BRIEF SUMMARY ~~OF THE INVENTION~~

[0012] To address such needs and others, the present disclosure~~invention~~ provides a stable glucagon rescue formulation as well as methods of using this stable glucagon formulation to treat severe hypoglycemia. Advantageously, the glucagon is stabilized in the formulations of the present invention so as to allow for long-term storage and/or delivery over a prolonged period of time. As such, the glucagon formulations of the present invention are stable at nonrefrigerated temperatures for extended periods of time, are simple to administer, without the need for reconstitution, and are functional over a range of temperatures, including temperatures ranging from 0°C – 30°C.

[0013] Importantly, the formulation technology of the present invention is widely applicable for the delivery of numerous other peptides that, like glucagon, have poor or limited stability and solubility in an aqueous environment. In fact, it is now clear that the formulation of peptides with an aprotic polar solvent such as DMSO, NMP, ethyl acetate, or a mixture thereof into high concentration, nonaqueous solutions is a valuable delivery platform for this important class of peptide therapeutics. Additionally, the formulation technology of the present invention is widely applicable for the delivery of two or more peptides in the same solution.

[0013a] In one aspect, there is provided a stable solution for parenteral injection, comprising:

(a) a peptide or a salt thereof, wherein the peptide has been dried in a non-volatile buffer, and wherein the dried peptide has a pH memory that is equal to the pH of the peptide in the non-volatile buffer; and

(b) an aprotic polar solvent, wherein the peptide or salt thereof is in the aprotic polar solvent in an amount from 0.1 mg/mL up to the solubility limit of the peptide or salt thereof;

wherein the moisture content of the solution is less than 5%, and wherein the dried peptide maintains the pH memory in the aprotic polar solvent.

[0013b] In another aspect, there is provided a stable solution for parenteral injection, comprising:

(a) glucagon, wherein the glucagon has been dried in a non-volatile buffer, and wherein the dried glucagon has a pH memory that is equal to the pH of the glucagon in the non-volatile buffer selected from a glycine buffer, a citrate buffer, a phosphate buffer, and mixtures thereof, wherein the pH memory of the dried glucagon is from 2.0 to 3.0; and

(b) an aprotic polar solvent selected from dimethylsulfoxide (DMSO), n-methyl pyrrolidone (NMP), ethyl acetate, and mixtures thereof, wherein the glucagon is in the aprotic polar solvent in an amount from 0.1 mg/mL up to the solubility limit of the glucagon;

wherein the moisture content of the solution is less than 1%, and wherein the dried glucagon maintains the pH memory in the aprotic polar solvent.

[0013c] In another aspect, there is provided stable solution for parenteral injection, comprising:

(a) a first peptide or a salt thereof, wherein the first peptide has been dried in a first non-volatile buffer, and wherein the first dried peptide has a first pH memory that is equal to the pH of the first peptide in the first non-volatile buffer;

(b) a second peptide or a salt thereof, wherein the second peptide has been dried in a second non-volatile buffer, and wherein the second dried peptide has a second pH memory that is equal to the pH of the second peptide in the second non-volatile buffer; and

(c) an aprotic polar solvent, wherein the first peptide or a salt thereof is in the aprotic polar solvent in an amount from 0.1 mg/mL up to the solubility limit of the first peptide or a salt thereof, and wherein the second peptide or a salt thereof is in the aprotic polar solvent in an amount from 0.1 mg/mL up to the solubility limit of the second peptide or salt thereof;

wherein the moisture content of the solution is less than 5%, wherein the first dried peptide maintains the first pH memory in the aprotic polar solvent, and wherein the second dried peptide maintains the second pH memory in the aprotic polar solvent.

[0013d] In another aspect, there is provided stable solution for parenteral injection, comprising:

(a) insulin, wherein the insulin has been dried in a first non-volatile buffer selected from a glycine buffer, a citrate buffer, a phosphate buffer, and mixtures thereof, and wherein the dried insulin has a first pH memory that is equal to the pH of the insulin in the first non-volatile buffer, wherein the first pH memory is from 1.5 to 2.5 or from 6.0 to 8.0;

(b) pramlintide, wherein the pramlintide has been dried in a second non-volatile buffer selected from a glycine buffer, a citrate buffer, a phosphate buffer, and mixtures thereof, and wherein the dried pramlintide has a second pH memory that is equal to the pH of the pramlintide in the second non-volatile buffer, wherein the second pH memory is from 3.0 to 5.0; and

(c) an aprotic polar solvent selected from dimethylsulfoxide (DMSO), n-methyl pyrrolidone (NMP), ethyl acetate, and mixtures thereof;

wherein the moisture content of the solution is less than 1%, wherein the dried insulin maintains the first pH memory that is equal to the pH of the insulin in the first non-volatile buffer when the dried insulin is reconstituted in the aprotic polar solvent, and wherein the dried pramlintide maintains the second pH memory that is equal to the pH of the pramlintide in the second non-volatile buffer when the dried pramlintide is reconstituted in the aprotic polar solvent.

