



(51) International Patent Classification:

A61K 33/30 (2006.01) A61K 47/26 (2006.01)

A61K 38/28 (2006.01) A61K 9/08 (2006.01)

A61K 47/12 (2006.01) A61P 3/10 (2006.01)

A61K 47/18 (2017.01)

TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(21) International Application Number:

PCT/GB2018/051179

(22) International Filing Date:

03 May 2018 (03.05.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

1707188.7 05 May 2017 (05.05.2017) GB

(71) Applicant: ARECOR LIMITED [GB/GB]; Chesterford Research Park, Little Chesterford, Saffron Walden, Cambridge Cambridgeshire CB10 1XL (GB).

(72) Inventors: JEZEK, Jan; c/o Arecor Limited, Chesterford Research Park, Little Chesterford, Saffron Walden, Cambridge Cambridgeshire CB10 1XL (GB). GERRING, David; c/o Arecor Limited, Chesterford Research Park, Little Chesterford, Saffron Walden, Cambridge Cambridgeshire CB10 1XL (GB). HOWELL, Sarah; c/o Arecor Limited, Chesterford Research Park, Little Chesterford, Saffron Walden, Cambridge Cambridgeshire CB10 1XL (GB). ZAKRZEWSKI, Leon; c/o Arecor Limited, Chesterford Research Park, Little Chesterford, Saffron Walden, Cambridge Cambridgeshire CB10 1XL (GB).

(74) Agent: TEUTEN, Andrew et al.; Sagittarius IP, Three Globeside, Fieldhouse Lane, Marlow Buckinghamshire SL7 1HZ (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,

Published:

— with international search report (Art. 21(3))

— with sequence listing part of description (Rule 5.2(a))

(54) Title: STABLE INSULIN FORMULATIONS

(57) Abstract: The present invention relates *inter alia* to an aqueous liquid pharmaceutical formulation comprising (i) an insulin compound other than insulin glargine, (ii) ionic zinc, (iii) a zinc binding species selected from diethylenetriamine (DETA) and triethylenetriamine (TETA), and (iv) a non-ionic surfactant. It also provides related methods, uses and pharmaceutical compositions.



WO 2018/203061 A1

STABLE INSULIN FORMULATIONS

FIELD OF THE INVENTION

This invention relates *inter alia* to rapid acting aqueous liquid formulations of insulin and insulin analogues. Such formulations are suitable for the treatment of subjects suffering from diabetes mellitus, especially Type 1 diabetes mellitus.

BACKGROUND OF THE INVENTION

Diabetes mellitus ("diabetes") is a metabolic disorder associated with poor control of blood sugar levels leading to hypo or hyperglycemia. Untreated diabetes can lead to serious microvascular and macrovascular complications including coronary artery disease, peripheral artery disease, stroke, diabetic nephropathy, neuropathy and retinopathy. The two main types of diabetes are (i) Type 1 diabetes resulting from the pancreas not producing insulin for which the usual treatment is insulin replacement therapy and (ii) Type 2 diabetes where patients either produce insufficient insulin or have insulin resistance and for which treatments include insulin sensitising agents (such as metformin or pioglitazone), traditional insulin secretagogues (such as sulfonylureas), SGLT2 inhibitors (such as dapagliflozin, canagliflozin and empagliflozin) which reduce glucose absorption in the kidneys and so promote glucose excretion, GLP-1 agonists (such as exenatide and dulaglutide) which stimulate insulin release from pancreatic beta cells and DPPIV inhibitors (such as sitagliptin or vildagliptin) which inhibit breakdown of GLP-1 leading to increased insulin secretion. Patients with Type 2 diabetes may eventually require insulin replacement therapy.

For patients requiring insulin replacement therapy, a range of therapeutic options are possible. The use of recombinant human insulin has in recent times been overtaken by use of insulin analogues which have modified properties, for example, are longer acting or faster acting than normal insulin. Thus, a common regimen for a patient involves receiving a long acting basal insulin supplemented by a rapid acting insulin around mealtimes.

Insulin is a peptide hormone formed of two chains (A chain and B chain, respectively 21 and 30 amino acids in length) linked via disulfide bridges. Insulin

normally exists at neutral pH in the form of a hexamer, each hexamer comprising three dimers bound together by zinc ions. Histidine residues on the insulin are known to be involved in the interaction with the zinc ions. Insulin is stored in the body in the hexameric form but the monomer form is the active form. Traditionally, therapeutic compositions of insulin have also been formulated in hexameric form in the presence of zinc ions. Typically, there are approximately three zinc cations per one insulin hexamer. It has been appreciated that the hexameric form is absorbed from the injection site considerably more slowly than the monomeric and dimeric form.

Therefore, a faster onset of insulin action can be achieved if the hexameric form is destabilised allowing a more rapid dissociation of the zinc-bound hexamer into dimers and monomers in the subcutaneous space following injection. Three insulin analogues have been genetically engineered with this principle in mind. A first is insulin lispro (Humalog®) in which residues 28 and 29 of the B chain (Pro and Lys respectively) are reversed, a second is insulin aspart (NovoLog®) in which residue 28 of the B chain, normally Pro, is replaced by Asp and a third is insulin glulisine (Apidra®) in which residue 3 of the B chain, normally Asn, is replaced by Lys and residue 29 of the B chain, normally Lys, is replaced by Glu.

Whilst the existing rapid acting insulin analogues can achieve a more rapid onset of action, it has been appreciated that an even more rapid acting ("ultra rapid acting") insulins can be achieved by removing the zinc cations from insulin altogether. Unfortunately, the consequence of the hexamer dissociation, e.g. by adding EDTA, is typically a considerable impairment in insulin stability both with respect to physical stability (e.g. stability to aggregation) and chemical stability (e.g. stability to deamidation). For example, monomeric insulin or insulin analogues having a rapid onset of action are known to aggregate and become physically unstable very rapidly because the formulation of insoluble aggregates proceeds via monomers of insulin. Various approaches to addressing this problem have been described in the art:

US5,866,538 (Norup) describes insulin preparations of superior chemical stability comprising human insulin or an analogue or derivative thereof, glycerol and/or mannitol and 5 to 100mM of a halogenide (e.g. NaCl).

US7,205,276 (Boderke) addresses the stability problems associated with preparing zinc free formulations of insulin and insulin derivatives and analogues and describes an aqueous liquid formulation comprising at least one insulin derivative, at least one surfactant, optionally at least one preservative and optionally at least one of
5 an isotonicizing agent, a buffer and an excipient, wherein the formulation is stable and free from or contains less than 0.4% (e.g. less than 0.2%) by weight of zinc based on the insulin content of the formulation. The preferred surfactant appears to be polysorbate 20 (polyoxyethylene (20) sorbitan monolaurate).

US2008/0194461 (Maggio) describes formulations of peptides and
10 polypeptides including insulin which contain an alkylglycoside, which component is said to reduce aggregation and immunogenicity.

WO2012/006283 (Pohl) describes formulations containing insulin together with a zinc chelator such as ethylenediaminetetracetate (EDTA). Modulating the type and quantity of EDTA is said to change the insulin absorption profile. Calcium EDTA is
15 the preferred form of EDTA since it is said to be associated with reduced pain at the injection site and is less likely to remove calcium from the body. Preferred formulations also contain citrate which is said to further enhance absorption and to improve the chemical stability of the formulation.

US2010/0227795 (Steiner) describes a composition comprising insulin, a
20 dissociating agent such as citric acid or sodium citrate, and a zinc chelator such as EDTA wherein the formulation has a physiological pH and is a clear aqueous solution. The formulations are said to have improved stability and rapid onset of action.

WO2015/120457 (Wilson) describes stabilized ultra-rapid acting insulin
25 formulations comprising insulin in combination with a zinc chelator such as EDTA, a dissolution/stabilization agent such as citric acid, a magnesium salt, a zinc compound and optionally additional excipients.

Further approaches to accelerating the absorption and effect of insulin through the use of specific accelerating additives have been described:

WO91/09617 (Jørgensen) reports that nicotinamide or nicotinic acid or a salt thereof increases the speed of absorption of insulin from aqueous preparations administered parenterally.

5 WO2010/149772 (Olsen) describes a formulation comprising insulin, a nicotinic compound and arginine. The presence of arginine is said to improve the chemical stability of the formulation.

WO2015/171484 (Christe) describes rapid acting formulations of insulin wherein onset of action and/or absorption of insulin is faster due to the presence of treprostinil.

10 US2013/0231281 (Soula) describes an aqueous solution composition comprising insulin or an insulin analogue and at least one oligosaccharide whose average degree of polymerisation is between 3 and 13 and whose polydispersity index is above 1.0, said oligosaccharide having partially substituted carboxyl functional groups, the unsubstituted carboxyl functional groups being salifiable. Such
15 a formulation is said to be rapid acting.

It would be desirable if analogues or formulations of insulin were available which were ultra-rapid acting, thus more closely matching the activity of physiological insulin. There also remains a need in the art to provide further, and preferably improved, formulations of insulin and insulin analogues which are rapid acting and
20 stable.

SUMMARY OF THE INVENTION

According to the invention there is provided an aqueous liquid pharmaceutical formulation comprising (i) an insulin compound other than insulin glargine, (ii) ionic
25 zinc, (iii) a zinc binding species selected from diethylenetriamine (DETA) and triethylenetetramine (TETA) and (iv) a non-ionic surfactant ("the formulation of the invention").

