speed of absorption, and onset of action of the Xeris Glucagon formulation (see Example 5) ("G-Pump™) in a Phase 2a, single-center, randomized, double-blind, crossover, outpatient comparative pharmacology study in fasted, healthy, type 1 diabetic volunteers (n = 19). The formulation was administered using an OmniPod® pump.

[00173] The experiments were conducted in 19 volunteers in a single-center, randomized, double-blind, crossover, outpatient, comparative, phase 2a study. Eligible subjects were otherwise healthy, fasted male and female patients with type 1 diabetes, between 18 and 65 years of age, on CSII (continuous subcutaneous insulin infusion) therapy. On Treatment Day 1, each subject received 3 doses of either Xeris or Novo glucagon at 0.3, 1.2 and 2.0 μg/kg via an OmniPod®. During the visit, CSII was suspended, patients were placed on an IV insulin clamp, and the infusion rate was fixed 30 min prior to the first glucagon dose with a goal of maintaining blood glucose of 80-140 mg/dl before the glucagon bolus infusion. A Hemocue was used to measure glucose every 10 min, and samples were drawn prior to, and at 2, 5, 10,

15, 20, 30, 40, 60, 80, 100, 120, and 150 minutes post-dose for measurement of glucagon by RIA. Each subject returned for a second treatment day, and received the same 3 doses of the other (Xeris or Novo glucagon) treatment via an OmniPod®.

[00174] The primary endpoints of the study were safety and tolerability assessments, including 12-lead ECGs, vital signs, adverse events (AEs) as well as serious adverse events (SAEs), body weight and laboratory tests.

[00175] The secondary Endpoints were also assessed for PD and PK. The PD parameters included: area under glucose vs. time curve (AUC); maximum concentration of glucose (C_{max}); time of maximum glucose concentration (T_{max}); The PK parameters included: area under the glucagon vs. time curve (AUC); maximum concentration of glucagon (C_{max}); time of maximum glucagon concentration (T_{max}).

Results

[00176] 19 subjects were randomized and 18 completed all treatment visits. The pharmacodynamics and pharmacokinetics for the G-Pump™ and GlucaGen® are presented in Tables 7 – 11.

Pharmacodynamics:

[00177] A Hemocue was used to measure glucose every 10 min. Glucose C_{max}, T_{max}, and $AUC_{0\text{-}60min}$, $AUC_{0\text{-}120min}$, and $AUC_{0\text{-}150min}$ were determined for all doses in both groups (Dose 0.3 = 0.3μg/kg, Dose 1.2 = 1.2μg/kg, Dose 2 = 2.0μg/kg). See Tables 7 through 11. The G-Pump™ glucagon effectively increased blood glucose levels in a dose-dependent fashion with C_{max} of 178, 198 and 213 mg/dl, respectively and a T_{max} of 38, 50, and 49 min. G-Pump™ glucose C_{max}, T_{max}, and $AUC_{0\text{-}60min}$, $AUC_{0\text{-}120min}$, and $AUC_{0\text{-}150min}$ were comparable to GlucaGen® See Table 7 through Table 11. As an example, a plot of mean plasma glucose and glucagon vs. time for the 2.0 μg/kg dose (0.15 mg in a 75 kg adult) showed little separation between treatment groups.

Pharmacokinetics:

[00178] Samples were drawn prior to dosing, and at 2, 5, 10, 15, 20, 30, 40, 60, 80, 100, 120, and 150 minutes post-dose for measurement of glucagon by RIA. Glucagon C_{max}, T_{max}, and $AUC_{0\text{-}60min}$, $AUC_{0\text{-}120min}$, and $AUC_{0\text{-}150min}$ were determined for all doses in both groups. See Tables 12 through 16. Comparison of the two treatment groups showed G-Pump™ was comparable to doses of GlucaGen® across the treatment groups for C_{max}, T_{max}, and $AUC_{0\text{-}60min}$, $AUC_{0\text{-}120min}$, and $AUC_{0\text{-}150min}$. As an example, the ratio between the 2.0μg/kg dose of G-

Pump™ and GlucaGen® for glucagon $AUC_{0\text{-}150min}$ is 1.01, 90% CI 0.85-1.21), but G-Pump™ exceeded the upper bound for C_{max} (1.11, 0.86-1.42) and T_{max} (1.10, 0.85-1.43). See Table 12 through Table 16.

TABLE 7

SUMMARY OF C_{max} OF GLUCOSE (mg/dl)

SUMMARY OF CMAX OF GLUCOSE (MG|DL) (GEOMETIC MEAN)			
	CATEGORY	XERIS	NOVO
Dose 0.3	N	19	16
	GEMean(SD)	177.6 (43.10)	162.0 (51.69)
	Min ~ Max	111.0~258.0	85.0~305.0
Dose 1.2	N	19	16
	GEMean(SD)	198.8 (49.37)	183.3 (66.15)
	Min ~ Max	123.0~281.0	79.0~280.0
Dose 2	N	19	16
	GEMean(SD)	212.6 (57.18)	200.6 (63.81)
	Min ~ Max	113.0~315.0	115.0~327.0
Overall	N	19	16
	GEMean(SD)	195.8 (43.37)	181.3 (56.26)
	Min ~ Max	127.8~273.1	102.6~303.4

TABLE 8
SUMMARY OF T_{max} OF GLUCOSE (min)

SUMMARY OF TMAX OF GLUCOSE (MIN) (GEOMETRIC MEAN)			
	CATEGORY	XERIS	NOVO
Dose 0.3	N	14	11
	GEMean(SD)	38.2 (29.62)	25.9 (28.38)
	Min ~ Max	5.0~150.0	2.0~150.0
Dose 1.2	N	18	16
	GEMean(SD)	49.5 (41.79)	31.2 (34.75)
	Min ~ Max	3.0~80.0	3.0~80.0
Dose 2	N	19	16
	GEMean(SD)	49.4 (40.59)	52.8 (20.21)
	Min ~ Max	2.0~80.0	20.0~100.0
Overall	N	19	16
	GEMean(SD)	46.5 (31.64)	37.4 (21.50)
	Min ~ Max	14.7~73.7	13.4~81.4

