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(54) Title: BIPHASIC PHARMACEUTICAL COMPOSITION OF INSULIN HUMAN

(57) Abstract: There is provided an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin, in-
sulin analogues or derivative thereof, wherein said composition comprises (i) a solution of insulin, insulin analogue or derivative
thereof, and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8µm and
20µm and optionally, (iii) one or more pharmaceutically acceptable excipients.



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BIPHASIC PHARMACEUTICAL COMPOSITION OF INSULIN HUMAN
PRIORITY DOCUMENTS

This application claims priority from the Indian provisional patent application No. IN 201621009801 filed on Mar 21, 2016.

TECHNICAL FIELD OF THE INVENTION

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The present invention provides an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin, insulin analogues or derivative thereof, wherein said composition comprises (i) solution of insulin, insulin analogue or derivative thereof, and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8 μ m and 20 μ m and optionally, (iii) one or more pharmaceutically acceptable excipients.

15

BACKGROUND OF THE INVENTION

Diabetes mellitus is a metabolic disorder in which the ability to utilize glucose is more or less completely lost. It is a major chronic illness found in humans with many consequences. Some complications arising from long-standing diabetes are blindness, kidney failure and limb amputations.

20

Insulin-dependent diabetes mellitus (IDDM) accounts for 10 to 15% of all cases of diabetes mellitus.

NPH insulin is the most widely-used insulin preparation, constituting from 50 to 70 percent of the insulin used worldwide. It is a suspension of a microcrystalline complex of insulin, zinc, protamine and one or more phenolic preservatives. NPH insulin preparations are commercially available incorporating human insulin, pork insulin, beef insulin, or mixtures thereof. Also, NPH-like preparations of a monomeric insulin analogue, LysB298, ProB29-human insulin analogue, are known in the art. Therapy using currently-available NPH insulin preparations fails to provide the ideal "flat" pharmacokinetics necessary to maintain optimal fasting blood glucose for an extended period of time between meals. Consequently, treatment with NPH insulin can result in undesirably high levels of insulin in the blood, which may cause life-threatening hypoglycemia.

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In absence of an ideal flat pharmacokinetics profile, the duration of action of NPH insulin is also not ideal. In particular, a major problem with NPH therapy is the "dawn phenomenon" which is hyperglycemia that results from loss of effective glucose control overnight while the patient is sleeping. These deficiencies in glycemic control contribute to serious long-term medical complications of diabetes and impose considerable inconvenience and reduction in quality-of-life of the patient.

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PCT publication No. WO2003053460 discloses a pharmaceutical composition comprising crystals of insulin and protamine for control of blood glucose. PCT publication No. WO1999/048520 discloses

5 an injectable, protracted acting human insulin preparation. PCT publication No. WO1988/002633 discloses protamine zinc insulin preparation. PCT publication No. WO 2011/156476 discloses a basal insulin formulation comprising a solution or suspension of recombinant human insulin at a pH of between 3.5 and 4.5. US Patent No. 5,461,031 discloses various parenteral pharmaceutical formulations, which comprise: a monomeric insulin analog, zinc, protamine, and phenolic derivative.
10 US Patent No. 5,547,930 discloses crystals of human insulin with protamine. US Patent No. 8,263,551 discloses a pharmaceutical formulation which is a solution comprising an insulin, an insulin analog or an insulin derivative or a mixture thereof and a protamine salt.

The medicinal product Huminsulin 30/70 100IU/mL marketed by Eli Lilly is a biphasic isophane insulin injection containing 30% soluble insulin and 70% isophane insulin. The medicinal product
15 Mixtard marketed by Novo Nordisk is a dual-acting, biphasic formulation consisting of a premix of soluble fast-acting insulin human and isophane long-acting insulin.

A considerable number of patients, in particular those with increased insulin resistance due to obesity and other physiological disorder, require higher doses of insulin to lower glucose levels. The marketed formulation Huminsulin 30/70 contains 100IU of human insulin in 1ml, which confer some
20 discomfort as the patient need to administer several injections of insulin each day. Although this treatment regimen is accepted as effective, it has limitations. The repeated injection cause painful administration of insulin which leads to inconvenience to patients. As a result, patients don't comply adequately with the prescribed treatment regimens and are often improperly medicated.