The formulations of the invention provide insulin in a form which is rapid or ultra-rapid acting with good physical and chemical stability. As noted in the
30 background discussion above, use of EDTA to chelate zinc ions in hexameric insulin does increase the rapidity of action but at the cost of greatly reduced stability. The

present inventors have appreciated and demonstrated that the use of species which bind zinc less strongly, specifically DETA and TETA, can achieve good speed of action of insulin and their moderately destabilising effects can be reduced or eliminated by using a non-ionic surfactant.

- 5 Formulations of the invention may be used in the treatment of subjects suffering from diabetes mellitus, particularly Type 1 diabetes mellitus especially for administration at meal times.

DESCRIPTION OF THE SEQUENCE LISTING

- 10 SEQ ID NO: 1: A chain of human insulin
SEQ ID NO: 2: B chain of human insulin
SEQ ID NO: 3: B chain of insulin lispro
SEQ ID NO: 4: B chain of insulin aspart
SEQ ID NO: 5: B chain of insulin glulisine

15

FIGURES

Fig. 1. Pharmacodynamic profile of Formulations 1-3 of Example 2 in a validated diabetic Yucatan miniature pig model.

20 DETAILED DESCRIPTION OF THE INVENTION

As used herein, "insulin compound" refers to insulin and insulin analogues.

- As used herein, "insulin" refers to native human insulin having an A chain and a B chain as set out in SEQ ID NOs. 1 and 2 and containing and connected by disulfide bridges as in the native molecule (Cys A6-Cys A11, Cys B7 to Cys A7 and
25 Cys-B19-Cys A20). Insulin is suitably recombinant insulin.

- "Insulin analogue" refers to an analogue of insulin which is an insulin receptor agonist and has a modified amino acid sequence, such as containing 1 or 2 amino acid changes in the sequence of the A or B chain (especially the B chain). Desirably such amino acid modifications are intended to reduce affinity of the molecule for zinc
30 and thus increase speed of action. Thus, desirably an insulin analogue has a speed of action which is the same as or preferably greater than that of insulin. The speed of

action of insulin or an insulin analogue may be determined in the Diabetic Pig Pharmacokinetic/Pharmacodynamic Model (see Examples, General Methods). Exemplary insulin analogues include faster acting analogues such as insulin lispro, insulin aspart and insulin glulisine. These forms of insulin have the human insulin A chain but variant B chains – see SEQ ID NOs. 3-5. Further faster acting analogues are described in EP0214826, EP0375437 and EP0678522 the contents of which are herein incorporated by reference in their entirety.

In one embodiment the insulin compound is recombinant human insulin. In another embodiment it is insulin lispro. In another embodiment it is insulin aspart. In another embodiment it is insulin glulisine.

The term “aqueous pharmaceutical formulation”, as used herein, refers to a formulation suitable for therapeutic use in which the aqueous component is or comprises water, preferably distilled water, deionized water, water for injection, sterile water for injection or bacteriostatic water for injection. The aqueous pharmaceutical formulations of the invention are solution formulations in which all components are dissolved in water.

The concentration of insulin compound in the formulation will typically be in the range 10-1000 U/ml, such as 50-500 U/ml e.g. 50-200 U/ml. An exemplary formulation contains insulin compound at a concentration of 100 U/ml (around 3.6 mg/ml). Another range of interest is 500-1000 U/ml e.g. 800-1000 U/ml and another exemplary formulation contains insulin compound at a concentration of 1000 U/ml (around 36 mg/ml).

The formulations of the invention contain ionic zinc i.e. Zn^{2+} ions. The source of the ionic zinc will typically be a water soluble zinc salt such as ZnCl_2 , ZnO , ZnSO_4 , $\text{Zn}(\text{NO}_3)_2$ or $\text{Zn}(\text{acetate})_2$ and most suitably ZnCl_2 or ZnO .

The concentration of the ionic zinc in the formulation will typically be more than 0.05% e.g. more than 0.1% e.g. more than 0.2%, more than 0.3% or more than 0.4% by weight of zinc based on the weight of insulin compound in the formulation. Thus the concentration of the ionic zinc in the formulation may be more than 0.5% by weight of zinc based on the weight of insulin compound in the formulation, for example 0.5-1%, e.g. 0.5-0.75%, e.g. 0.5-0.6% by weight of zinc based on the weight

of insulin compound in the formulation. For the purpose of the calculation the weight of the counter ion to zinc is excluded.

In a formulation e.g. containing 100 U/ml of insulin compound the concentration of the ionic zinc will typically be more than 0.015 mM e.g. more than 0.03 mM e.g. more than 0.06 mM, more than 0.09 mM or more than 0.12 mM. Thus concentration of the ionic zinc in the formulation may be more than 0.15 mM, for example 0.15-0.60 mM, e.g. 0.20-0.45 mM, e.g. 0.25-0.35 mM.

In a formulation e.g. containing 1000 U/ml of insulin compound the concentration of the ionic zinc will typically be more than 0.15 mM e.g. more than 0.3 mM e.g. more than 0.6 mM, more than 0.9 mM or more than 1.2 mM. Thus concentration of the ionic zinc in the formulation may be more than 1.5 mM, for example 1.5-6.0 mM, e.g. 2.0-4.5 mM, e.g. 2.5-3.5 mM.

The formulations of the invention contain a zinc binding species, specifically selected from DETA or TETA. Preferably the zinc binding species is TETA. DETA has a logK metal binding stability constant of 8.88 with respect to zinc binding, and TETA has a logK metal binding stability constant of 11.95 with respect to zinc binding, both as determined at 25 °C. Metal binding stability constants are listed in the National Institute of Standards and Technology reference database 46 (Critically Selected Stability Constants of Metal Complexes) and are typically determined at 25 °C..

Suitably the concentration of the zinc binding species selected from DETA and TETA is about 0.05 mM or more e.g. in the range 0.05-5 mM.

For example, the concentration of the zinc binding species selected from DETA and TETA in the formulation may typically be in the range 0.05-2 mM, more preferably 0.1-1.5 mM e.g. 0.2-1.0 mM e.g. 0.25-0.75 mM, more preferably around 0.5 mM, especially when the zinc binding species is TETA.

In an embodiment, the concentration of the zinc binding species selected from DETA and TETA is 0.25 mM or more.

DETA and TETA may be employed as the free base or a salt form, such as a chloride or acetate salt form.

A mixture of DETA and TETA may be employed. When a mixture is employed, the concentration of zinc binding species refers to the combined, total concentration of DETA and TETA.

Suitably the molar ratio of ionic zinc to the zinc binding species selected from DETA and TETA in the formulation is in the range 10:1 to 1:10 e.g. 5:1 to 1:10 e.g. 2:1 to 1:10 or 1:1 to 1:4 e.g. 1:1 to 1:3

For example, a formulation containing 100 U/ml of insulin compound may contain around 0.3 mM of ionic zinc (i.e. around 19.7 µg/ml of ionic zinc, i.e. around 0.54% by weight of zinc based on the weight of insulin compound in the formulation) and around 0.25-0.75 mM zinc binding species selected from DETA and TETA (especially TETA).

The formulations of the invention are suitably substantially free of zinc binding species which have a logK metal binding stability constant with respect to zinc binding of more than 12.3 as determined at 25 °C. Specifically, the formulations of the invention are suitably substantially free of EDTA (logK = 14.5). Further examples of zinc binding species which have a logK metal binding stability constant with respect to zinc binding of more than 12.3 to be suitably avoided include EGTA (logK = 12.6). In general, formulations of the invention will suitably be substantially free of tetradentate ligands or ligands of higher denticity. "Substantially free" means that the concentration of zinc binding species which have a logK metal binding stability constant with respect to zinc binding of more than 12.3 as determined at 25 °C (such as EDTA) is less than 0.1 mM, such as less than 0.05 mM or less than 0.01 mM.

The formulations of the invention contain a non-ionic surfactant.

A suitable class of non-ionic surfactants is the alkyl glycosides, especially dodecyl maltoside. Other alkyl glycosides include dodecyl glucoside, octyl glucoside, octyl maltoside, decyl glucoside, decyl maltoside, tridecyl glucoside, tridecyl maltoside, tetradecyl glucoside, tetradecyl maltoside, hexadecyl glucoside, hexadecyl maltoside, sucrose monooctanoate, sucrose mono decanoate, sucrose monododecanoate, sucrose monotridecanoate, sucrose monotetradecanoate and sucrose monohexadecanoate.

Another suitable class of non-ionic surfactants is the polysorbates (fatty acid esters of ethoxylated sorbitan), such as polysorbate 80 or polysorbate 20.

Polysorbate 80 is polysorbate 80 is a mono ester formed from oleic acid and polyoxyethylene (20) sorbitan in which the number 20 indicates the number of oxyethylene groups in the molecule. Polysorbate 80 is known under a range of brand names including in particular Tween 80, and also Alkest TW 80. In one embodiment, the non-ionic surfactant is a polysorbate surfactant other than polysorbate 80.

Polysorbate 20 is a mono ester formed from lauric acid and polyoxyethylene (20) sorbitan in which the number 20 indicates the number of oxyethylene groups in the molecule. Polysorbate 20 is known under a range of brand names including in particular Tween 20, and also Alkest TW 20. Other suitable polysorbates include polysorbate 40 and polysorbate 60.