TABLE 9
SUMMARY OF $AUC_{0\text{-}60min}$ OF GLUCOSE ([mg/dl]*min)

SUMMARY OF GLUAUC60 (MG|DL*MIN) (GEOMETIC MEAN)			
	CATEGORY	XERIS	NOVO
Dose 0.3	N	19	16
	GEMean(SD)	9587 (2820)	9032 (2983)
	Min ~ Max	5210~15033	4708~17994
Dose 1.2	N	19	16
	GEMean(SD)	10431 (3637)	9791 (3584)
	Min ~ Max	6391~15553	4049~16279
Dose 2	N	19	16
	GEMean(SD)	10609 (2763)	9943 (2692)
	Min ~ Max	6180~17899	6299~15735
Overall	N	19	16
	GEMean(SD)	10196 (2374)	9580 (2789)
	Min ~ Max	6472~14530	5632~15804

TABLE 10
SUMMARY OF $AUC_{0\text{-}120min}$ OF GLUCOSE ([mg/dl]*min)

SUMMARY OF GLUAUC120 (MG|DL*MIN) (GEOMETIC MEAN)			
	CATEGORY	XERIS	NOVO
Dose 0.3	N	19	16
	GEMean(SD)	18472 (6189)	17374 (6300)
	Min ~ Max	8881~29793	7968~35304
Dose 1.2	N	19	16
	GEMean(SD)	21406 (5563)	19687 (7600)
	Min ~ Max	12971~30763	7979~32519
Dose 2	N	19	16
	GEMean(SD)	22310 (6339)	21057 (6653)
	Min ~ Max	11230~34829	11678~33265
Overall	N	19	16
	GEMean(SD)	20663 (5424)	19312 (6307)
	Min ~ Max	11074~29890	10403~33489

TABLE 11

SUMMARY OF $AUC_{0\text{-}150min}$ OF GLUCOSE ([mg/dl]*min)

SUMMARY OF GLUAUC150 (MG|DL*MIN) (GEOMETIC MEAN)			
	CATEGORY	XERIS	NOVO
Dose 0.3	N	19	16
	GEMean(SD)	22296 (7998)	21005 (7931)
	Min ~ Max	9736~36978	9138~43629
Dose 1.2	N	19	16
	GEMean(SD)	26251 (7110)	24079 (9348)
	Min ~ Max	15431~37797	10019~40349
Dose 2	N	19	16
	GEMean(SD)	27360 (8190)	25845 (8624)
	Min ~ Max	13045~42104	13643~41805
Overall	N	19	16
	GEMean(SD)	25206 (7097)	23557 (7975)
	Min ~ Max	12607~37240	12286~41906

TABLE 12

SUMMARY OF C_{max} OF GLUCAGON (pg/dl)

SUMMARY OF CMAX OF GLUCAGON (PG|DL) (GEOMETIC MEAN)			
	CATEGORY	XERIS	NOVO
Dose 0.3	N	19	16
	GEMean(SD)	168.1 (57.66)	140.2 (53.69)
	Min ~ Max	105.6~300.0	77.2~303.2
Dose 1.2	N	19	16
	GEMean(SD)	328.3 (127.9)	229.3 (161.6)
	Min ~ Max	163.2~664.2	52.4~601.0
Dose 2	N	19	16
	GEMean(SD)	440.6 (310.9)	446.9 (220.4)
	Min ~ Max	56.6~1253	177.7~1212
Overall	N	19	16
	GEMean(SD)	289.7 (95.19)	243.1 (94.04)
	Min ~ Max	158.7~543.5	120.0~422.5

TABLE 13
SUMMARY OF T_{max} OF GLUCAGON (min)

SUMMARY OF TMAX OF GLUCAGON (MIN) (GEOMETIC MEAN)			
	CATEGORY	XERIS	NOVO
Dose 0.3	N	19	16
	GEMean(SD)	24.5 (23.42)	23.3 (10.71)
	Min - Max	2.0-150.0	15.0-80.0
Dose 1.2	N	19	16
	GEMean(SD)	27.3 (12.93)	23.9 (6.31)
	Min - Max	10.0-60.0	15.0-40.0
Dose 2	N	19	16
	GEMean(SD)	31.9 (15.93)	23.9 (8.74)
	Min - Max	15.0-150.0	10.0-40.0
Overall	N	19	16
	GEMean(SD)	27.7 (12.10)	23.7 (5.92)
	Min - Max	9.3-45.8	15.9-41.6

TABLE 14
SUMMARY OF $AUC_{0\text{-}60min}$ OF GLUCAGON ([pg/dl]*min)

SUMMARY OF AUC60 (PG|DL*MIN) (GEOMETIC MEAN)			
	CATEGORY	XERIS	NOVO
Dose 0.3	N	19	16
	GEMean(SD)	7022 (2572)	6187 (2217)
	Min ~ Max	3120~12319	3576~13701
Dose 1.2	N	19	16
	GEMean(SD)	13062 (4886)	9704 (6112)
	Min ~ Max	5979~21680	2481~19255
Dose 2	N	19	16
	GEMean(SD)	17451 (11487)	18132 (8481)
	Min ~ Max	2444~39209	8196~43117
Overall	N	19	16
	GEMean(SD)	11697 (3490)	10287 (3457)
	Min ~ Max	6780~19316	5684~16949

TABLE 15
SUMMARY OF $AUC_{0\text{-}120min}$ OF GLUCAGON ([pg/dl]*min)

SUMMARY OF AUC120 (PG|DL*MIN) (GEOMETIC MEAN)			
	CATEGORY	XERIS	NOVO
Dose 0.3	N	19	16
	GEMean(SD)	10542 (3281)	9395 (3162)
	Min ~ Max	4070-18041	4597-18967
Dose 1.2	N	19	16
	GEMean(SD)	19460 (5909)	14266 (7703)
	Min ~ Max	10078~30730	4150~35199
Dose 2	N	19	16
	GEMean(SD)	25561 (14459)	25439 (10635)
	Min ~ Max	4486~45459	12045~48951
Overall	N	19	16
	GEMean(SD)	17373 (4467)	15051 (4392)
	Min ~ Max	10133~25200	8741~23924