So it is an object of present invention to develop a pharmaceutical composition of insulin human, which reduces the number of injection that a patient needs to administer. It is also an object of the present invention to provide specific concentrated insulin formulations for treating insulin resistant diabetic patients which modulate the pharmacokinetics and pharmacodynamics of injectable insulin compositions by increasing the rate of absorption from the site of subcutaneous injection. Further the formulation allows patients to inject the same number of units of human insulin at half the volume of
30 injection.

SUMMARY OF THE INVENTION

In one aspect of the invention, there is provided an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin, insulin analogues or derivative thereof, wherein said composition comprises: (i) a solution of insulin, insulin analogue or derivative thereof, and (ii)
35 crystals of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8µm and 20µm and optionally, (iii) one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml insulin composition comprising (i) a solution of insulin, insulin analogue or derivative thereof and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine,

5 when administered to a subject at the same dose. In an embodiment of this aspect, the pharmaceutical composition has a pH in the range of 6.0 and 8.5. In another embodiment of this aspect, the solution of insulin, insulin analogue or derivative thereof and the crystals of insulin, insulin analogue or derivative thereof with protamine are present in a volume proportion ranging from about 99:1 to 1:99. In another embodiment of this aspect, the solution of insulin, insulin analogue or derivative thereof
10 and the crystals of insulin, insulin analogue or derivative thereof with protamine are present in the volume proportion of about 30:70. In another embodiment of this aspect, the insulin, insulin analogue or derivative thereof are selected from the group comprising insulin human, insulin isophane (NPH), insulin lispro, insulin glulisine, insulin aspart, Gly(A21) human insulin, Gly(A21) Lys(B28) human insulin, Gly(A21) Lys(B28) Pro(B29) human insulin, Gly(A21) Asp(B28). In another embodiment of
15 this aspect, the insulin, insulin analogues or derivative thereof is insulin human. In another embodiment of this aspect, the one or more pharmaceutically acceptable excipients comprise isotonic agent, complexing agent, buffer, preservative, pH modifying agent, stabilizing agent or mixture thereof. In another embodiment of this aspect, the pharmaceutical composition is administered through subcutaneous, intravenous or intramuscular route to a subject. In another embodiment of this
20 aspect, the pharmaceutical composition is for use in the treatment of type I and type II diabetes mellitus.

In another aspect of the invention, there is provided an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin human, wherein said pharmaceutical composition
25 comprises: (i) a solution of insulin human; (ii) crystals of insulin human with protamine having particle size in range of 8 μ m and 15 μ m; and (iii) pharmaceutically acceptable excipient comprising m-cresol, phenol, glycerol and zinc or salts thereof; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml insulin composition comprising (i) a solution of insulin human and (ii) crystals of insulin human with
30 protamine, when administered to a subject at the same dose.

In another aspect of the invention, there is provided a process of preparing an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin, insulin analogues or derivative thereof, wherein said pharmaceutical composition comprises: (i) a solution of insulin, insulin
35 analogue or derivative thereof and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8 μ m and 20 μ m and optionally, (iii) one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml insulin composition comprising (i) a solution of insulin, insulin analogue or derivative thereof and (ii) crystals of insulin,
40 insulin analogue or derivative thereof with protamine, when administered to a subject at the same dose; wherein said process comprises steps of:

- 5 a. preparing a suspension of protamine and insulin by dissolving the insulin, insulin analogue or derivative thereof, protamine, one or more pharmaceutically acceptable excipients and adjusting a pH of the suspension in a range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH;
- 10 b. preparing the solution of insulin by dissolving insulin, insulin analogue or derivative thereof, one or more pharmaceutically acceptable excipients and adjusting the pH of the solution in the range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH; and
- c. mixing about 70% suspension of step (a) with the about 30% solution of step (b) to obtain an aqueous injectable biphasic pharmaceutical composition.
- 15 In another aspect of the invention, there is provided a process of preparing an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin human, wherein said pharmaceutical composition comprises: (i) a solution of insulin human; (ii) crystals of insulin human with protamine having particle size in range of 8 μ m and 15 μ m; and (iii) pharmaceutically acceptable excipient comprising m-cresol, phenol, glycerol and zinc or salts thereof, wherein said pharmaceutical
- 20 composition provides similar onset of action and longer duration of action as compared to 100IU/ml insulin composition comprising (i) a solution of insulin human and (ii) crystals of insulin human with protamine, when administered to a subject at the same dose; wherein said process comprises steps of:
- a. preparing a suspension of protamine and insulin human by dissolving insulin human, protamine, zinc or salts thereof, meta-cresol, phenol in water for injection and
- 25 adjusting a pH of the suspension in a range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH;
- b. preparing the solution of insulin human by dissolving insulin human, phenol, m-cresol, glycerol and disodium hydrogen phosphate in water for injection and adjusting the pH of solution in the range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH; and
- 30 c. mixing about 70% of suspension of step (a) with 30% of solution of step (b) to obtain the aqueous injectable biphasic pharmaceutical composition.

In another aspect of the invention, there is provided an aqueous injectable pharmaceutical composition in the form of a solution, wherein the pharmaceutical composition comprises 200IU/ml

35 of insulin, insulin analogues or derivative thereof and one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml composition of insulin, insulin analogues or derivative thereof and one or more pharmaceutically acceptable excipients, when administered to a subject at the same dose. In one embodiment of this aspect, the pharmaceutical composition has a pH in the range of

40 6.0 and 8.5. In another embodiment of this aspect, the insulin, insulin analogue or derivative thereof are selected from the group comprising insulin human, insulin isophane (NPH), insulin lispro, insulin

5 glulisine, insulin aspart, Gly(A21) human insulin, Gly(A21) Lys(B28) human insulin, Gly(A21) Lys(B28) Pro(B29) human insulin, Gly(A21) Asp(B28). In another embodiment of this aspect, the insulin, insulin analogues or derivative thereof is insulin human. In another embodiment of this aspect, the one or more pharmaceutically acceptable excipients comprise isotonic agent, complexing agent, buffer, preservative, pH modifying agent, stabilizing agent or mixture thereof. In another
10 embodiment of this aspect, the pharmaceutical composition is administered through subcutaneous, intravenous or intramuscular route to a subject. In another embodiment of this aspect, the pharmaceutical composition is for use in the treatment of type I and type II diabetes mellitus.

In another aspect of the invention, there is provided an aqueous injectable pharmaceutical
15 composition in the form of a suspension, wherein the pharmaceutical composition comprises 200IU/ml of crystals of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8 μ m and 20 μ m and one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml composition of crystals of insulin, insulin analogue or derivative thereof with
20 protamine and one or more pharmaceutically acceptable excipients, when administered to a subject at the same dose. In another embodiment of this aspect, the pharmaceutical composition has a pH in the range of 6.0 and 8.5. In another embodiment of this aspect, the insulin, insulin analogue or derivative thereof are selected from the group comprising insulin human, insulin isophane (NPH), insulin lispro, insulin glulisine, insulin aspart, Gly(A21) human insulin, Gly(A21) Lys(B28) human insulin,
25 Gly(A21) Lys(B28) Pro(B29) human insulin, Gly(A21) Asp(B28). In another embodiment of this aspect, the insulin, insulin analogues or derivative thereof is insulin human. In another embodiment of this aspect, the one or more pharmaceutically acceptable excipients comprise isotonic agent, complexing agent, buffer, preservative, pH modifying agent, stabilizing agent or mixture thereof. In another embodiment of this aspect, the pharmaceutical composition is administered through
30 subcutaneous, intravenous or intramuscular route to a subject. In another embodiment of this aspect, the pharmaceutical composition is for use in the treatment of type I and type II diabetes mellitus.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is the line graph showing blood glucose levels (mg/dL) after administration of conventional
35 100 IU/mL product (Huminsulin 30/70) and Example 1 composition (200 IU/mL) to beagle dogs.