Another suitable class of non-ionic surfactants is the class of block copolymers of polyethylene glycol and polypropylene glycol, also known as poloxamers, especially poloxamer 188, poloxamer 407, poloxamer 171 and poloxamer 185. Poloxamers are also known under brand names Pluronic or Koliphors. For example, poloxamer 188 is marketed as Pluronic F-68.

Another suitable class of non-ionic surfactants is the class of alkyl ethers of polyethylene glycol, especially those known under a brand name Brij, such as selected from polyethylene glycol (2) hexadecyl ether (Brij 52), polyethylene glycol (2) oleyl ether (Brij 93) and polyethylene glycol (2) dodecyl ether (Brij L4). Other suitable Brij surfactants include polyethylene glycol (4) lauryl ether (Brij 30), polyethylene glycol (10) lauryl ether (Brij 35), polyethylene glycol (20) hexadecyl ether (Brij 58) and polyethylene glycol (10) stearyl ether (Brij 78).

Another suitable class of non-ionic surfactants is the class of alkylphenyl ethers of polyethylene glycol, especially 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol, also known under a brand name Triton X-100.

Particularly suitable are non-ionic surfactants with molecular weight of less than 1000 g/mole, especially less than 600 g/mole, such as 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol (Triton X-100) (647 g/mole), dodecyl

maltoside (511 g/mole), octyl glucoside (292 g/mole), polyethylene glycol (2) dodecyl ether (Brij L4) (362 g/mole), polyethylene glycol (2) oleyl ether (Brij 93) (357 g/mole) and polyethylene glycol (2) hexadecyl ether (Brij 52) (330 g/mole).

5 The concentration of the non-ionic surfactant in the formulation will typically be in the range 1-1000 µg/ml, e.g. 5-500 µg/ml, e.g. 10-200 µg/ml, such as 10-100 µg/ml especially around 50 µg/ml.

Suitably the pH of the aqueous formulations of the invention is in the range 5.5-9.0 especially 6.5-8.0 e.g. 7.0-7.8. e.g. 7.0-7.5. In order to minimise injection pain the pH is preferably close to physiological pH (around pH 7.4). Another pH
10 range of interest is 7.6-8.0 e.g. around 7.8.

Suitably, the formulation of the invention comprises a buffer in order to stabilise the pH of the formulation, which can also be selected to enhance protein stability. In one embodiment, a buffer is selected to have a pKa close to the pH of the formulation; for example histidine is suitably employed as a buffer when the pH of
15 the formulation is in the range 5.0-7.0. Such a buffer may be employed in a concentration of 0.5-20 mM e.g. 2-5 mM. As another example, phosphate is suitably employed as a buffer when the pH of the formulation is in the range 6.1-8.1. Such a buffer may be employed in a concentration of 0.5-20 mM e.g. 2-5 mM. Alternatively, in another embodiment, the formulation of the invention is further stabilised as
20 disclosed in WO2008/084237 (herein incorporated by reference in its entirety), which describes a formulation comprising a protein and one or more additives, characterised in that the system is substantially free of a conventional buffer, i.e. a compound with an ionisable group having a pKa within 1 unit of the pH of the formulation at the intended temperature range of storage of the formulation, such as
25 25 °C. In this embodiment, the pH of the formulation is set to a value at which the formulation has maximum measurable stability with respect to pH; the one or more additives (displaced buffers) are capable of exchanging protons with the insulin compound and have pKa values at least 1 unit more or less than the pH of the formulation at the intended temperature range of storage of the formulation. The
30 additives may have ionisable groups having pKa between 1 to 5 pH units, preferably between 1 to 3 pH units, most preferably from 1.5 to 2.5 pH units, of the pH of the

aqueous formulation at the intended temperature range of storage of the formulation (e.g. 25 °C). Such additives may typically be employed at a concentration of 0.5-10 mM e.g. 2-5 mM.

The aqueous formulations of the present invention cover a wide range of osmolarity, including hypotonic, isotonic and hypertonic formulations. Preferably, the formulations of the invention are substantially isotonic. Suitably the osmolarity of the formulation is selected to minimize pain according to the route of administration e.g. upon injection. Preferred formulations have an osmolarity in the range of about 200 to about 500 mOsm/L. Preferably, the osmolarity is in the range of about 250 to about 350 mOsm/L. More preferably, the osmolarity is about 300 mOsm/L.

Tonicity of the formulation may be adjusted with a tonicity modifying agent. Thus according to a further aspect of the invention the formulation further comprises a tonicity modifying agent. Tonicity modifying agents may be charged or uncharged. Examples of charged tonicity modifying agents include salts such as a combination of sodium, potassium, magnesium or calcium ions, with chloride, sulfate, carbonate, sulfite, nitrate, lactate, succinate, acetate or maleate ions (especially sodium chloride or sodium sulphate, particularly sodium chloride). Amino acids such as arginine, glycine or histidine may also be used for this purpose. Charged tonicity modifying agent is preferably used at a concentration of 100 – 300 mM, e.g. around 150 mM. Examples of uncharged tonicity modifying agents include sugars, sugar alcohols and other polyols, such as trehalose, sucrose, mannitol, glycerol, 1,2-propanediol, raffinose, lactose, dextrose, sorbitol or lactitol (especially trehalose, mannitol, glycerol or 1,2-propanediol, particularly glycerol). Uncharged tonicity modifying agent is preferably used at a concentration of 200 – 500 mM, e.g. around 300 mM.

When the insulin compound is insulin lispro, the tonicity is suitably adjusted using an uncharged tonicity modifying agent, preferably at a concentration of 200 – 500 mM, e.g. around 300 mM. In this embodiment, the uncharged tonicity modifying agent is suitably selected from the group consisting of trehalose, mannitol, glycerol and 1,2-propanediol (most suitably glycerol). When the insulin compound is insulin aspart at a concentration of 500 U/ml or less (e.g. 100 U/ml), the tonicity is suitably adjusted using a charged tonicity modifying agent, especially sodium chloride,

preferably at a concentration of 100 – 300 mM, e.g. around 150 mM. When the insulin compound is insulin aspart at a concentration of > 500 U/ml (e.g. 1000 U/ml), the tonicity is suitably adjusted using an uncharged tonicity modifying agent, preferably at a concentration of 200 – 500 mM, e.g. around 300 mM. In this

5 embodiment, the uncharged tonicity modifying agent is suitably selected from the group consisting of trehalose, mannitol, glycerol and 1,2-propanediol (most suitably glycerol).

The ionic strength of a formulation may be calculated according to the formula:

$$I = 0.5 \times \sum_{x=1}^n c_x z_x^2$$

10 in which c_x is molar concentration of ion x (mol L⁻¹), z_x is the absolute value of the charge of ion x and the sum covers all ions (n) present in the formulation. The contribution of the insulin compound itself should be ignored for the purposes of the calculation. For zwitterions the absolute value of the charge is the total charge excluding polarity, e.g. for glycine the possible ions have absolute charge of 0, 1 or 2

15 and for aspartate the possible ions have absolute charge of 0, 1, 2 or 3.

In general, the ionic strength of the formulation is suitably in the range of around 5 mM up to around 500 mM.

When the insulin compound is insulin lispro, the ionic strength of the formulation is suitably kept to a minimum level since higher ionic strength

20 formulations are less stable than lower ionic strength formulations. Suitably the ionic strength taking account of ions in the formulation except for the zinc binding species and the insulin compound is less than 40 mM, e.g. less than 20 mM, e.g. less than 10 mM such as 5-10 mM.

When the insulin compound is insulin aspart at a concentration of > 500 U/ml

25 (e.g. 1000 U/ml), the ionic strength of the formulation is suitably kept to a minimum level since higher ionic strength formulations are less stable than lower ionic strength formulations. Suitably the ionic strength taking account of ions in the formulation except for the zinc binding species and the insulin compound is less than 40 mM, e.g. less than 20 mM, e.g. less than 10 mM.

When the insulin compound is insulin aspart at a concentration of 500 U/ml or less (e.g. 100 U/ml), the ionic strength of the formulation may be high. Suitably the ionic strength taking account of ions in the formulation except for the zinc binding species and the insulin compound is more than 50 mM, e.g. more than 100 mM, e.g. 50-500 mM or 100-500 mM or 100-300 mM such as around 150 mM.

The formulations of the invention can optionally include preservative, preferably phenol, m-cresol, chlorocresol, benzyl alcohol, propylparaben, methylparaben, benzalkonium chloride or benzethonium chloride.

The formulations of the invention may optionally comprise nicotinamide. The presence of nicotinamide may further increase the speed of onset of action of insulin formulated in formulations of the invention. Suitably, the concentration of nicotinamide is in the range 10-150 mM, preferably in the range 20-100 mM, such as around 80 mM.

The formulations of the invention may optionally comprise nicotinic acid or a salt thereof. The presence of nicotinic acid or a salt thereof may also further increase the speed of onset of action of insulin formulated in formulations of the invention. Suitably, the concentration of nicotinic acid or a salt thereof is in the range 10-150 mM, preferably in the range 20-100 mM, such as around 80 mM. Example salts include metal salts such as sodium, potassium and magnesium salts.

In an embodiment, one of nicotinamide and nicotinic acid (or as salt thereof) is included in the formulation but not both. In an embodiment, a mixture of nicotinamide and nicotinic acid (or as salt thereof) is included in the formulation.

Formulations of the invention may optionally include other beneficial components including stabilising agents. For example amino acids such as arginine or proline may be included which may have stabilising properties. Thus in one embodiment, the formulations of the invention comprise arginine.