TABLE 16

SUMMARY OF $AUC_{0\text{-}150min}$ OF GLUCAGON ([pg/dl]*min)

SUMMARY OF AUC150 (PG|DL*MIN) (GEOMETIC MEAN)			
	CATEGORY	XERIS	NOVO
Dose 0.3	N	19	16
	GEMean(SD)	13104 (3944)	11070 (3032)
	Min ~ Max	4878~19618	7050~20467
Dose 1.2	N	19	16
	GEMean(SD)	21177 (6139)	15781 (7874)
	Min ~ Max	11201~33489	5108~36806
Dose 2	N	19	16
	GEMean(SD)	27595 (14530)	37355 (10757)
	Min ~ Max	5664~47947	13390~50603
Overall	N	19	16
	GEMean(SD)	19196 (4866)	16844 (4520)
	Min ~ Max	11303~38099	10218~25643

Safety and Tolerability:

[00179] No SAEs were reported and no unexpected safety or tolerability issues with the glucagon treatments were observed. Adverse events reported generally included known issues related to administration of glucagon.

5 Conclusions:

[00180] The G-Pump™ was comparable to doses of GlucaGen® across the treatment groups and effectively increased blood glucose levels in a dose-dependent fashion. The G-Pump™ glucagon formulation was comparable to the Novo Nordisk GlucaGen® glucagon formulation when administered from an OmniPod® pump.

10

EXAMPLE 7
OPEN-LOOP CLINICAL STUDY

[00181] This example concerns data obtained in an open-loop clinical study where hypoglycemia was induced prior to administering the glucagon formulation. Adult male or 15 female patients with post-bariatric hypoglycemia (PBH) syndrome were recruited from the hypoglycemia clinic at the Joslin Diabetes Center (Boston, MA). After providing written informed consent, study subjects underwent a history and physical examination, with blood and urine samples obtained for laboratory testing, including HbA1c (glycated hemoglobin), serum chemistry, urinalysis, and pregnancy test (if applicable).

20 **[00182]** Subjects meeting all eligibility criteria had two continuous glucose monitor sensors (Dexcom® G4 Platinum) placed on the anterior abdominal wall (to ensure sensor availability and calibration for the subsequent visit). Participants were provided a glucometer and instructed in both sensor insertion and calibration techniques. After allowing sensors to calibrate at 24-72 hours, subjects returned to the study site for a mixed-meal-tolerance test 25 (MMTT).

[00183] An OmniPod® pump (Insulet Corporation; MA, USA) filled with 1 mL of glucagon solution (a 5 mg/mL concentration prepared as outlined in Example 15 above) was applied to the abdomen of the subject. Each subject was instructed drink a mixed-meal beverage containing 60 g of carbohydrates (e.g., Boost® Nutritional Drink) over 10 minutes, 30 producing an increase in blood glucose followed by a rapid decline, which is characteristic of PBH syndrome (See FIG. 6).

[00184] The open-loop system was set to recognize impending sensor glucose values <75 mg/dL. This triggered an alert to the monitoring physician, who then delivered a bolus dose of 150 μg of glucagon (corresponding to a 30 μL volume) via the OmniPod® pump, with the goal of preventing a further decline in glucose values. Note that while the algorithm provided an alert as to when glucagon should be dosed to prevent hypoglycemia, there was no automation of dosing, and it was left to the attending physician to decide when to initiate glucagon dosing via the OmniPod® controller. Plasma glucose was measured with a 2300 Stat Plus™ glucose analyzer (YSI Incorporated; OH, USA) just prior to dosing and at 5, 10, 20, 30, 45, 60, 90, and 120 minutes after glucagon administration.

[00185] For both subjects the administered dose of glucagon arrested hypoglycemia and raised the blood glucose back to normal levels (i.e. > 70 mg/dL) (FIG. 6). In addition, symptoms of hypoglycemia, which appeared as blood glucose levels decreased, were reversed following administration of glucagon. Further, blood glucose remained well below 180 mg/dL during the 120 minutes post-treatment monitoring window, indicating the prevention of rebound hyperglycemia, a problem commonly associated with treatment of hypoglycemia.

* * * * * *

[00186] All of the compositions and/or methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this disclosure have been described in terms of some embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit, and scope of the disclosure. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope, and concept of any invention as defined by the appended claims.

[00187] Embodiments of the present disclosure have been described in an illustrative manner, and it is to be understood that the particular embodiments depicted in the figures and the terminology which has been used has been intended in a nature of words of description rather than of limitation. It is to be further understood that any combination of the

ingredients/therapeutic agents described in the foregoing paragraphs are deemed to be encompassed by the appended claims. It is to be further understood that all specific embodiments of the delivery apparatus are deemed to be encompassed by the appended claims. Many modifications and variations of the present disclosure are possible in light of
5 the above teachings. It is therefore to be understood that the obvious modifications are deemed to be encompass within the appended claims.

[00188] The above specification and examples provide a complete description of the structure and use of illustrative embodiments. Although certain embodiments have been described above with a certain degree of particularity, or with reference to one or more
10 individual embodiments, those skilled in the art could make numerous alterations to the disclosed embodiments without departing from the scope of this disclosure. As such, the various illustrative embodiments of the methods and systems are not intended to be limited to the particular forms disclosed. Rather, they include all modifications and alternatives falling within the scope of the claims, and embodiments other than the one shown may include some
15 or all of the features of the depicted embodiment. For example, elements may be omitted or combined as a unitary structure, and/or connections may be substituted. Further, where appropriate, aspects of any of the examples described above may be combined with aspects of any of the other examples described to form further examples having comparable or different properties and/or functions, and addressing the same or different problems.
20 Similarly, it will be understood that the benefits and advantages described above may relate to one embodiment or may relate to several embodiments.