DETAILED DESCRIPTION OF THE INVENTION

In general aspect of the invention, there is provided an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin, insulin analogues or derivative thereof, wherein said

- 5 composition comprises solution of insulin, insulin analogue or derivative thereof and crystals of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8µm and 20µm and optionally, (iii) one or more pharmaceutically acceptable excipients.

The term “insulin” used herein includes mammalian insulin, human insulin, insulin analogues or derivatives.

- 10 The term “insulin analogue” or “derivative” includes insulin human, insulin isophane (NPH), insulin lispro, insulin glulisine, insulin aspart, Gly(A21) human insulin, Gly(A21) Lys(B28) human insulin, Gly(A21) Lys(B28) Pro(B29) human insulin, Gly(A21) Asp(B28).

- The term “biphasic” herein relates to a composition of insulin, insulin analogue or derivative thereof
15 wherein the composition is a mixture of solution of insulin, insulin analogue or derivative thereof and suspension of crystals of insulin, insulin analogue or derivative thereof.

“Pharmaceutical composition” refers to the combination of one or more active ingredients and one or more excipients.

- 20 The term “pharmaceutically acceptable excipients” herein relates to non-active pharmaceutical ingredients which are within the scope of sound medical judgment suitable for use in pharmaceutical products.

The term “onset of action” refers to the duration of time it takes for a drug's effects to come to prominence upon administration.

- 25 The term “duration of action” refers to the length of time of a drug for which the drug remains effective.

The term “dose” refers to the quantity of drug to be administered at one time, as a specified amount of medication.

- 30 The term “subject” refers to living mammals which includes human or animal.

- In one embodiment of the invention, there is provided an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin, insulin analogues or derivative thereof, wherein said composition comprises: (i) a solution of insulin, insulin analogue or derivative thereof and (ii) crystals
35 of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8µm and 20µm and optionally, (iii) one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as

5 compared to 100IU/ml insulin composition comprising (i) a solution of insulin, insulin analogue or derivative thereof and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine, when administered to a subject at the same dose.

10 In another embodiment of the invention, there is provided an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin, insulin analogues or derivative thereof, wherein said composition comprises: (i) a solution of insulin, insulin analogue or derivative thereof and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8 μ m and 20 μ m and optionally, (iii) one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of
15 action as compared to 100IU/ml insulin composition comprising (i) a solution of insulin, insulin analogue or derivative thereof and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine, when administered to a subject at the same dose, and wherein the pharmaceutical composition has a pH in the range of 6.0 and 8.5, and wherein the solution of insulin, insulin analogue or derivative thereof and the crystals of insulin, insulin analogue or derivative thereof with protamine
20 are present in a volume proportion ranging from about 99:1 to 1:99 or present in a volume proportion of about 30:70, and wherein the insulin, insulin analogue or derivative thereof are selected from the group comprising insulin human, insulin isophane (NPH), insulin lispro, insulin glulisine, insulin aspart, Gly(A21) human insulin, Gly(A21) Lys(B28) human insulin, Gly(A21) Lys(B28) Pro(B29) human insulin, Gly(A21) Asp(B28), and wherein the insulin, insulin analogues or derivative thereof is
25 insulin human, and wherein the one or more pharmaceutically acceptable excipients comprise isotonic agent, complexing agent, buffer, preservative, pH modifying agent, stabilizing agent or mixture thereof, and wherein the pharmaceutical composition is administered through subcutaneous, intravenous or intramuscular route to a subject, and wherein the pharmaceutical composition is for use in the treatment of type I and type II diabetes mellitus.