In an embodiment of the invention the formulations are free of acids selected from glutamic acid, ascorbic acid, succinic acid, aspartic acid, maleic acid, fumaric acid, adipic acid and acetic acid and are also free from the corresponding ionic forms of these acids.

In an embodiment of the invention the formulations are free of arginine.

In an embodiment of the invention the formulations are free of protamine and protamine salts.

In an embodiment of the invention the formulations are free of magnesium ions.

5 In an embodiment of the invention the formulations are free of calcium ions.

Suitably the formulations of the invention are sufficiently stable that the concentration of high molecular weight species remains low upon extended storage. The term “high molecular weight species” as used herein, refers to any irreversibly formed component of the protein content which has an apparent molecular weight at least about double the molecular weight of the parent insulin compound, as detected by a suitable analytical method, such as size-exclusion chromatography. That is, high molecular weight species are multimeric aggregates of the parent insulin compound. The multimeric aggregates may comprise the parent protein molecules with considerably altered conformation or they may be an assembly of the parent protein units in the native or near-native conformation. The determination of high molecular weight species can be done using methods known in the art, including size exclusion chromatography, electrophoresis, analytical ultracentrifugation, light scattering, dynamic light scattering, static light scattering and field flow fractionation.

Suitably the formulations of the invention are sufficiently stable that they remain substantially free of visible particles after storage at 30°C for at least one, two or three months. Visible particles are suitably detected using the 2.9.20. European Pharmacopoeia Monograph method (Particulate Contamination: Visible Particles). For example, a formulation is substantially free of visible particles if it has a Visual score of 1, 2 or 3, especially 1 or 2 according to the definition given in the Examples section.

Suitably the formulations of the invention are sufficiently stable that the concentration of related species remains low upon extended storage. The term “related species” as used herein, refers to any component of the protein content formed by a chemical modification of the parent insulin compound, particularly desamido or cyclic imide forms of insulin. Related species are suitably detected by RP-HPLC.

In a preferred embodiment, the formulation of the invention retains at least 95%, e.g. at least 96%, e.g. at least 97%, e.g. at least 98%, e.g. at least 99% parent insulin compound (by weight of total protein) after storage at 30°C for one, two or three months. The percentage of insulin compound (by weight of total protein) may be determined by size-exclusion chromatography or RP-HPLC.

In a preferred embodiment, the formulation of the invention comprises no more than 4% (by weight of total protein), preferably no more than 2% high molecular weight species after storage at 30°C for one, two or three months.

In a preferred embodiment, the formulation of the invention comprises no more than 4% (by weight of total protein), preferably no more than 2%, preferably no more than 1% A-21 desamido form of the insulin compound after storage at 30°C for one, two or three months.

In preferred embodiments, a formulation of the present invention should exhibit an increase in high molecular weight species during storage which is at least 10% lower, preferably at least 25% lower, more preferably at least 50% lower, than a formulation lacking the non-ionic surfactant but otherwise identical, following storage under the same conditions (e.g. 30°C) and length of time (e.g. one, two or three months).

In preferred embodiments, a formulation of the present invention should exhibit an increase in related species during storage which is at least 10% lower, preferably at least 25% lower, more preferably at least 50% lower, than a formulation lacking the non-ionic surfactant but otherwise identical, following storage under the same conditions (e.g. 30°C) and length of time (e.g. one, two or three months).

The speed of action of a formulation of the invention may be determined in the Diabetic Pig Pharmacokinetic/Pharmacodynamic Model (see Examples, General Methods). In preferred embodiments, a formulation of the present invention should exhibit a $T_{\max}$ (i.e. time to peak insulin concentration) that is at least 10% shorter, preferably at least 20% shorter, more preferably at least 30% shorter than a formulation lacking the zinc binding species selected from DETA and TETA but otherwise identical, using the model. In preferred embodiments, a formulation of the present invention should exhibit an area under the curve on the pharmacodynamics

profile within the first 45 minutes after injection that is at least 10% greater, preferably at least 20% greater, more preferably at least 30% greater than a formulation lacking the zinc binding species selected from DETA and TETA but otherwise identical, using the model.

5 According to further aspects of the invention, there is provided a formulation of the invention for use in the treatment of a subject suffering from diabetes mellitus. There is also provided a method of treatment of diabetes mellitus which comprises administering to a subject in need thereof an effective amount of a formulation of the invention.

10 A typical dose of the formulation of the invention is 2-30 U, e.g. 5-15 U. Administration should suitably occur in the window between 15 minutes before eating (i.e. before start of a meal) and 15 minutes after eating (i.e. after end of a meal).

 An aspect of the invention is a container e.g. made of plastics or glass containing one dose or a plurality of doses of the formulation of the invention. The
15 container can, for example, be a cartridge designed to be a replaceable item for use with an injection device.

 The formulations of the invention may suitably be packaged for injection, especially sub-cutaneous or intramuscular injection. Sub-cutaneous injection is preferred. Injection may be by conventional syringe or more preferably via a pen
20 device adapted for use by diabetic subjects. Exemplary pen devices include the Kwikpen® device and the Flexpen® device.

 an aspect of the invention is an injection device, particularly a device adapted for subcutaneous or intramuscular injection, for single or multiple use comprising a container containing one dose or a plurality of doses of the formulation of the
25 invention together with an injection needle. In an embodiment the container is a replaceable cartridge which contains a plurality of doses. In an embodiment, the needle is replaceable e.g. after each occasion of use.

 Another aspect of the invention is a medical device comprising a reservoir comprising plurality of doses of the formulation of the invention and a pump adapted
30 for automatic or remote operation such that upon automatic or remote operation one or more doses of the formulation of the invention is administered to the body e.g.

subcutaneously or intramuscularly. Such devices may be worn on the outside of the body or implanted in the body.

Formulations of the invention may be prepared by mixing the ingredients. For example, the insulin compound may be dissolved in an aqueous formulation

5 comprising the other components. Alternatively, the insulin compound may be dissolved in a strong acid (typically HCl), after dissolution diluted with an aqueous formulation comprising the other components, and then pH adjusted to the desired pH with addition of alkali (e.g. NaOH). As a variation on this method, a step of neutralising the acid solution may be performed before the dilution step and it may
10 then not be necessary to adjust the pH after the dilution step (or a small adjustment only may be necessary).

According to another aspect of the invention there is provided a dry solid pharmaceutical composition suitable for reconstitution with an aqueous medium which comprises (i) an insulin compound other than insulin glargine, (ii) ionic zinc
15 e.g. at a concentration of 0.05% or more e.g. 0.5% or more by weight of zinc based on the weight of insulin compound in the formulation, (iii) a zinc binding species selected from DETA and TETA e.g. at a concentration of 0.05 mM or more, and (iv) a non-ionic surfactant. Thus a formulation of the invention may be prepared by dissolving such a dry solid pharmaceutical composition in an aqueous medium e.g.
20 water or saline. Such a dry solid pharmaceutical composition may be prepared by dehydrating (e.g. freeze drying) a formulation of the invention. The invention also provides a container containing one dose or a plurality of doses of such a dry solid pharmaceutical composition.

Further aspects of the invention include:

- 25
- A method of improving the storage stability of an aqueous liquid pharmaceutical formulation comprising (i) an insulin compound other than insulin glargine, (ii) ionic zinc e.g. at a concentration of 0.05% or more e.g. 0.5% or more by weight of zinc based on the weight of insulin compound in the formulation and (iii) a zinc binding species selected from DETA and TETA e.g.
30 at a concentration of 0.05 mM or more; which comprises adding a non-ionic surfactant to the formulation; and

- Use of a non-ionic surfactant to improve the storage stability of an aqueous liquid pharmaceutical formulation comprising (i) an insulin compound other than insulin glargine, (ii) ionic zinc e.g. at a concentration of 0.05% or more e.g. 0.5% or more by weight of zinc based on the weight of insulin compound in the formulation and (iii) a zinc binding species selected from DETA and TETA e.g. at a concentration of 0.05 mM or more.

Formulations of the invention are expected to have one or more of the following advantageous properties:

- rapid speed of action, typically faster than normal human insulin, upon administration to a subject;
- good physical stability upon storage, especially as measured by the amount of HMWS or visual detection of particles;
- good chemical stability upon storage, especially as measured by the amount of related products e.g. products of deamidation.

Further aspects of the invention are defined by the following clauses:

Clause 1. An aqueous liquid pharmaceutical formulation comprising (i) an insulin compound other than insulin glargine, (ii) ionic zinc, (iii) a zinc binding species selected from diethylenetriamine (DETA) and triethylenetetramine (TETA), and (iv) a non-ionic surfactant.

Clause 2. The formulation according to clause 1 wherein the insulin compound is insulin lispro.

Clause 3. The formulation according to clause 1 wherein the insulin compound is insulin aspart.

Clause 4. The formulation according to clause 1 wherein the insulin compound is insulin glulisine.

Clause 5. The formulation according to clause 1 wherein the insulin compound is recombinant human insulin.

Clause 6. The formulation according to any one of clauses 1 to 5 wherein the formulation is substantially free of ethylenediaminetetraacetate (EDTA) and any other

zinc binding species having a logK with respect to zinc ion binding of more than 12.3 at 25 °C.

Clause 7. The formulation according to any one of clauses 1 to 6, wherein the insulin compound is present at a concentration of 10-1000 U/ml.