[00189] The claims are not intended to include, and should not be interpreted to include, means-plus- or step-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase(s) “means for” or “step for,” respectively.

CLAIMS

1. A glucagon delivery apparatus comprising:

   a reservoir containing a composition comprising glucagon, a glucagon analogue, or a salt form of either thereof;

   a sensor configured to measure a patient's blood glucose level; and

   an electronic pump configured to intradermally, subcutaneously or intramuscularly deliver at least a portion of the composition to a patient based on the patient's measured blood glucose level.

2. The glucagon delivery apparatus of claim 1, where the sensor is configured to transmit data wirelessly, via radio frequency, or via a wired connection, to a processor configured to control operation of the electronic pump.

3. The glucagon delivery apparatus of claim 2, where the processor is configured to control operation of the pump based at least in part on the data obtained by the sensor.

4. The glucagon delivery apparatus of claim 3, where the processor is configured to control operation of the pump to intradermally, subcutaneously or intramuscularly inject at least a portion of the composition if the data obtained by the sensor indicates a glucose level below a defined threshold.

5. The glucagon delivery apparatus of any one of claims 1 to 4, further comprising a monitor configured to communicate information indicative of the patient's glucose level.

6. The glucagon delivery apparatus of claim 5, where the monitor comprises a speaker or a display device, or both.

7. The glucagon delivery apparatus of any of claims 5 to 6, where the monitor is configured to communicate an alert when a glucose level of the patient is estimated to be at a defined threshold.

8. The glucagon delivery apparatus of any of claims 1 to 7, where the apparatus is configured to allow manual adjustment of at least one of a delivery rate and a dose of

the composition intradermally, subcutaneously or intramuscularly delivered by the pump.

9. The glucagon delivery apparatus of any of claims 1 to 8, where the composition does not include a drug capable of decreasing the blood glucose level in the patient and/or where the apparatus is not capable of injecting a composition comprising a drug capable of decreasing the blood glucose level in the patient.

10. The glucagon delivery apparatus of claims 1 to 8, where the composition further includes a drug capable of decreasing the blood glucose level in the patient and/or where the apparatus is capable of injecting a composition comprising a drug capable of decreasing the blood glucose level in the patient.

11. The glucagon delivery apparatus of any one of claims 9 to 10, where the drug capable of decreasing the blood glucose level in the patient is insulin, an insulin mimetic peptide, incretin, or an incretin mimetic peptide.

12. The glucagon delivery apparatus of any one of claims 1 to 11, wherein the apparatus is a closed-loop system for delivering glucagon to the patient.

13. The glucagon delivery apparatus of any one of claims 1 to 11, wherein the apparatus is an open-loop system for delivering glucagon to the patient.

14. The glucagon delivery apparatus of any one of claims 1 to 11, wherein the apparatus is a no-loop system for delivering glucagon to the patient.

15. The glucagon delivery apparatus of any one of claims 1 to 14, wherein the composition is a single-phase solution comprising the glucagon, glucagon analogue, or a salt form of either thereof, dissolved in a non-aqueous solvent.

16. The glucagon delivery apparatus of claim 15, wherein the composition comprises glucagon, glucagon analogue, or a salt form of either thereof solubilized in an aprotic polar solvent.

17. The glucagon delivery apparatus of claim 16, wherein the composition further comprises an ionization stabilizing excipient, wherein (i) the glucagon, glucagon analogue, or salt thereof is dissolved in the aprotic solvent in an amount from about

0.1 mg/mL up to the solubility limit of the glucagon, glucagon analogue, or salt thereof, and (ii) the ionization stabilizing excipient is dissolved in the aprotic solvent in an amount to stabilize the ionization of the glucagon peptide or salt thereof.

18. The glucagon delivery apparatus of claim 17, wherein the ionization stabilizing excipient is at a concentration of 0.1 mM to less than 100 mM.

19. The glucagon delivery apparatus of claim 17, wherein the ionization stabilizing excipient is a mineral acid.

20. The glucagon delivery apparatus of claim 19, wherein the mineral acid is hydrochloric acid.

21. The glucagon delivery apparatus of claim 17, wherein the aprotic solvent is DMSO.

22. The glucagon delivery apparatus of claim 17, wherein the aprotic solvent is a deoxygenated aprotic solvent.

23. The glucagon delivery apparatus of claim 17, wherein the ionization stabilizing excipient is HCl and the aprotic solvent is DMSO.

24. The glucagon delivery apparatus of claim 17, wherein the composition has a moisture content of less than 10, 5, or 3 %.

25. The glucagon delivery apparatus of claim 17, wherein the composition further comprises a preservative at less than 10, 5, or 3% w/v.

26. The glucagon delivery apparatus of claim 25, wherein the preservative is benzyl alcohol.

27. The glucagon delivery apparatus of claim 17, wherein the composition further comprises a sugar alcohol at less than 10, 5, or 3% w/v.

28. The glucagon delivery apparatus of claim 27, wherein the sugar alcohol is mannitol.

29. The glucagon delivery apparatus of claim 16, wherein the composition further comprises a carbohydrate, an amphoteric molecule, and optionally an acid.

30. The glucagon delivery apparatus of claim 29, wherein the aprotic polar solvent is DMSO, the carbohydrate is trehalose, the amphoteric molecule is glycine, and the optional acid is hydrochloric acid.

31. The glucagon delivery apparatus of any one of claims 29 to 30, wherein the composition comprises at least 80 wt.% of the aprotic polar solvent, 3 to 7 wt. % of the carbohydrate, 0.001 to 0.1 wt. % of the amphoteric molecule, and 0 wt. % to less than 0.1 wt. % of the acid.

32. The glucose delivery apparatus of any one of claims 29 to 31, where the composition comprises, consists essentially of, or consists of glucagon, the glucagon analogue, or the salt form of either thereof, the aprotic polar solvent, the amphoteric molecule, the carbohydrate, and optionally the acid.