[0013e] In another aspect, there is provided the use of the stable solution as defined herein in the manufacture of a medicament for treating a disease, condition, or disorder that may be treated, alleviated, or prevented by administration of the medicament.

[0013f] In another aspect, there is provided the use of the stable solution as defined herein in the manufacture of a medicament for treating hypoglycemia.

[0013g] In another aspect, there is provided the use of the stable solution as defined herein in the manufacture of a medicament for treating diabetes.

[0013h] In another aspect, there is provided a method of making a stable solution for parenteral injection, the method comprising:

(a) drying a mixture comprising a peptide or salt thereof in a non-volatile buffer to a dried peptide powder, wherein the dried peptide powder has a pH memory that is equal to the pH of the peptide in the non-volatile buffer; and

(b) reconstituting the dried peptide powder in an aprotic polar solvent, wherein the peptide or salt thereof is reconstituted in the aprotic polar solvent in an amount from 0.1 mg/mL up to the solubility limit of the peptide or salt thereof;

wherein the moisture content of the solution is less than 5%, and wherein the dried peptide powder maintains the pH memory in the aprotic polar solvent.

[0013i] In another aspect, there is provided a method of making a stable solution for parenteral injection, the method comprising:

(a) drying a first mixture comprising a first peptide or salt thereof in a first non-volatile buffer to a first dried peptide powder, wherein the first dried peptide powder has a first pH memory that is equal to the pH of the first peptide in the first non-volatile buffer;

(b) drying a second mixture comprising a second peptide or salt thereof in a second non-volatile buffer to a second dried peptide powder, wherein the second dried peptide powder has a second pH memory that is equal to the pH of the second peptide in the second non-volatile buffer; and

(c) reconstituting the first dried peptide powder and the second dried peptide powder in an aprotic polar solvent, wherein the first peptide or salt thereof is reconstituted in the aprotic polar solvent in an amount from 0.1 mg/mL up to the solubility limit of the first peptide or salt thereof, and wherein the second peptide or salt thereof is reconstituted in the aprotic polar solvent in an amount from 0.1 mg/mL up to the solubility limit of the second peptide or salt thereof;

wherein the moisture content of the solution is less than 5%, wherein the first dried peptide powder maintains the first pH memory in the aprotic polar solvent, and wherein the second dried peptide powder maintains the second pH memory in the aprotic polar solvent.

[0014] Thus, in one embodiment~~aspect~~, the present disclosure~~invention~~ provides a stable formulation for parenteral injection, the formulation comprising: (a) a peptide or a salt thereof, wherein the peptide has been dried in a non-volatile buffer, and wherein the dried peptide has a pH memory that is about equal to the pH of the peptide in the non-volatile buffer; and (b) an aprotic polar solvent; wherein the moisture content of the formulation is less than 5%, and wherein the dried peptide maintains the pH memory that is about equal to the pH of the peptide in the non-volatile buffer when the dried peptide is reconstituted in the aprotic polar solvent.

[0015] In another embodiment~~aspect~~, the present disclosure~~invention~~ provides a stable formulation for parenteral injection, the formulation comprising: (a) a first peptide or a salt thereof, wherein the first peptide has been dried in a first non-volatile buffer, and wherein the first dried peptide has a first pH memory that is about equal to the pH of the first peptide in the first non-volatile buffer; (b) a second peptide or a salt thereof, wherein the second peptide has been dried in a second non-volatile buffer, and wherein the second dried peptide has a second pH memory that is about equal to the pH of the second peptide in the second non-volatile buffer; and (c) an aprotic polar solvent; wherein the moisture content of the formulation is less than 5%,

wherein the first dried peptide maintains the first pH memory that is about equal to the pH of the first peptide in the first non-volatile buffer when the first dried peptide is reconstituted in the aprotic polar solvent, and wherein the second dried peptide maintains the second pH memory that is about equal to the pH of the second peptide in the second non-volatile buffer when the second dried peptide is reconstituted in the aprotic polar solvent.

[0016] In another embodiment~~aspect~~, the present disclosure~~invention~~ provides a stable formulation for parenteral injection, the formulation comprising: a peptide or a salt thereof (such as a hydrochloride or acetate salt thereof); and an aprotic polar solvent, wherein the moisture content of the formulation is less than 5%.