- 5 Clause 8. The formulation according to any one of clauses 1 to 7, wherein the ionic zinc is present at a concentration of more than 0.05% by weight of zinc based on the weight of insulin compound in the formulation.

Clause 9. The formulation according to clause 8, wherein the ionic zinc is present at a concentration of more than 0.5% by weight of zinc based on the weight of insulin
10 compound in the formulation.

Clause 10. The formulation according to clause 9, wherein the ionic zinc is present at a concentration of 0.5-1% by weight of zinc based on the weight of insulin compound in the formulation.

Clause 11. The formulation according to any one of clauses 1 to 10, wherein the
15 zinc binding species is TETA.

Clause 12. The formulation according to any one of clauses 1 to 11, wherein the zinc binding species is present at a concentration of about 0.05 mM or more e.g. 0.05-5 mM e.g. 0.05-2 mM.

Clause 13. The formulation according to clause 11, wherein the molar ratio of ionic
20 zinc to the zinc binding species is 2:1 to 1:10.

Clause 14. The formulation according to any one of clauses 1 to 13 wherein the non-ionic surfactant is an alkyl glycoside.

Clause 15. The formulation according to clause 14 wherein the alkyl glycoside is dodecyl maltoside.

25 Clause 16. The formulation according to any one of clauses 1 to 13 wherein the non-ionic surfactant is a polysorbate surfactant other than polysorbate 80.

Clause 17. The formulation according to clause 16 wherein the polysorbate surfactant is polysorbate 20.

Clause 18. The formulation according to any one of clauses 1 to 13 wherein the
30 non-ionic surfactant is an alkyl ether of polyethylene glycol.

Clause 19. The formulation according to clause 18 wherein the alkyl ether of polyethylene glycol is selected from polyethylene glycol (2) dodecyl ether, polyethylene glycol (2) oleyl ether and polyethylene glycol (2) hexadecyl ether.

5 Clause 20. The formulation according to any one of clauses 1 to 13 wherein the non-ionic surfactant is a block copolymer of polyethylene glycol and polypropylene glycol.

Clause 21. The formulation according to clause 20 wherein the block copolymer of polyethylene glycol and polypropylene glycol is poloxamer 188, poloxamer 407, poloxamer 171 or poloxamer 185.

10 Clause 22. The formulation according to any one of clauses 1 to 13 wherein the non-ionic surfactant is an alkylphenyl ether of polyethylene glycol.

Clause 23. The formulation according to clause 22 wherein the alkylphenyl ether of polyethylene glycol is 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol.

15 Clause 24. The formulation according to any one of clauses 1 to 23 wherein the non-ionic surfactant is present at a concentration of 1-1000 µg/ml.

Clause 25. The formulation according to any one of clauses 1 to 24, further comprising a tonicity modifying agent.

20 Clause 26. The formulation according to clause 25 wherein the insulin compound is insulin lispro and the tonicity modifying agent is an uncharged tonicity modifying agent or wherein the insulin compound is insulin aspart at a concentration of > 500 U/ml and the tonicity modifying agent is an uncharged tonicity modifying agent.

Clause 27. The formulation according to clause 26, wherein the uncharged tonicity modifying agent is selected from the group consisting of trehalose, mannitol, glycerol and 1,2-propanediol.

25 Clause 28. The formulation according to clause 27, wherein the uncharged tonicity modifying agent is glycerol.

30 Clause 29. The formulation according to any one of clauses 1, 2 and 6-28 wherein the insulin compound is insulin lispro and the ionic strength of the formulation taking account of ions in the formulation excluding the zinc binding species and the insulin compound is less than 40 mM or wherein the insulin compound is insulin aspart at a concentration of > 500 U/ml and the ionic strength of the formulation taking account

of ions in the formulation excluding the zinc binding species and the insulin compound is less than 40 mM.

Clause 30. The formulation according to clause 25 wherein the insulin compound is insulin aspart at a concentration of 500 U/ml or less and the tonicity modifier is a charged tonicity modifier.

Clause 31. The formulation according to clause 30, wherein the charged tonicity modifier is sodium chloride.

Clause 32. The formulation according to any one of clauses 1, 3 and 6-28 wherein the insulin compound is insulin aspart at a concentration of 500 U/ml or less and the ionic strength of the formulation taking account of ions in the formulation excluding the zinc binding species and the insulin compound is more than 50 mM.

Clause 33. The formulation according to any one of clauses 1 to 32, wherein the formulation is substantially isotonic.

Clause 34. The formulation according to any one of clauses 1 to 33, wherein the pH is in the range 5.5 to 9.0.

Clause 35. The formulation according to any of clauses 1 to 34, further comprising a preservative.

Clause 36. The formulation according to clause 35, wherein the preservative is selected from the group consisting of phenol, m-cresol, chlorocresol, benzyl alcohol, propylparaben, methylparaben, benzalkonium chloride and benzethonium chloride.

Clause 37. The formulation according to any one of clauses 1 to 36, further comprising nicotinamide.

Clause 38. The formulation according to any one of clauses 1 to 36, further comprising nicotinic acid or a salt thereof.

Clause 39. The formulation according to any one of clauses 1 to 37 for use in the treatment of a subject suffering from diabetes mellitus.

Clause 40. A method of treatment of diabetes mellitus which comprises administering to a subject in need thereof an effective amount of a formulation according to any one of clauses 1 to 38.

Clause 41. A container containing one dose or a plurality of doses of the formulation according to any one of clauses 1 to 38.

Clause 42. An injection device for single or multiple use comprising a container containing one dose or a plurality of doses of the formulation according to any one of clauses 1 to 38 together with an injection needle.

Clause 43. A medical device comprising a reservoir comprising plurality of doses of the formulation according to any one of clauses 1 to 38 and a pump adapted for automatic or remote operation such that upon automatic or remote operation one or more doses of the formulation is administered to the body.

Clause 44. A dry solid pharmaceutical composition suitable for reconstitution with an aqueous medium which comprises (i) an insulin compound other than insulin glargine, (ii) ionic zinc, (iii) a zinc binding species selected from DETA and TETA, and (iv) a non-ionic surfactant.

Clause 45. A method of preparing a formulation according to any one of clauses 1 to 38 which comprises dissolving a dry solid pharmaceutical composition according to clause 44 in an aqueous medium.

Clause 46. A method of improving the storage stability of an aqueous liquid pharmaceutical formulation comprising (i) an insulin compound other than insulin glargine, (ii) ionic zinc and (iii) a zinc binding species selected from DETA and TETA; which comprises adding a non-ionic surfactant to the formulation.

Clause 47. Use of a non-ionic surfactant to improve the storage stability of an aqueous liquid pharmaceutical formulation comprising (i) an insulin compound other than insulin glargine, (ii) ionic zinc and (iii) a zinc binding species selected from DETA and TETA.

ABBREVIATIONS

25	EDTA	ethylenediaminetetraacetate
	EGTA	ethyleneglycoltetraacetate
	DETA	diethylenetriamine
	TETA	triethylenetetramine
	HPLC	high performance liquid chromatography
30	HMWS	high molecular weight species
	RP	reverse phase

SEC size-exclusion chromatography
PD pharmacodynamic

EXAMPLES

5 General Methods

(a) The Diabetic Pig Pharmacokinetic/Pharmacodynamic Model: Method for determining speed of action:

10 male diabetic Yucatan miniature pigs are used. Pigs are injected subcutaneously with a sample of the test formulation and blood is taken (1 or 2 ml) at various time-
10 points (min) with respect to the injection e.g. at the time points (min): -30 (or -15), 0, 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210 and 240. For pharmacodynamics profile, serum is analysed for glucose (using a commercially available glucometer). For pharmacokinetic profile, insulin concentration is determined in the serum using an immunoassay.

15

(b) Visual assessment

Visible particles are suitably detected using the 2.9.20. European Pharmacopoeia Monograph (Particulate Contamination: Visible Particles). The apparatus required consists of a viewing station comprising:

- 20 • a matt black panel of appropriate size held in a vertical position
- a non-glare white panel of appropriate size held in a vertical position next to the black panel
- an adjustable lampholder fitted with a suitable, shaded, white-light source and with a suitable light diffuser (a viewing illuminator containing two 13 W
25 fluorescent tubes, each 525 mm in length, is suitable). The intensity of illumination at the viewing point is maintained between 2000 lux and 3750 lux.

Any adherent labels are removed from the container and the outside washed and dried. The container is gently swirled or inverted, ensuring that air bubbles are not introduced, and observed for about 5 s in front of the white panel. The procedure is
30 repeated in front of the black panel. The presence of any particles is recorded.

The visual scores are ranked as follows:

Visual score 1: Clear solution, virtually free of particles

Visual score 2: ~ 5 very small particles

Visual score 3: ~10-20 very small particles

Visual score 4: 20-50 particles, including larger particles

5 Visual score 5: >50 particles, including larger particles

Whilst the particles in samples with visual scores 4 and 5 are clearly detectable on casual visual assessment under normal light, samples with visual score 1-3 generally appear as clear solutions on the same assessment. Samples with visual scores 1-3 are considered to be "Pass"; samples with visual score 4-5 are considered to be
10 "Fail".