33. The glucagon delivery apparatus of any one of claims 1 to 32, where the composition has a water content of 0 to less than 15 wt. %, 0 to less than 3 wt. %, 3 to 10 wt. %, or 5 to 8 wt. %.

34. The glucagon delivery apparatus of any one of claims 16 to 33, where the glucagon, glucagon analogue, or salt form of either thereof, has been previously dried from a buffer, wherein the dried glucagon, glucagon analogue, or salt form of either thereof, has a first ionization profile that corresponds to an optimal stability and solubility for the glucagon, glucagon analogue, or salt form thereof, wherein the dried glucagon, glucagon analogue, or salt form of either thereof, is reconstituted into an aprotic polar solvent and has a second ionization profile in the aprotic polar solvent, and wherein the first and second ionization profiles are within 1 pH unit of one another.

35. The glucagon delivery apparatus of claim 34, where the first or second or both ionization profiles correspond to the ionization profile of glucagon when solubilized in an aqueous solution having a pH range of about 1 to 4 or 2 to 3.

36. The glucagon delivery apparatus of any one of claims 16 to 35, where the composition is capable of providing a T_{max} for glucagon or the glucagon analogue of 1 minutes to 160 minutes after subcutaneous administration to the subject, and/or is capable of providing a C_{max} for glucagon or the glucagon analogue of 50 pg/dL to 1300 pg/dL after subcutaneous administration to the subject.

37. The glucagon delivery apparatus of claim 36, where the composition is capable of providing a T_{max} for glucagon or the glucagon analogue of 5 minutes to 50 minutes after subcutaneous administration to the subject and/or wherein the composition is capable of providing a C_{max} for glucagon or the glucagon analogue of 150 pg/dL to 550 pg/dL after subcutaneous administration to the subject.

38. The glucagon delivery apparatus of claim 37, where the composition is capable of providing an AUC_{60} for glucagon or the glucagon analogue of 2000 pg min./dL to 40000 pg min./dL.

39. The glucagon delivery apparatus of any one of claims 1 to 14, where the composition is a two-phase mixture of a powder dispersed in a liquid that is a non-solvent to the solid, where the powder comprises the glucagon, glucagon analogue, or a salt form of either thereof, and where the liquid is a pharmaceutically acceptable carrier, where the powder is homogeneously contained within a pharmaceutically acceptable carrier.

40. The glucagon delivery apparatus of claim 39, where the composition is a paste, slurry, or suspension.

41. The glucagon delivery apparatus of any one of claims 39 to 40, where the powder has a mean particle size ranging from 10 nanometers (0.01 microns) to about 100 microns, with no particles being larger than about 500 microns.

42. The glucose delivery apparatus of any one of claims 1 to 41, where the composition has been stored in the reservoir for at least 1, 2, 3, 4, 5, 6, 7, 14, 21, 30, 45, or 60 days.

43. The glucose delivery apparatus of any one of claims 1 to 42, where the composition remains stable after being stored for one month or 6 months or 12 months or 18 months at room temperature.

44. A method of treating hypoglycemia in a patient, the method comprising using a glucagon delivery apparatus of any of claims 1-43 to intradermally, subcutaneously or intramuscularly deliver at least a portion of the composition to the patient after the sensor measures the patient's blood glucose level, and data containing the patient's blood glucose level is transmitted to the processor.

45. The method of claim 44, where the patient's measured blood glucose level is from 0 mg/dL to less than 50 mg/dL or the data shows an indication of impending hypoglycemia before delivery of the composition, and where the patient has a blood glucose level from 50 mg/dL to 180 mg/dL within 1 to 20 minutes after delivery of the composition.

46. The method of any one of claims 44 to 45, wherein the patient has been diagnosed with Type 1, Type II, or gestational diabetes.

47. The method of any one of claims 44 to 46, wherein the subject has been diagnosed with a hyperinsulinemic hypoglycemia disorder.

48. The method of claim 47, wherein the hyperinsulinemic hypoglycemia disorder is congenital hyperinsulinism, insulinoma, reactive hypoglycemia, or drug-induced hypoglycemia.