[0017] The stable formulations described herein are useful for the parenteral injection of any peptide that has limited or poor stability or solubility in an aqueous environment. Thus, in some embodiments, the peptide (or each of the first and second peptides) or salt thereof is selected from the group consisting of glucagon, pramlintide, insulin, leuprolide, an LHRH agonist, parathyroid hormone (PTH), amylin, botulinum toxin, hematide, an amyloid peptide, cholecystikinin, a conotoxin, a gastric inhibitory peptide, an insulin-like growth factor, a growth hormone releasing factor, an anti-microbial factor, glatiramer, glucagon-like peptide-1 (GLP-1), a GLP-1 agonist, exenatide, analogs thereof, and mixtures thereof. In a preferred embodiment, the peptide is glucagon or a glucagon analog or a glucagon peptidomimetic. In another embodiment, the peptide is parathyroid hormone. In yet another embodiment, the peptide is leuprolide. In still another embodiment, the peptide is glatiramer. In yet another embodiment, the first peptide is pramlintide and the second peptide is insulin. In still another embodiment, the first peptide is glucagon and the second peptide is exenatide.

[0018] The peptide (or, in embodiments where the formulation comprises two or more peptides, each of the peptides) is mixed with a non-volatile buffer and dried to a dry peptide powder. Suitable non-volatile buffers include, but are not limited to, glycine buffers, citrate buffers, phosphate buffers, and mixtures thereof. In one preferred embodiment, the non-volatile buffer is a glycine buffer. In another preferred embodiment, the non-volatile buffer is a mixture of citrate buffer and phosphate buffer. In some embodiments, wherein the formulation comprises two or more peptides, the first non-volatile buffer and the second non-volatile buffer are the same. In some embodiments, wherein the formulation comprises two or more peptides, the first non-volatile buffer and the second non-volatile buffer are different.

[0019] In some formulations of the present disclosure~~invention~~, the peptide is mixed with a non-volatile buffer and a stabilizing excipient, and then dried to a dry peptide powder. Suitable

mixtures thereof) and a co-solvent that depresses the freezing point of the formulation. In some embodiments, the co-solvent depresses the freezing point of the formulation by at least about 5°C, at least about 10°C, at least about 15°C, or at least about 20°C. In some embodiments, the 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. In some embodiments, the co-solvent is a polar protic solvent. In preferred embodiments, the co-solvent is selected from ethanol, propylene glycol (PG), glycerol, and mixtures thereof. 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).

[0022] Importantly, the formulations of the present disclosure~~invention~~ have very little residual moisture and, thus, the peptides in such formulations remain stable over extended periods of time. In preferred embodiments, the moisture content of the formulation of the present invention is less than about 4%, preferably, less than about 3%, preferably, less than about 2%, and even more preferably, less than about 1%, preferably, less than about 0.5%, preferably, less than about 0.25%, preferably, less than about 0.2%, preferably, less than about 0.15%, or preferably, less than about 0.1%. In other preferred embodiments, the moisture content of the formulation of the present invention is from about 0.01% to about 4%, preferably, from about 0.01% to about 3%, preferably, from about 0.01% to about 2%, preferably, from about 0.01% to about 1%, preferably, from about 0.1% to about 4%, preferably, from about 0.1% to about 3%, preferably, from about 0.1% to about 2%, preferably, from about 0.1% to about 1%, preferably, from about 0.25% to about 4%, preferably, from about 0.25% to about 3%, preferably, from about 0.25% to about 2%, preferably, from about 0.25% to about 1%, or preferably, from about 0.5% to about 1%.

[0023] When the peptide is mixed with a nonvolatile buffer, the nonvolatile buffer is selected such that the peptide has a pH of maximal stability, maximal solubility, and minimal degradation in the aqueous environment. Once dried, the peptide will have a pH memory of maximal stability, maximal solubility, and minimal degradation and will retain that pH memory when dissolved in or reconstituted in the aprotic polar solvent. As such, in preferred embodiments, the peptide in the formulation will have a pH memory of about 2.0 to about 3.0 to ensure maximal stability, maximal solubility, and minimal degradation. In other embodiments, the peptide in the formulation will have a pH memory of about 3.0 to about 5.0

[0025] In some embodiments, the formulations of the present disclosure~~invention~~ further comprise an antioxidant. In other embodiments, the formulations further comprise a chelator. In still other embodiments, the formulations of the present disclosure~~invention~~ further comprise a preservative.