Example 1 – Example formulations

The following example formulations may be prepared:

15 Example A:

Insulin lispro 100 U/ml

Sodium phosphate 2 mM

phenol 15.9 mM

m-cresol 15.9 mM

20 Ionic zinc (as ZnCl_2) 19.7 $\mu\text{g/ml}$ (0.3 mM), equals 0.55% (w/w) based on the weight of insulin compound in the formulation

TETA 0.5 mM

Glycerol 174 mM

Surfactant Selected from A1, A2 or A3 (see below)

25 Water for injection qs

Residual NaCl Acidification and subsequent neutralisation during preparation results in formation of 2-4 mM NaCl

pH adjusted to 7.4

30 Example A1: surfactant = dodecyl maltoside (0.05 mg/ml)

Example A2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)

Example A3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)

Example B:

	Insulin aspart	100 U/ml
5	Sodium phosphate	2 mM
	phenol	15.9 mM
	m-cresol	15.9 mM
	Ionic zinc (as ZnCl_2)	19.7 $\mu\text{g/ml}$ (0.3 mM), equals 0.55% (w/w) based on the weight of insulin compound in the formulation
10	TETA	0.5 mM
	NaCl	150 mM
	Surfactant	Selected from B1, B2 or B3 (see below)
	Water for injection	qs
	Residual NaCl	Acidification and subsequent neutralisation during
15		preparation results in formation of 2-4 mM NaCl
	pH adjusted to 7.4	

Example B1: surfactant = dodecyl maltoside (0.05 mg/ml)

Example B2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)

20 Example B3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)

Example C:

	Insulin aspart	1000 U/ml
	Sodium phosphate	2 mM
25	phenol	15.9 mM
	m-cresol	15.9 mM
	Ionic zinc (as ZnCl_2)	19.7 $\mu\text{g/ml}$ (0.3 mM), equals 0.55% (w/w) based on the weight of insulin compound in the formulation
	TETA	5 mM
30	Glycerol	174 mM
	Surfactant	Selected from C1, C2 or C3 (see below)

Water for injection qs
 Residual NaCl Acidification and subsequent neutralisation during
 preparation results in formation of 2-4 mM NaCl
 pH adjusted to 7.4

5

Example C1: surfactant = dodecyl maltoside (0.05 mg/ml)

Example C2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)

Example C3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)

10 Example D:

Insulin lispro 100 U/ml

Sodium phosphate 2 mM

phenol 15.9 mM

m-cresol 15.9 mM

15 Ionic zinc (as ZnCl_2) 19.7 $\mu\text{g/ml}$ (0.3 mM), equals 0.55% (w/w) based on the
 weight of insulin compound in the formulation

TETA 0.5 mM

Glycerol 174 mM

Surfactant Selected from D1, D2 or D3 (see below)

20 Water for injection qs

Residual NaCl Acidification and subsequent neutralisation during
 preparation results in formation of 2-4 mM NaCl

pH adjusted to 7.8

25 Example D1: surfactant = dodecyl maltoside (0.05 mg/ml)

Example D2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)

Example D3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)

Example E:

30 Insulin aspart 100 U/ml

Sodium phosphate 2 mM

	phenol	15.9 mM
	m-cresol	15.9 mM
	Ionic zinc (as ZnCl ₂)	19.7 µg/ml (0.3 mM), equals 0.55% (w/w) based on the weight of insulin compound in the formulation
5	TETA	0.5 mM
	NaCl	150 mM
	Surfactant	Selected from E1, E2 or E3 (see below)
	Water for injection	qs
	Residual NaCl	Acidification and subsequent neutralisation during preparation results in formation of 2-4 mM NaCl
10	pH adjusted to 7.8	
Example E1: surfactant = dodecyl maltoside (0.05 mg/ml)		
Example E2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)		
15	Example E3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)	
Example F:		
	Insulin aspart	1000 U/ml
	Sodium phosphate	2 mM
20	phenol	15.9 mM
	m-cresol	15.9 mM
	Ionic zinc (as ZnCl ₂)	19.7 µg/ml (0.3 mM), equals 0.55% (w/w) based on the weight of insulin compound in the formulation
	TETA	5 mM
25	Glycerol	174 mM
	Surfactant	Selected from F1, F2 or F3 (see below)
	Water for injection	qs
	Residual NaCl	Acidification and subsequent neutralisation during preparation results in formation of 2-4 mM NaCl
30	pH adjusted to 7.8	

Example F1: surfactant = dodecyl maltoside (0.05 mg/ml)

Example F2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)

Example F3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)

5 Example G:

Insulin lispro 100 U/ml

Sodium phosphate 2 mM

phenol 15.9 mM

m-cresol 15.9 mM

10 Ionic zinc (as ZnCl_2) 19.7 $\mu\text{g/ml}$ (0.3 mM), equals 0.55% (w/w) based on the weight of insulin compound in the formulation

DETA 5 mM

Glycerol 174 mM

Surfactant Selected from G1, G2 or G3 (see below)

15 Water for injection qs

Residual NaCl Acidification and subsequent neutralisation during preparation results in formation of 2-4 mM NaCl

pH adjusted to 7.4

20 Example G1: surfactant = dodecyl maltoside (0.05 mg/ml)

Example G2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)

Example G3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)

Example H:

25 Insulin aspart 100 U/ml

Sodium phosphate 2 mM

phenol 15.9 mM

m-cresol 15.9 mM

30 Ionic zinc (as ZnCl_2) 19.7 $\mu\text{g/ml}$ (0.3 mM), equals 0.55% (w/w) based on the weight of insulin compound in the formulation

DETA 5 mM

	NaCl	150 mM
	Surfactant	Selected from H1, H2 or H3 (see below)
	Water for injection	qs
	Residual NaCl	Acidification and subsequent neutralisation during
5		preparation results in formation of 2-4 mM NaCl
	pH adjusted to 7.4	

Example H1: surfactant = dodecyl maltoside (0.05 mg/ml)

Example H2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)

10 Example H3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)

Example I:

	Insulin aspart	1000 U/ml
	Sodium phosphate	2 mM
15	phenol	15.9 mM
	m-cresol	15.9 mM
	Ionic zinc (as ZnCl ₂)	19.7 µg/ml (0.3 mM), equals 0.55% (w/w) based on the
		weight of insulin compound in the formulation
	DETA	5 mM
20	Glycerol	174 mM
	Surfactant	Selected from I1, I2 or I3 (see below)
	Water for injection	qs
	Residual NaCl	Acidification and subsequent neutralisation during
		preparation results in formation of 2-4 mM NaCl
25	pH adjusted to 7.4	

Example I1: surfactant = dodecyl maltoside (0.05 mg/ml)

Example I2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)

Example I3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)

30

Example J:

	Insulin lispro	100 U/ml
	Sodium phosphate	2 mM
	phenol	15.9 mM
5	m-cresol	15.9 mM
	Ionic zinc (as ZnCl ₂)	19.7 µg/ml (0.3 mM), equals 0.55% (w/w) based on the weight of insulin compound in the formulation
	DETA	0.5 mM
	Glycerol	174 mM
10	Surfactant	Selected from J1, J2 or J3 (see below)
	Water for injection	qs
	Residual NaCl	Acidification and subsequent neutralisation during preparation results in formation of 2-4 mM NaCl
	pH adjusted to 7.8	

15

Example J1: surfactant = dodecyl maltoside (0.05 mg/ml)

Example J2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)

Example J3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)

20 Example K:

	Insulin aspart	100 U/ml
	Sodium phosphate	2 mM
	phenol	15.9 mM
	m-cresol	15.9 mM
25	Ionic zinc (as ZnCl ₂)	19.7 µg/ml (0.3 mM), equals 0.55% (w/w) based on the weight of insulin compound in the formulation
	TETA	0.5 mM
	NaCl	150 mM
	Surfactant	Selected from K1, K2 or K3 (see below)
30	Water for injection	qs

Residual NaCl Acidification and subsequent neutralisation during
preparation results in formation of 2-4 mM NaCl

pH adjusted to 7.8

5 Example K1: surfactant = dodecyl maltoside (0.05 mg/ml)

Example K2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)

Example K3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)

Example L:

10	Insulin aspart	1000 U/ml
	Sodium phosphate	2 mM
	phenol	15.9 mM
	m-cresol	15.9 mM
	Ionic zinc (as ZnCl ₂)	19.7 µg/ml (0.3 mM), equals 0.55% (w/w) based on the
15		weight of insulin compound in the formulation
	TETA	5 mM
	Glycerol	174 mM
	Surfactant	Selected from L1, L2 or L3 (see below)
	Water for injection	qs
20	Residual NaCl	Acidification and subsequent neutralisation during preparation results in formation of 2-4 mM NaCl
		pH adjusted to 7.8

Example L1: surfactant = dodecyl maltoside (0.05 mg/ml)

25 Example L2: surfactant = polysorbate 20 (Tween 20) (0.05 mg/ml)

Example L3: surfactant = polyethylene glycol (2) dodecyl ether (Brij L4) (0.05 mg/ml)

Method for preparation for the above formulations:

Insulin powder is added to water and HCl is added until the powder is fully dissolved
30 (pH has to be <3 in order to achieve full dissolution). ZnCl₂ is added to the required
level. Once dissolved, pH is adjusted to approximately 7 and volume is adjusted with

water so that the insulin concentration is 2× the required concentration. The formulation is then mixed 1:1 (v/v) with a mixture of additional excipients (all at 2× the required concentration).