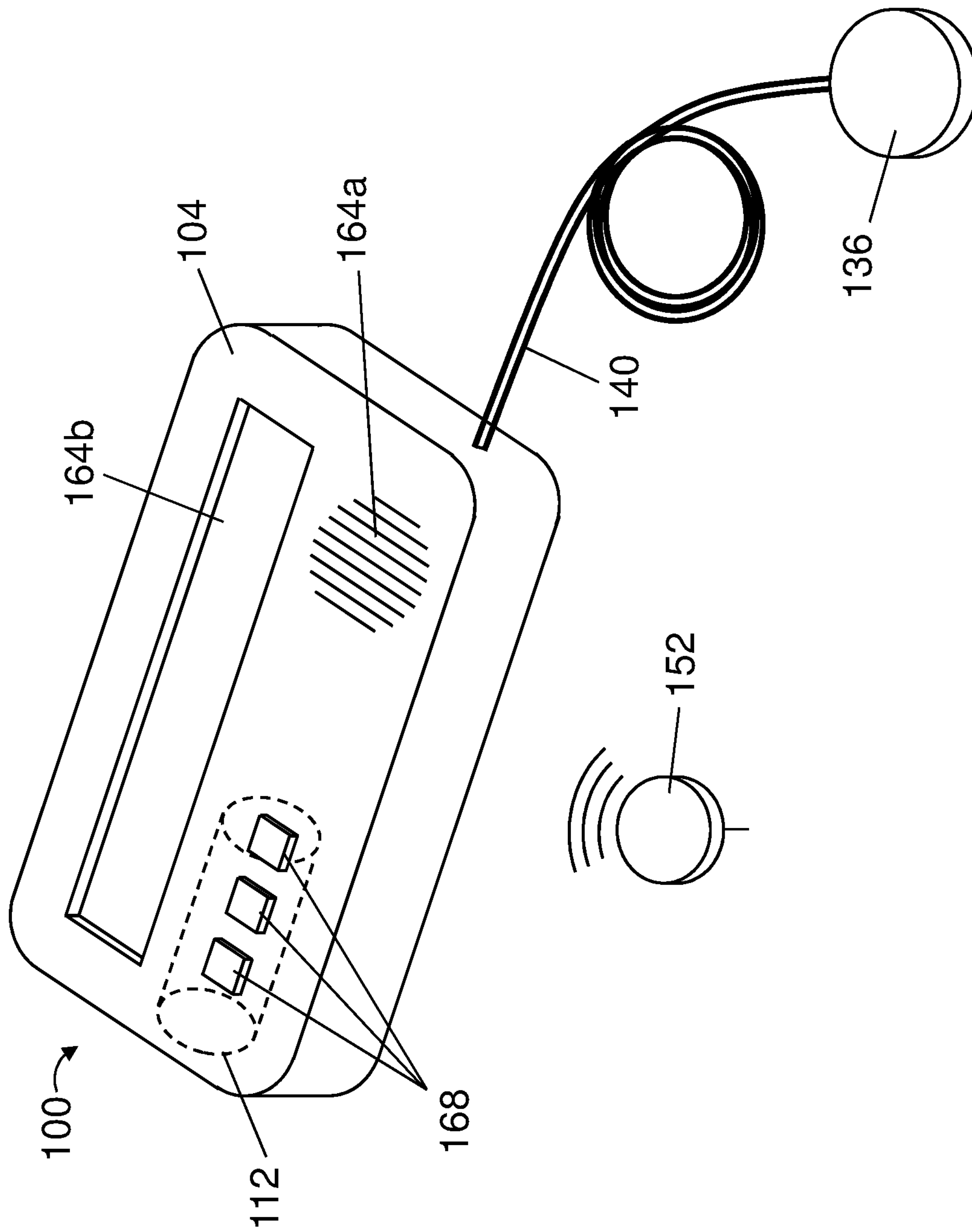

FIG. 1

FIG. 2

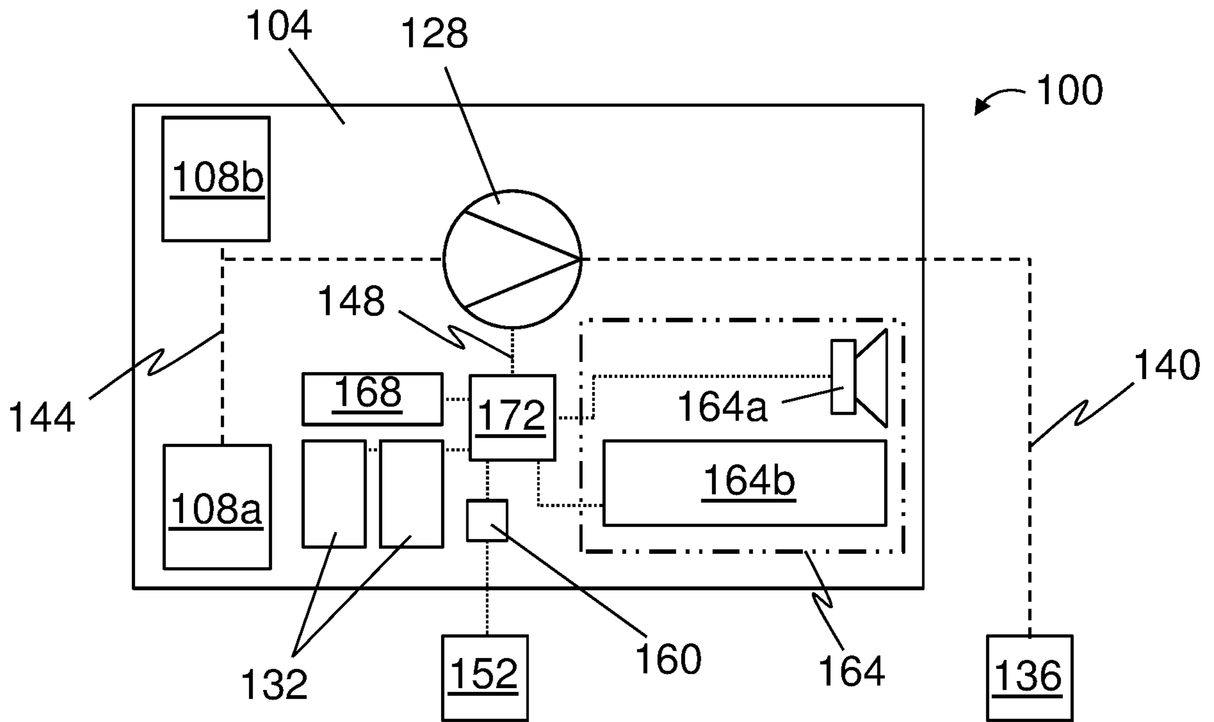

FIG. 3

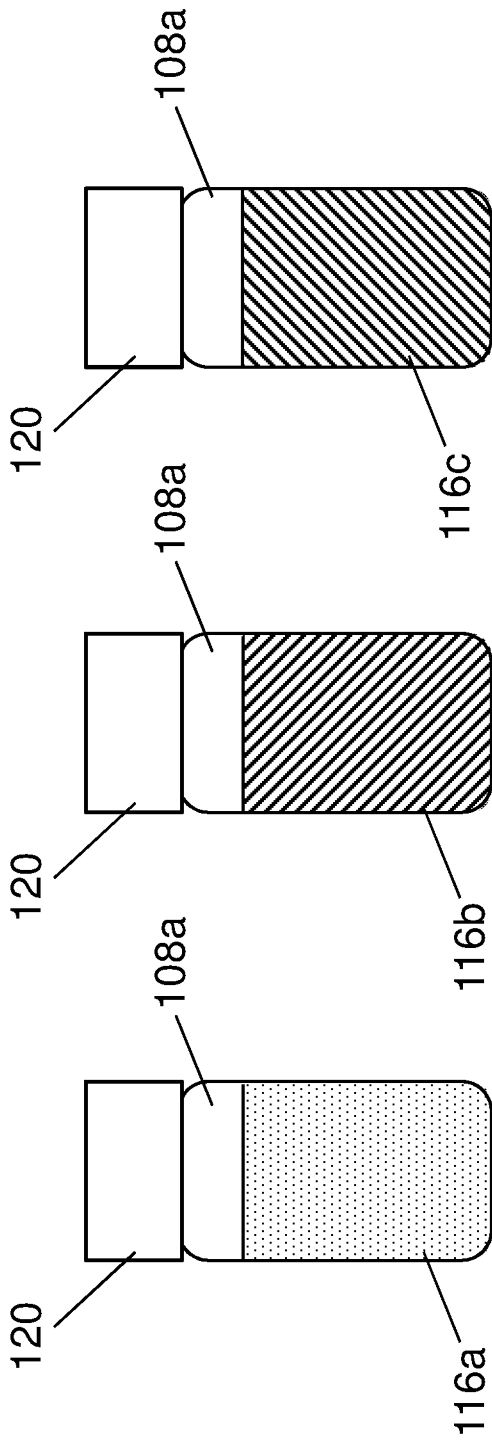

FIG. 4C

FIG. 4B

FIG. 4A

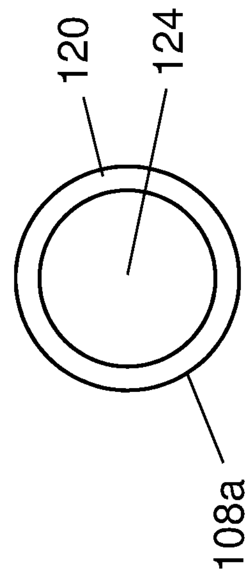

FIG. 4D

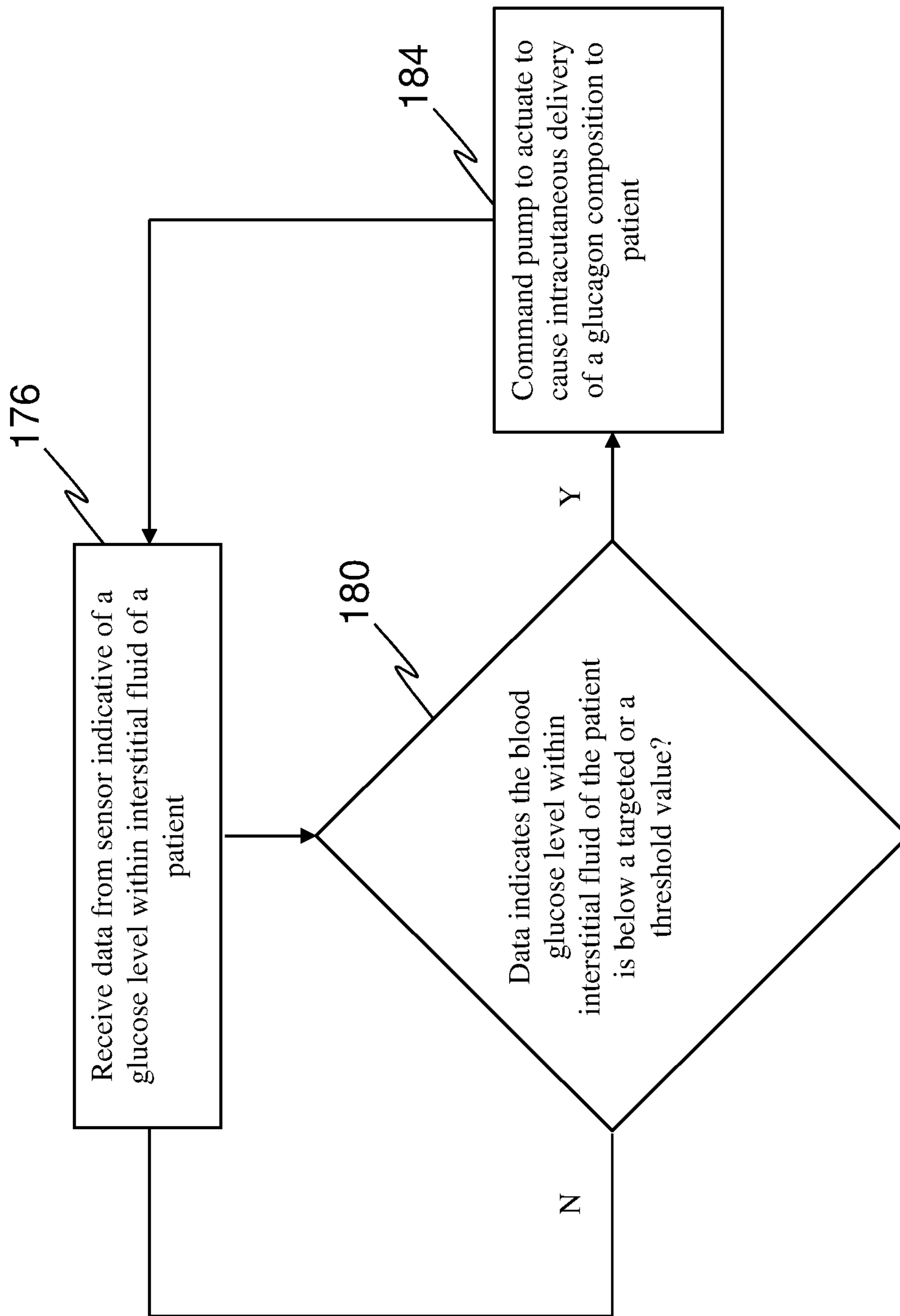

FIG. 5

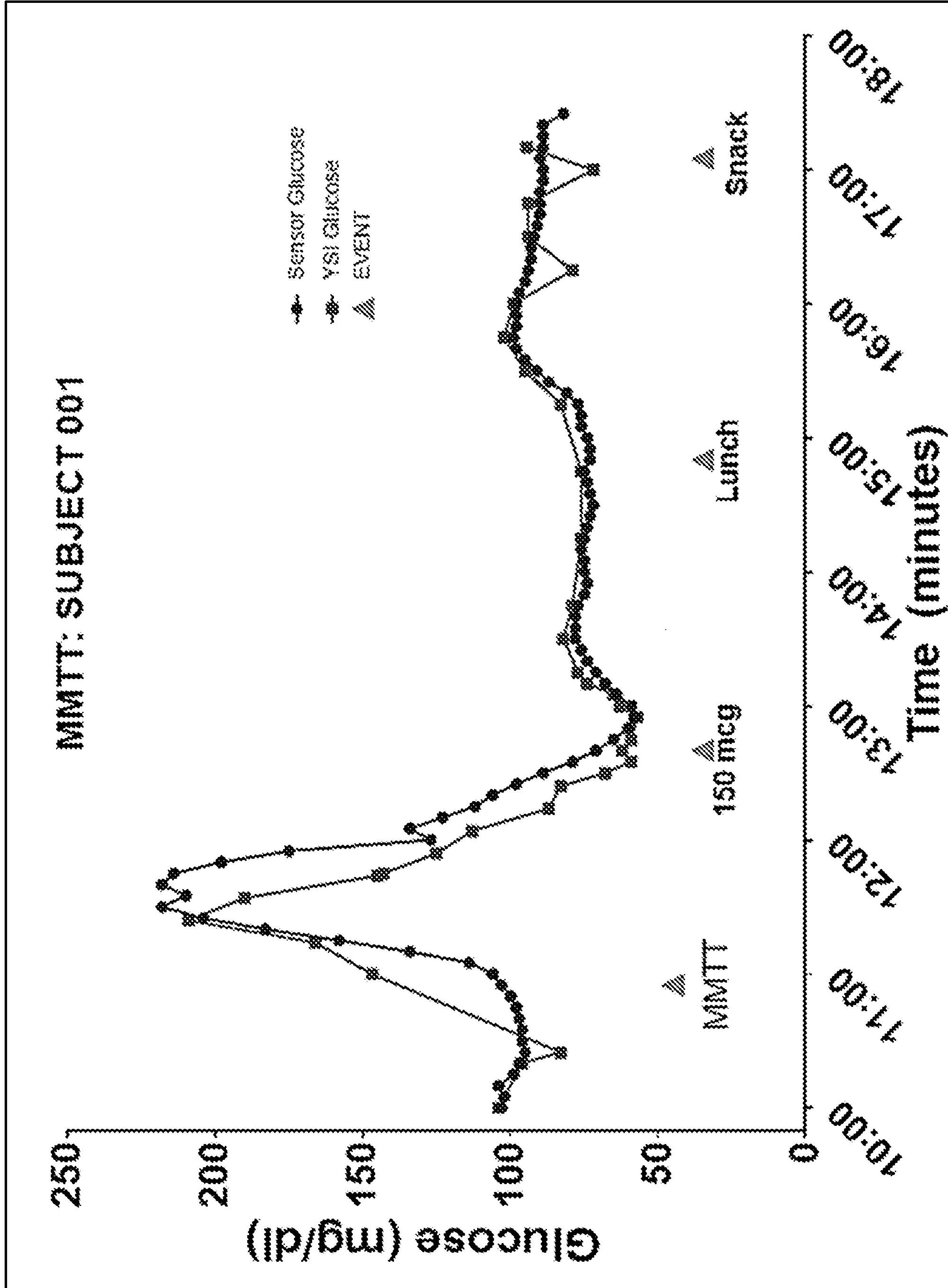

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/035792

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/00 A61K9/00 A61M5/142 A61K47/26
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61M

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/060908 A2 (ROCHE DIAGNOSTICS GMBH [DE]; HOFFMANN LA ROCHE [CH]) 26 May 2011 (2011-05-26) (par. 0025; 0034; fig. 6-7; claims 1-15; abstract; whole document -----	1-48