[0026] In another embodiment~~aspect~~, the present disclosure~~invention~~ provides a method for treating a disease, condition or disorder that may be treated, alleviated, or prevented by administering to a subject a stable peptide formulation as described herein in an amount effective to treat, alleviate or prevent the disease, condition, or disorder. In some embodiments, the disease, condition, or disorder is hypoglycemia. In some embodiments, wherein the disease, condition, or disorder is hypoglycemia, the method comprises administering a stable glucagon formulation of the present invention in an amount effective to treat the hypoglycemia. In some embodiments, the disease, condition, or disorder is diabetes. In some embodiments, wherein the disease, condition, or disorder is diabetes, the method comprises administering a stable insulin and pramlintide formulation of the present invention in an amount effective to treat the diabetes.

[0027] In yet another embodiment~~aspect~~, the present disclosure~~invention~~ provides a process for making a stable formulation for parenteral injection, the process comprising: drying a peptide and a nonvolatile buffer to a dry peptide powder; and reconstituting the dry peptide powder with an aprotic polar solvent, thereby making the stable formulation, wherein the moisture content of the stable formulation is less than 5%. In some embodiments, the dried peptide powder has a pH memory that is about equal to the pH of the peptide in the non-volatile buffer, and the dried peptide powder maintains the pH memory that is about equal to the pH of the peptide in the non-volatile buffer when the dried peptide powder is reconstituted in the aprotic polar solvent.

[0028] In still another embodiment~~aspect~~, the present disclosure~~invention~~ provides kits for treating a disease, condition or disorder, the kit comprising: a stable formulation comprising one or more peptides or salts thereof, wherein the peptide(s) has been dried in a non-volatile buffer, and wherein the dried peptide(s) has a pH memory that is about equal to the pH of the peptide(s) in the non-volatile buffer; and an aprotic polar solvent; wherein the moisture content of the formulation is less than 5%, and wherein the dried peptide(s) maintains the pH memory that is about equal to the pH of the peptide(s) in the non-volatile buffer when the dried peptide(s) is reconstituted in the aprotic polar solvent; and a syringe for administration of the stable formulation to the subject.

62. Use of the stable solution of any of claims 1-61 in the manufacture of a medicament for treating a disease, condition, or disorder that may be treated, alleviated, or prevented by administration of the medicament.

63. Use of the stable solution of any of claims 35-38 in the manufacture of a medicament for treating hypoglycemia.

64. Use of the stable solution of any of claims 56-61 in the manufacture of a medicament for treating diabetes.

65. The use in accordance with any of claims –62-64, wherein the stable solution is to be administered via the use of a syringe.

66. The use in accordance with any of claims –62-64, wherein the stable solution is to be administered via the use of a pen injection device.

67. The use in accordance with any of claims –62-64, wherein the stable solution is to be administered via the use of an auto-injector device.

68. The use in accordance with any of claims –62-64, wherein the stable solution is to be administered via the use of a pump.

69. A method of making a stable solution for parenteral injection, the method comprising:

wherein the moisture content of the solution is less than 5%, and wherein the dried peptide powder maintains the pH memory in the aprotic polar solvent.

70. The method in accordance with claim 69, wherein the peptide or salt thereof is selected from the group consisting of glucagon, pramlintide, insulin, leuprolide, an LHRH agonist, parathyroid hormone (PTH), amylin, botulinum toxin, hematide, an amyloid peptide, cholecystikinin, a conotoxin, a gastric inhibitory peptide, an insulin-like growth factor, a growth hormone releasing factor, an anti-microbial factor, glatiramer, exenatide, analogs thereof, and mixtures thereof.

71. The method in accordance with claim 70, wherein the peptide is glucagon.

72. The method in accordance with claim 69, wherein the non-volatile buffer is selected from a glycine buffer, a citrate buffer, a phosphate buffer, and mixtures thereof.

73. The method in accordance with claim 69, wherein the aprotic polar solvent is selected from dimethylsulfoxide (DMSO), n-methyl pyrrolidone (NMP), ethyl acetate, and mixtures thereof.

74. The method in accordance with claim 69, further comprising drying the peptide and the non-volatile buffer with a stabilizing excipient, wherein the stabilizing excipient is selected from sugars, starches, and mixtures thereof.

75. The method in accordance with claim 69, wherein step (b) comprises reconstituting the dried peptide powder in a solution comprising an aprotic polar solvent and a co-solvent that depresses the freezing point of the solution, wherein the co-solvent is selected from ethanol, propylene glycol, glycerol, and mixtures thereof.

76. The method in accordance with claim 69, wherein the pH of the peptide in the non-volatile buffer is such that the dried peptide powder has a pH memory of 2.0 to 3.0.