5 Example 2 – Comparison of pharmacodynamic profiles of insulin aspart formulations in the presence of (a) TETA, (b) EDTA and (c) nicotinamide

Pharmacodynamic profile of the following formulations was tested:

Formulation 1: insulin aspart (100 U/ml), NaCl (10 mM), TRIS (7 mM), glycerol (83.6 mM), arginine (30 mM), nicotinamide (80 mM), phenol (16 mM), m-cresol (16 mM),
10 zinc (from ZnCl₂, 19.6 µg/ml with respect to zinc), pH 7.4

Formulation 2: insulin aspart (100 U/ml), NaCl (150 mM), sodium phosphate (2 mM), EDTA (0.5 mM), dodecyl maltoside (0.05 mg/ml), phenol (16 mM), m-cresol (16 mM), zinc (from ZnCl₂, 19.6 µg/ml with respect to zinc), pH 7.4

Formulation 3: insulin aspart (100 U/ml), NaCl (150 mM), sodium phosphate (2 mM),
15 TETA (0.5 mM), dodecyl maltoside (0.05 mg/ml), phenol (16 mM), m-cresol (16 mM), zinc (from ZnCl₂, 19.6 µg/ml with respect to zinc), pH 7.4

Formulation 1 is identical to Formulation K in Example 1 of WO2010/149772, which was shown to have a significantly more rapid onset of action compared with that of commercially available NovoRapid® product (Formulation A in Example 1 of
20 WO2010/149772) – see Figures 4 and 5 of WO2010/149772.

Results are shown in Figure 1. It was shown that the formulation comprising TETA (Formulation 3) resulted in a comparable PD profile to that of the formulation comprising nicotinamide (Formulation 1). The decline in glucose concentration appeared to be slightly more rapid in the TETA-based formulation in the first 50
25 minutes after injection, but it appeared to slow down beyond that point.

Formulation comprising EDTA (Formulation 2) resulted in a more rapid glucose decrease compared with both the TETA-based and the nicotinamide-based formulation. However, as shown below, this formulation is very unstable and therefore not suitable for a viable pharmaceutical product.

30

Example 3 – Stability of insulin aspart formulations in the presence of (a) TETA and (b) EDTA

The effect TETA and EDTA on stability of insulin aspart was investigated. The stability was compared to that of the ultra-rapid acting formulation disclosed in WO2010/149772 (Formulation K in Example 1 of WO2010/149772). All formulations tested comprised insulin aspart (100 U/ml), phenol (16 mM), m-cresol (16 mM) and zinc (from ZnCl₂, 19.6 µg/ml with respect to zinc). The additional components of each formulation are listed in Table 1.

Table 1: Additional components in formulations of insulin aspart tested.

	Na phosphate (mM)	TRIS (mM)	NaCl (mM)	Glycerol (mM)	EDTA (mM)	TETA (mM)	Nicotinamide (mM)	Arginine (mM)	Dodecyl maltoside (mg/ml)
Formulation 3A (NovoRapid control)	13.2		10	174					
Formulation 3B (= Formulation K in WO2010/149772)		7	10	83.6			80	30	
Formulation 3C	2		150		0.5				
Formulation 3D	2		150		2				
Formulation 3E	2		150		0.5				0.05
Formulation 3F	2		150		2				0.05
Formulation 3G	2		150			0.5			
Formulation 3H	2		150			2			
Formulation 3I	2		150			0.5			0.05
Formulation 3J	2		150			2			0.05

Stability of insulin aspart was tested by visual assessment. Results are shown in Table 2. The formulation of NovoRapid remained clear and particle-free following 4 weeks storage at 30°C. The nicotinamide-based formulation (Formulation K in Example 1 of WO2010/149772) also showed good stability over 4 weeks at 30°C, although slight particle formation was observed at the 4 weeks time-point. Significant precipitation was observed in the EDTA-based formulations. Whilst the presence of dodecyl maltoside appeared to delay the precipitation, significant particle formation was still observed at the 4 weeks time-point. Slow precipitation was also observed in the TETA-based formulation. However, in the presence of dodecyl maltoside, the TETA-based formulations remained clear and particle-free following 4 weeks storage at 30°C

Table 2: Visual scores of insulin aspart formulations following storage at 30 °C.

Visual score 1: clear solution, virtually free of particles; visual score 2: ~ 5 very small particles; visual score 3: ~10-20 very small particles; visual score 4: 20-50 particles, including larger particles; visual score 5: >50 particles, including larger particles.

	0 weeks	1 week	2 weeks	4 weeks
Formulation 3A (NovoRapid control)	1	1	1	1
Formulation 3B (= Formulation K in WO2010/149772)	1	1	1	2
Formulation 3C	1	3	4	5
Formulation 3D	1	4	5	5
Formulation 3E	1	1	3	4
Formulation 3F	1	3	4	5
Formulation 3G	1	1	2	3
Formulation 3H	1	2	3	5
Formulation 3I	1	1	1	1
Formulation 3J	1	1	1	1

Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer, step, group of integers or group of steps but not to the exclusion of any other integer, step, group of integers or group of steps.

The term "and/or" as used in a phrase such as "A and/or B" herein is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

All publications, patents, patent applications, internet sites, and accession numbers/database sequences (including both polynucleotide and polypeptide sequences) cited are herein incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent

application, internet site, or accession number/database sequence were specifically and individually indicated to be so incorporated by reference.

SEQUENCE LISTING:

5

SEQ ID NO: 1: GIVEQCCTSICSLYQLENYCN

SEQ ID NO: 2: FVNQHLCGSHLVEALYLVCGERGFFYTPKT

10 SEQ ID NO: 3: FVNQHLCGSHLVEALYLVCGERGFFYTKPT

SEQ ID NO: 4: FVNQHLCGSHLVEALYLVCGERGFFYTDKT

SEQ ID NO: 5: FVKQHLCGSHLVEALYLVCGERGFFYTPET

15

CLAIMS

1. An aqueous liquid pharmaceutical formulation comprising (i) an insulin compound other than insulin glargine, (ii) ionic zinc, (iii) a zinc binding species
5 selected from diethylenetriamine (DETA) and triethylenetetramine (TETA), and (iv) a non-ionic surfactant.
2. The formulation according to claim 1 wherein the insulin compound is insulin lispro.
3. The formulation according to claim 1 wherein the insulin compound is insulin
10 aspart.
4. The formulation according to claim 1 wherein the insulin compound is insulin glulisine.
5. The formulation according to claim 1 wherein the insulin compound is recombinant human insulin.
- 15 6. The formulation according to any one of claims 1 to 5 wherein the formulation is substantially free of ethylenediaminetetraacetate (EDTA) and any other zinc binding species having a logK with respect to zinc ion binding of more than 12.3 at 25 °C.
7. The formulation according to any one of claims 1 to 6, wherein the insulin
20 compound is present at a concentration of 10-1000 U/ml.
8. The formulation according to any one of claims 1 to 7, wherein the ionic zinc is present at a concentration of more than 0.05% by weight of zinc based on the weight of insulin compound in the formulation.
9. The formulation according to claim 8, wherein the ionic zinc is present at a
25 concentration of more than 0.5% by weight of zinc based on the weight of insulin compound in the formulation.
10. The formulation according to claim 9, wherein the ionic zinc is present at a concentration of 0.5-1% by weight of zinc based on the weight of insulin compound in the formulation.
- 30 11. The formulation according to any one of claims 1 to 10, wherein the zinc binding species is TETA.

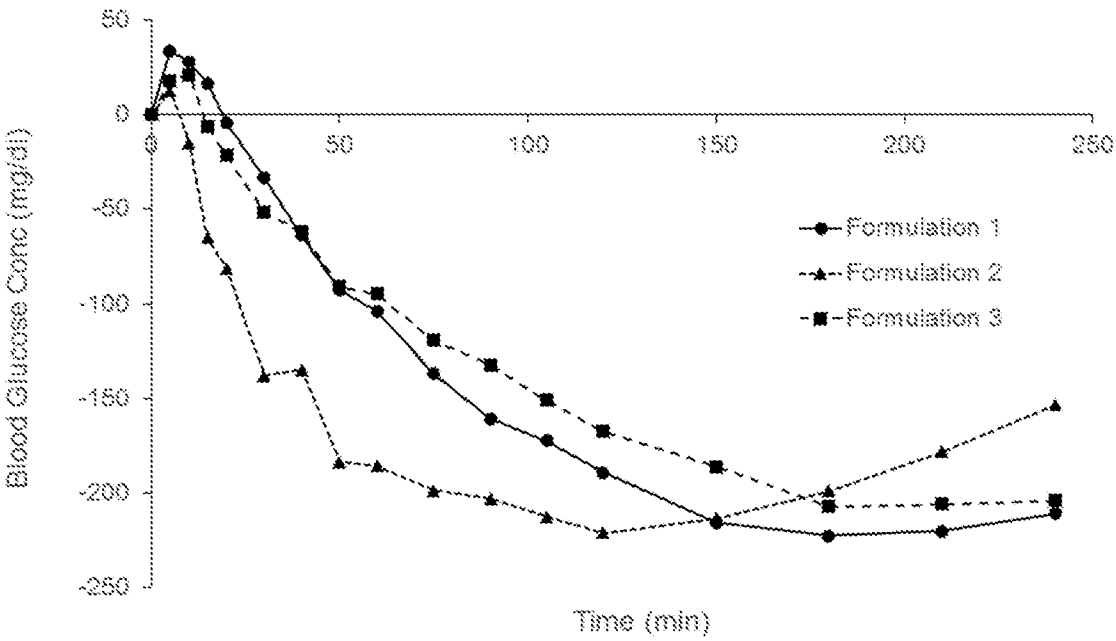
12. The formulation according to any one of claims 1 to 11, wherein the zinc binding species is present at a concentration of about 0.05 mM or more e.g. 0.05-5 mM e.g. 0.05-2 mM.
13. The formulation according to claim 11, wherein the molar ratio of ionic zinc to the zinc binding species is 2:1 to 1:10.
14. The formulation according to any one of claims 1 to 13 wherein the non-ionic surfactant is an alkyl glycoside.
15. The formulation according to claim 14 wherein the alkyl glycoside is dodecyl maltoside.
16. The formulation according to any one of claims 1 to 13 wherein the non-ionic surfactant is a polysorbate surfactant other than polysorbate 80.
17. The formulation according to claim 16 wherein the polysorbate surfactant is polysorbate 20.
18. The formulation according to any one of claims 1 to 13 wherein the non-ionic surfactant is an alkyl ether of polyethylene glycol.
19. The formulation according to claim 18 wherein the alkyl ether of polyethylene glycol is selected from polyethylene glycol (2) dodecyl ether, polyethylene glycol (2) oleyl ether and polyethylene glycol (2) hexadecyl ether.
20. The formulation according to any one of claims 1 to 13 wherein the non-ionic surfactant is a block copolymer of polyethylene glycol and polypropylene glycol.
21. The formulation according to claim 20 wherein the block copolymer of polyethylene glycol and polypropylene glycol is poloxamer 188, poloxamer 407, poloxamer 171 or poloxamer 185.
22. The formulation according to any one of claims 1 to 13 wherein the non-ionic surfactant is an alkylphenyl ether of polyethylene glycol.
23. The formulation according to claim 22 wherein the alkylphenyl ether of polyethylene glycol is 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol.
24. The formulation according to any one of claims 1 to 23 wherein the non-ionic surfactant is present at a concentration of 1-1000 µg/ml.

