will also have an effect on diffusion, so that the larger the volume, the slower the diffusion rate. It is this combination of factors that is primarily responsible for the latency in onset of action and peak plasma insulin levels.

[0007] To prevent fibrillation and degradation of insulin in aqueous solution while also promoting subcutaneous absorption, insulin analogs have been developed where the amino acid sequence has been changed to reduce the propensity for self-association while preserving receptor-binding affinity. These classes of insulin are often referred to as "monomeric" insulin, but they actually exist as weakly associated hexamers. Absorption of such preparations will still be delayed because it is dependent on the diffusion and subsequent reduction in subcutaneous concentration required for the hexamer to dissociate to the dimer/monomer. Insulin analogs with equilibrium in favor of the monomeric state (e.g. the insulin analog Lispro) have shown more rapid absorption and a shorter duration of action. However, these analog molecules are less stable and more prone to irreversible aggregation under thermal and mechanical stress compared to hexameric insulin. Moreover, these aggregates decrease not only the dose of insulin available, but can also induce irritation or immune reactions in patients. Concerns also have emerged in experimental and epidemiological studies with respect to prolonged signaling of the receptor machinery and the induction of tumor proliferation by some newer insulin analogs. Despite their shortfalls, insulin analogs are costly--about twice as much as regular human insulin.

SUMMARY ~~OF THE INVENTION~~

[0007a] In a first aspect there is provided a formulation for parenteral administration comprising:

(a) insulin that comprises a pH memory between 1 to 4 or between 6 to 8 and has been previously dried from a non-volatile buffer; and

(b) an aprotic polar solvent,

wherein the insulin is solubilized in the aprotic polar solvent, wherein the majority of the solubilized insulin comprises stable monomeric or dimeric forms of insulin or mixtures thereof, and

wherein the water content of the formulation is equal to or less than 15% w/v.

[0007b] In a second aspect there is provided a use of any one of the formulations as disclosed herein in the manufacture of a medicament for reducing blood glucose level, wherein the medicament is to be administered to a subject in need thereof in an amount effective to reduce the blood glucose level in the subject.

[0007c] In a third aspect there is provided a method of making the formulation as disclosed herein comprising:

(a) drying a mixture comprising insulin and a non-volatile buffer to obtain dried insulin, wherein the dried insulin has a pH memory between 1 to 4 or 6 to 8; and

(b) reconstituting the dried insulin in an aprotic polar solvent,

wherein the insulin is solubilized in the aprotic polar solvent, wherein the solubilized insulin comprises stable monomeric or dimeric forms of insulin or mixtures thereof, and

wherein the water content of the formulation is equal to or less than 15% w/v.

[0008] The present ~~invention~~ disclosure provides a solution to the current problems facing insulin formulations. The ~~invention~~ disclosure resides in drying insulin in a buffer to create a dried form of insulin that maintains a desired pH after it is reconstituted and solubilized in an aprotic polar solvent. The resulting formulation includes solubilized and stabilized monomeric and dimeric forms of insulin. Notably, the formulations can have relatively low amounts of water (20, 15, 10, 5, 4, 3, 2, 1, % or less) or can be non-aqueous, which further allows for increased amounts of insulin to be present in the formulation, thereby reducing the volume of the insulin containing formulation to be administered to a subject. Further, the ~~invention~~ disclosure allows for both non-modified or native and modified or analogue forms of insulin to be used. Stated another way, while insulin analogs can be used with the present ~~invention~~disclosure, non-modified/native insulin can also be used and remain stable in both its monomeric and dimeric forms.