X	US 2012/232001 A1 (PRESTRELSKI STEVEN [US] ET AL) 13 September 2012 (2012-09-13) paragraph [0117]; claim 72; examples 1-16 the whole document -----	1-48
A	WO 2013/067022 A1 (XERIS PHARMACEUTICALS INC [US]) 10 May 2013 (2013-05-10) the whole document -----	15-48
X,P	WO 2015/153728 A1 (XERIS PHARMACEUTICALS INC [US]) 8 October 2015 (2015-10-08) the whole document -----	1-44

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2016	03/08/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Schindler-Bauer, P

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/035792

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011060908 A2	26-05-2011	CN 102648470 A	22-08-2012
		EP 2502172 A2	26-09-2012
		US 2011118578 A1	19-05-2011
		WO 2011060908 A2	26-05-2011
US 2012232001 A1	13-09-2012	AU 2012225268 A1	26-09-2013
		CA 2829400 A1	13-09-2012
		CN 103442695 A	11-12-2013
		EA 201370178 A1	28-02-2014
		EP 2683364 A2	15-01-2014
		JP 2014507484 A	27-03-2014
		KR 20140041460 A	04-04-2014
		SG 193389 A1	30-10-2013
		US 2012232001 A1	13-09-2012
		US 2014171362 A1	19-06-2014
		US 2014179599 A1	26-06-2014
		US 2014179600 A1	26-06-2014
		WO 2012122535 A2	13-09-2012
		ZA 201306836 B	29-04-2015
WO 2013067022 A1	10-05-2013	AU 2012332556 A1	19-06-2014
		CA 2853942 A1	10-05-2013
		CN 103930096 A	16-07-2014
		DK 2773331 T3	14-03-2016
		EA 201490913 A1	30-09-2014
		EP 2773331 A1	10-09-2014
		ES 2574761 T3	21-06-2016
		JP 2014532667 A	08-12-2014
		KR 20140107228 A	04-09-2014
		SG 11201401921Y A	29-05-2014
		US 2014349926 A1	27-11-2014
		WO 2013067022 A1	10-05-2013
WO 2015153728 A1	08-10-2015	NONE	