25. The formulation according to any one of claims 1 to 24, further comprising a tonicity modifying agent.
26. The formulation according to claim 25 wherein the insulin compound is insulin lispro and the tonicity modifying agent is an uncharged tonicity modifying agent or wherein the insulin compound is insulin aspart at a concentration of > 500 U/ml and the tonicity modifying agent is an uncharged tonicity modifying agent.
27. The formulation according to claim 26, wherein the uncharged tonicity modifying agent is selected from the group consisting of trehalose, mannitol, glycerol and 1,2-propanediol.
28. The formulation according to claim 27, wherein the uncharged tonicity modifying agent is glycerol.
29. The formulation according to any one of claims 1, 2 and 6-28 wherein the insulin compound is insulin lispro and the ionic strength of the formulation taking account of ions in the formulation excluding the zinc binding species and the insulin compound is less than 40 mM or wherein the insulin compound is insulin aspart at a concentration of > 500 U/ml and the ionic strength of the formulation taking account of ions in the formulation excluding the zinc binding species and the insulin compound is less than 40 mM.
30. The formulation according to claim 25 wherein the insulin compound is insulin aspart at a concentration of 500 U/ml or less and the tonicity modifier is a charged tonicity modifier.
31. The formulation according to claim 30, wherein the charged tonicity modifier is sodium chloride.
32. The formulation according to any one of claims 1, 3 and 6-28 wherein the insulin compound is insulin aspart at a concentration of 500 U/ml or less and the ionic strength of the formulation taking account of ions in the formulation excluding the zinc binding species and the insulin compound is more than 50 mM.
33. The formulation according to any one of claims 1 to 32, wherein the formulation is substantially isotonic.

34. The formulation according to any one of claims 1 to 33, wherein the pH is in the range 5.5 to 9.0.
35. The formulation according to any of claims 1 to 34, further comprising a preservative.
- 5 36. The formulation according to claim 35, wherein the preservative is selected from the group consisting of phenol, m-cresol, chlorocresol, benzyl alcohol, propylparaben, methylparaben, benzalkonium chloride and benzethonium chloride.
37. The formulation according to any one of claims 1 to 36, further comprising
10 nicotinamide.
38. The formulation according to any one of claims 1 to 36, further comprising nicotinic acid or a salt thereof.
39. The formulation according to any one of claims 1 to 37 for use in the treatment of a subject suffering from diabetes mellitus.
- 15 40. A method of treatment of diabetes mellitus which comprises administering to a subject in need thereof an effective amount of a formulation according to any one of claims 1 to 38.
41. A container containing one dose or a plurality of doses of the formulation according to any one of claims 1 to 38.
- 20 42. An injection device for single or multiple use comprising a container containing one dose or a plurality of doses of the formulation according to any one of claims 1 to 38 together with an injection needle.
43. A medical device comprising a reservoir comprising plurality of doses of the formulation according to any one of claims 1 to 38 and a pump adapted for
25 automatic or remote operation such that upon automatic or remote operation one or more doses of the formulation is administered to the body.
44. A dry solid pharmaceutical composition suitable for reconstitution with an aqueous medium which comprises (i) an insulin compound other than insulin glargine, (ii) ionic zinc, (iii) a zinc binding species selected from DETA and
30 TETA, and (iv) a non-ionic surfactant.

45. A method of preparing a formulation according to any one of claims 1 to 38 which comprises dissolving a dry solid pharmaceutical composition according to claim 44 in an aqueous medium.
46. A method of improving the storage stability of an aqueous liquid pharmaceutical formulation comprising (i) an insulin compound other than insulin glargine, (ii) ionic zinc and (iii) a zinc binding species selected from DETA and TETA; which comprises adding a non-ionic surfactant to the formulation.
47. Use of a non-ionic surfactant to improve the storage stability of an aqueous liquid pharmaceutical formulation comprising (i) an insulin compound other than insulin glargine, (ii) ionic zinc and (iii) a zinc binding species selected from DETA and TETA.

Fig. 1



INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2018/051179

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K33/30 A61K38/28 A61K47/12 A61K47/18 A61K47/26
A61K9/08 A61P3/10

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 A61P

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, BIOSIS, CHEM ABS Data, EMBASE, 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,P	WO 2018/060736 A1 (ARECOR LTD [GB]) 5 April 2018 (2018-04-05) figures G-J the whole document	1-47
Y,P	WO 2017/191464 A1 (ARECOR LTD [GB]) 9 November 2017 (2017-11-09) examples 1a-1c claims 1-38 page 7, line 17 - page 8, line 7 ----- -/--	1-47



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"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

4 July 2018

Date of mailing of the international search report

23/07/2018

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Collura, Alessandra

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2018/051179

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2015/120457 A1 (BIODEL INC [US]) 13 August 2015 (2015-08-13) cited in the application tables 8, 6 page 3, lines 19-21 page 6, lines 1-5 page 12 claims -----	1-47
Y	WO 2012/006283 A1 (BIODEL INC [US]; POHL RODERIKE [US]; STEINER SOLOMON S [US]; HAUSER RO) 12 January 2012 (2012-01-12) cited in the application claims page 4, lines 13-22 page 8, lines 9-13 -----	1-47
Y	COOPER GARTH J S: "Therapeutic potential of copper chelation with triethylenetetramine in managing diabetes mellitus and Alzheimer's disease", MEDLINE, US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US, 9 July 2011 (2011-07-09), XP002774649, abstract -----	1-47
Y	HADI MAHMOUDI MOGHADDAM ET AL: "Evaluation of Insulin Stability in the Presence of Nonionic Surface Active Agents (Polysorbate Groups) by Circular Dichroism and Fluorescence Spectroscopy", ASIAN JOURNAL OF BIOCHEMISTRY, vol. 10, no. 1, 1 January 2015 (2015-01-01), pages 17-30, XP055384029, ISSN: 1815-9923, DOI: 10.3923/ajb.2015.17.30 abstract -----	1-47
Y	LOUGHEED W D ET AL: "PHYSICAL STABILITY OF INSULIN FORMULATIONS", DIABETES, AMERICAN DIABETES ASSOCIATION, US, vol. 32, no. 5, 1 May 1983 (1983-05-01), pages 424-432, XP001096187, ISSN: 0012-1797, DOI: 10.2337/DIABETES.32.5.424 abstract ----- -/--	1-47

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2018/051179

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RODERIKE POHL ET AL: "Ultra-Rapid Absorption of Recombinant Human Insulin Induced by Zinc Chelation and Surface Charge Masking Author Affiliations", JOURNAL OF DIABETES SCIENCE AND TECHNOLOGY, vol. 6, no. 4, 4 July 2012 (2012-07-04), pages 755-763, XP055206316, the whole document -----	1-47

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2018/051179

Patent document cited in search report			Publication date	Patent family member(s)			Publication date
WO 2018060736	A1	05-04-2018	WO	2018060735	A1	05-04-2018	
			WO	2018060736	A1	05-04-2018	

WO 2017191464	A1	09-11-2017	US	2018078645	A1	22-03-2018	
			WO	2017191464	A1	09-11-2017	

WO 2015120457	A1	13-08-2015	US	2015273022	A1	01-10-2015	
			WO	2015120457	A1	13-08-2015	

WO 2012006283	A1	12-01-2012	CA	2805031	A1	12-01-2012	
			EP	2590667	A1	15-05-2013	
			US	2012178675	A1	12-07-2012	
			WO	2012006283	A1	12-01-2012	