[0009] In one ~~aspect~~ embodiment of the present ~~invention~~disclosure, there is disclosed a formulation comprising insulin that has a pH memory between 1 to 4 (or 1 to 3 or about 2) or between 6 to 8 (or 6.5 to 7.5 or about 7) and an aprotic polar solvent, wherein the insulin can be solubilized in the aprotic polar solvent, wherein the solubilized insulin can include stable monomeric or dimeric forms of insulin or mixtures thereof, and wherein the water content of the formulation can be equal to or less than 20, 15, 10, 5, 4, 3, 2, 1% w/v or w/w or less (*e.g.*, anhydrous). The formulation can be used for parenteral administration. In certain ~~aspects~~embodiments, the aprotic polar solvent can be dimethylsulfoxide (DMSO), n-methyl pyrrolidone (NMP), ethyl acetate, dimethylformamide (DMF), dimethylacetamide (DMA), or propylene carbonate, or mixtures thereof. In certain ~~aspects~~embodiments, the aprotic polar solvent can be dimethylsulfoxide (DMSO). In some ~~aspects~~embodiments, the formulation comprises 3 mg/ml to 50 mg/ml, 3 mg/ml to 10 mg/ml, or 10 mg/ml to 50 mg/ml of insulin. In others, it can include 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80. 90, 100 mg/mL, or more or as needed, or any range therein. In some ~~aspects~~embodiments, the majority of the insulin within the formulation is monomeric form or dimeric form or a combination of monomeric and dimeric forms. The formulation can further include ingredients that are capable of reducing the aggregation of monomeric or dimeric forms of insulin. Non-limiting examples of such ingredients include urea, guanidinium chloride, an amino acid, a sugar, a polyol, a polymer, an acid, or a surfactant, or mixtures thereof. In certain ~~aspects~~embodiments, the acid can be acetic acid, ascorbic acid, citric acid, glutamic acid, aspartic acid, succinic acid, fumaric acid, maleic acid, or adipic acid, or mixtures thereof. The formulation can include a co-solvent. One non-limiting example of a co-solvent is water. In some ~~aspects~~embodiments, the formulation does not include zinc, includes low amounts of zinc and/or includes zinc that is bound to a chelating agent so as to reducing the likelihood of hexamer formation. In certain ~~aspects~~embodiments, the insulin can be previously dried in a non-volatile buffer, said buffer can have a pH range between 1 to 4 or between 1 to 3 or about 2 or between 6 to 8 or 6.5 to 7.5 or about 7. Examples of non-volatile buffers can be a glycine buffer, a citrate buffer, or a phosphate buffer, or mixtures thereof. In some ~~aspects~~embodiments, the buffer can include a chelating agent. Non-limiting examples of chelating agents include ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), tartaric acid, glycerin, or citric acid or any combination thereof. The formulation can also include an ingredient that is capable of depressing the freezing point of the aprotic polar solvent to about 0°C, and non-limiting examples of such an ingredient include water, a sugar, a sugar alcohol, or mixtures thereof.

In some instances, the insulin can be non-modified or native human insulin. In other ~~aspects~~embodiments,

the composition can further include an insulin adjuvant such as an amylin analog. The amylin analog can be solubilized in the formulation. A non-limiting example of an amylin analog is pramlintide. The pramlintide can be processed such that it has a pH memory between 1 to 5, or 2, 3, or 4, or about 2. In some particular instances of the co-formulation, the insulin pH memory can be about 2 and the pramlintide pH memory can be about 2. In some ~~aspects~~embodiments, the processing of the pramlintide can include drying said pramlintide in a non-volatile buffer, said buffer having a pH range between 1 to 5, or 2, 3, or 4, or about 2. In formulations that include insulin and pramlintide, the water content can be between 5 to 20% w/v or w/w or 5 to 15% w/v or w/w or 7 to 12% w/v or w/w, or 8 to 10% w/v or w/w, or about 9% w/v or w/w. The formulation can be in liquid form. The formulation can be a solution. In certain ~~aspects~~embodiments, least 50, 60, 70, 80, or 90% or more of the insulin within the formulation can remain chemically and physically stable when the formulation is stored at room temperature for one month. In some ~~aspects~~embodiments, the formulation can be comprised within a container. The container can be a syringe, a pen injection device, an auto-injector device, a pump, or a perfusion bag. In certain ~~aspects~~embodiments, the aprotic polar solvent can be the continuous phase of the formulation. The formulation can include at least 75, 80, 85, 90, 95, 96, 97, 98, 99 or % w/v or w/w of the aprotic polar solvent. The insulin within the formulation can be meta-stable.

[0010] Also disclosed is a method for reducing blood glucose level comprising administering to a subject in need thereof any one of the formulations of the present invention in an amount effective to reduce the blood glucose level in the subject. The subject can be human (adult or child), an animal (*e.g.*, chimpanzee, horse, cow, pig, rabbit, rat, mouse, *etc.*). In certain ~~aspects~~embodiments, the blood glucose level in the subject is reduced within 10, 20, 30 minutes, 60 minutes, or 90 minutes after administration. In some instances, the early ½ Tmax blood insulin level in the subject occurs within 10, 20, 30, 40, 50, or 60 minutes after administration or within 30 to 60 minutes after administration. The subject can have already been diagnosed with Type-I or Type-II diabetes or can be susceptible to developing Type-I or II diabetes. In some instances, the formulation can be administered within 30, 20, 15, 10 minutes, 5, minutes, or 1 minute before ingestion of food by the subject, or within 1 minutes, 5 minutes, 10, 15, 20, or 30 minutes after ingestion of food by the subject.

[0011] Also disclosed is a method of making the formulations of the present ~~invention~~disclosure. The method can include drying a mixture comprising insulin and a non-volatile

buffer to obtain dried insulin, wherein the dried insulin can have a pH memory between 1 to 4 (or 2 to 3 or about 2) or 6 to 8 (or 6.5 to 7.5 or about 7) and subsequently reconstituting the dried insulin in an aprotic polar solvent, wherein the insulin can be solubilized in the aprotic polar solvent, wherein the solubilized insulin can include stable monomeric or dimeric forms of insulin or mixtures thereof, and wherein the water content of the formulation can be equal to or less than 20, 15, 10, 5, 4, 3, 2, 1% w/v or w/w or less (*e.g.*, anhydrous). The method can further include drying a mixture comprising an amylin analog and a second non-volatile buffer to obtain a dried amylin analog and reconstituting the dried amylin analog in the aprotic polar solvent along with the dried insulin, wherein the dried amylin analog can be solubilized in the aprotic polar solvent. As noted above, the amylin analog can be pramlintide and can be processed to have a pH memory between 1 to 5, or 2, 3, or 4, or in particular instances of about 2. In some particular instances of the co-formulation, the insulin pH memory can be about 2 and the pramlintide pH memory can be about 2. The second non-volatile buffer can have a pH range between 1 to 5 or about 2, 3, or 4, or more particularly about 2. This method can further include adding a co-solvent such as water to the formulation in amounts ranging from 5 to 20% w/v or w/w or 5 to 15% w/v or w/w or 7 to 12% w/v or w/w, or 8 to 10% w/v or w/w, or about 9% w/v or w/w.

[0012] Another unique ~~aspect~~ embodiment of the present formulation is that it can be contained in a container, be stored, and be immediately ready for parenteral administration on an as needed basis without having to reconstitute or dilute the formulation. Therefore, the container that the formulation can be stored in can be a syringe, a pen injection device, an auto-injector device, a pump, or a perfusion bag. Also contemplated for use in the formulations are additional ingredients/pharmaceutical excipients, non-limiting example of which include: antioxidants (examples include ascorbic acid, cysteine, methionine, monothioglycerol, sodium thiosulfate, sulfites, BHT, BHA, ascorbyl palmitate, propyl gallate, or vitamin E); chelating agents (examples include EDTA, EGTA, tartaric acid, glycerin, or citric acid); or preservatives (examples include alkyl alcohols, benzyl alcohol, a methyl paraben, or a propyl paraben or mixtures thereof). The formulation can be in liquid form, semi-solid form, or gel form. As discussed below, the formulation can have a desired viscosity range (in one non-limiting example, such a range could be between 0.5 to 15 cps).

[0013] It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the ~~invention~~disclosure, and *vice versa*. Furthermore, compositions of the ~~invention~~ disclosure can be used to achieve methods of the ~~invention~~disclosure.

[0014] "Insulin" means human, non-human, recombinant, purified, and/or synthetic (*e.g.*, modified insulin or insulin analogs) insulin. "Human insulin" means the human peptide hormone insulin that is secreted by the pancreas—it can be isolated, from a natural source, made from a genetically altered organism, manufactured *via* synthetic chemistry, purchased, *etc.* "Non-human insulin" is insulin derived from an animal (*e.g.*, pig, cow, *etc.*).

[0015] "Modified insulin" or "insulin analog" is an altered form of insulin, different from that in nature (*e.g.*, chemical modification, different structure, different amino acid sequence), but still available to a subject (*e.g.*, human) to perform the same function as natural/un-modified insulin. For instance, through genetic engineering of the coding of DNA, the amino acid sequence of insulin can be changed to alter its ADME (adsorption, distribution, metabolism, and/or excretion) characteristics. Examples of modified insulin or insulin analogs include Lispro®, Aspart®, Glulisine®, Detemir®, Degludec®, *etc.* Un-modified or native insulin includes the native or naturally occurring amino acid sequence.

[0016] "Stable insulin" means insulin within the formulation does not irreversibly aggregate within the formulation or otherwise lose its activity once the formulation is administered. The insulin retains its activity once absorbed into the blood. Without wishing to be bound by theory, it is believed that the insulin within the formulation of the present invention is "meta-stable" in that while the conformation of the solubilized insulin may change, the insulin reverts back to its native conformation once administered and absorbed into the blood. Further, it is believed that the conformational change of insulin within the formulation reduces the likelihood of aggregation with other insulin monomers and dimers or adjuvants such as amylin analogs present within the formation. Monomeric insulin form means insulin in its monomer form. Dimeric insulin form means insulin in its dimeric form (*e.g.*, two monomers associated or coupled together). Hexamer insulin form means insulin in its hexamer form (*e.g.*, three dimeric forms associated or coupled together).

[0017] "Zinc-free" or "low-zinc" means that the formulation includes about 0.6% or less (*e.g.*, 0.5, 0.4, 0.3, 0.2, 0.1, 0%) zinc relative to the insulin content or 3 zinc ions per 6 insulin monomers or less (*e.g*, 2, 1, 0).

[0032] Other objects, features and advantages of the present ~~invention~~ disclosure will become apparent from the following detailed description. It should be understood, however, that the

detailed description and the examples, while indicating specific embodiments of the ~~invention~~disclosure, are given by way of illustration only. Additionally, it is contemplated that changes and modifications within the spirit and scope of the ~~invention~~ disclosure will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented below.

[0034] **FIG. 1:** the FTIR spectra of DMSO/Insulin and Aqueous/Insulin formulations.

[0035] **FIG. 2:** Apparent molecular weight of DMSO/Insulin and Aqueous/Insulin formulations.

[0036] **FIG. 3:** Hydrodynamic radius distribution for Ins-E at 50 mg/mL in DMSO. The horizontal axis is a logarithmically-spaced grid of hydrodynamic radius values (with adjacent points differing by a factor of ~1.3). The analysis covers a range of radii from ~0.01 nm to ~20 μm. Peaks at 0.01-0.1 nm which are artifacts arising from after-pulsing of the photodetector have been suppressed.

[0037] **FIG. 4:** Hydrodynamic radius distribution for Ins-E at 30 mg/mL in DMSO (see above FIG. 3 for graph explanation).

[0038] **FIG. 5:** Hydrodynamic radius distribution for Ins-E at 10 mg/mL in DMSO (see above FIG. 3 for graph explanation).

[0039] **FIG. 6:** Hydrodynamic radius distribution for Ins-E at 3 mg/mL in DMSO (see above FIG. 3 for graph explanation).

[0040] **FIG. 7:** Hydrodynamic radius distribution for Ins-H_2O at 10 mg/mL in buffer E (see above FIG. 3 for graph explanation).

[0041] **FIG. 8:** Hydrodynamic radius distribution for Ins-H_2O at 10 mg/mL in buffer F (see above FIG. 3 for graph explanation).

23. The formulation of claim 22, wherein the pramlintide has a pH memory of about 2 or wherein the pramlintide has a pH memory of about 2 and the insulin has a pH memory of about 2.

24. The formulation of claim 23, wherein the pramlintide has been previously dried in a non-volatile buffer, said buffer having a pH of about 2.

25. The formulation of any one of claims 22-24, wherein the water content of the formulation is between 5 to 15% w/v, 7 to 12% w/v, or 8 to 10% w/v, or about 9% w/v.

26. The formulation of any one of claims 1-25, wherein the formulation is in liquid form.

27. The formulation of 26, wherein the formulation is a solution.

28. The formulation of any one of claims 1-27, wherein at least 90% of the insulin within the formulation remains chemically and physically stable when the formulation is stored at room temperature for one month.

29. The formulation of any one of claims 1-28, wherein the formulation is comprised within a container.

30. The formulation of claim 29, wherein the container is a syringe, a pen injection device, an auto-injector device, a pump, or a perfusion bag.

31. The formulation of any one of claims 1-30, wherein the aprotic polar solvent is the continuous phase of the formulation.

32. The formulation of any one of claims 1-31, wherein the formulation comprises at least 75, 80, 85, 90, or 95% w/v of the aprotic polar solvent.

33. The formulation of any one of claims 1-32, wherein the solubilized insulin is meta-stable.

34. ~~A method for reducing blood glucose level comprising administering to a subject in need thereof~~ Use of any one of the formulations of claims 1-33 in the manufacture of a medicament for reducing blood glucose level, wherein the medicament is to be administered to a subject in need thereof in an amount effective to reduce the blood glucose level in the subject.

35. The ~~method~~ use of claim 34, wherein the blood glucose level in the subject is reduced within 30 minutes, 60 minutes, or 90 minutes after administration.

36. The ~~method~~ use of any one of claims 34-35, wherein the early ½ T_{max} blood insulin level in the subject occurs within 30 to 60 minutes after administration.

37. The ~~method~~ use of any one of claims 34-36, wherein the subject has been diagnosed with Type-I or Type-II diabetes.

38. The ~~method~~ use of any one of claims 34-37, wherein the formulation is to be administered within 10 minutes, 5, minutes, or 1 minute before ingestion of food by the subject, or within 1 minutes, 5 minutes, or 10 minutes after ingestion of food by the subject.

39. A method of making the formulation of any one of claims 1-33 comprising:

(a) drying a mixture comprising insulin and a non-volatile buffer to obtain dried insulin, wherein the dried insulin has a pH memory between 1 to 4 or 6 to 8; and

(b) reconstituting the dried insulin in an aprotic polar solvent,

wherein the insulin is solubilized in the aprotic polar solvent, wherein the solubilized insulin comprises stable monomeric or dimeric forms of insulin or mixtures thereof, and

wherein the water content of the formulation is equal to or less than 15% w/v.

40. The method of claim 39, wherein the pH memory of the insulin is between 1 to 4 or between 1 to 3, or about 2.

41. The method of claim 39, wherein the pH memory of the insulin is between 6 to 8 or about 7.

42. The method of any one of claims 39-41, wherein the aprotic polar solvent is dimethylsulfoxide (DMSO), n-methyl pyrrolidone (NMP), ethyl acetate, dimethylformamide (DMF), dimethylacetamide (DMA), or propylene carbonate, or mixtures thereof.

43. The method of claim 42, wherein the aprotic polar solvent is dimethylsulfoxide (DMSO).