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In another embodiment of the invention, there is provided an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin, insulin analogues or derivative thereof, wherein said composition comprises: (i) a solution of insulin, insulin analogue or derivative thereof and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine having particle size
35 in range of 8 μ m and 15 μ m and optionally, (iii) one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml insulin composition comprising (i) a solution of insulin, insulin analogue or derivative thereof and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine, when administered to a subject at the same dose, and wherein the pharmaceutical
40 composition has a pH in the range of 6.5 and 8 or 7 and 7.5, and wherein the solution of insulin, insulin analogue or derivative thereof and the crystals of insulin, insulin analogue or derivative thereof

5 with protamine are present in a volume proportion ranging from about 99:1 to 1:99 or present in a volume proportion of about 90:10 or about 80:20 or about 70:30 or about 60:40 or about 50:50 or about 40:60 or about 30:70 or about 20:80 or about 10:90, and wherein the insulin, insulin analogues or derivative thereof is insulin human, and wherein the one or more pharmaceutically acceptable excipients comprise isotonic agent, complexing agent, buffer, preservative, pH modifying agent, 10 stabilizing agent or mixture thereof, and wherein the pharmaceutical composition is administered through subcutaneous, intravenous or intramuscular route to a subject, and wherein the pharmaceutical composition is for use in the treatment of type I and type II diabetes mellitus.

15 In another embodiment of the present invention provides a pharmaceutical composition, wherein the pharmaceutical composition may include one or more of the following features. For example, one or more pharmaceutically acceptable excipients comprising isotonic agent, complexing agents, buffer, zinc or salt thereof, preservatives, pH modifying agents, stabilizing agents and combination thereof.

20 An "isotonic agent" is a compound, such as glycerine, are commonly used for such purposes at known concentrations. Other possible isotonicity agents are selected from the group comprising glycerol, mannitol, sorbitol, lactose, dextrose, trehalose, sodium chloride, propylene glycol, dimethyl sulfone or mixture thereof.

25 A "complexing agents" as used herein include but not limited to one or more of ethylenediamine tetra-acetic acid or salts thereof, ethylene glycol tetra-acetic acid or salts thereof, protamine or salts thereof or mixture thereof. The protamine is added in the form of protamine salts, wherein the protamine salt is selected from the group comprising protamine sulphate, acetate, ascorbate, benzoate, citrate, formate, fumarate, glycolate and mesylate.

30 The "preservatives" as used herein include, but are not limited to, benzoic acid, butylparaben, ethyl paraben, methyl paraben, propylparaben, sodium benzoate, sodium propionate, benzalkonium chloride, benzethonium chloride, benzyl alcohol, cetylpyridinium chloride, chlorobutanol, phenol, phenylethyl alcohol, 2-Penoxyethanol, Phenyl mercuric nitrate, Thimerosal, meta-cresol or combinations thereof. In another embodiment of present invention, preservatives are selected from group comprising m-cresol and phenol. The m-cresol is present in the range of 1.20mg/ml to 35 2.10mg/ml. Further, the phenol is present in the range of 0.50mg/ml to 0.85mg/ml.

An "buffer" as used herein include, but are not limited to, phosphate, acetate, citrate, arginine, disodium hydrogen o-phosphate anhydrous, glycylglycine or TRIS (i.e. 2-amino-2-hydroxymethyl-1,3-propanediol) buffer and corresponding salts. The buffer is present in amount of 50mM to 600mM.

5 The zinc is added in the form of zinc salts as stabilizer, wherein the zinc salt is selected from the group comprising zinc acetate, zinc bromide, zinc chloride, zinc fluoride, zinc iodide, zinc oxide and zinc sulphate. Zinc is added in the form of zinc chloride and is present in the range of 0.040mg/ml to 0.08mg/ml.

10 The pH modifying agents as used herein refers to a combination of acid and alkali. The pH modifying agents can be selected from the group comprising of hydrochloric acid, o-phosphoric acid, citric acid, acetic acid, succinic acid, lactic acid, gluconic acid, tartaric acid, 1,2,3,4-butane tetracarboxylic acid, fumaric acid or malic acid. Alkali is selected from the group comprising of sodium hydroxide, potassium hydroxide, sodium hydroxide, ammonium hydroxide, magnesium oxide, calcium
15 hydroxide, calcium carbonate, magnesium carbonate, magnesium aluminum silicates, diethanolamine, monoethanolamine, sodium carbonate, sodium bicarbonate or triethanolamine and combination thereof.

In another embodiment of the invention, there is provided an aqueous injectable biphasic
20 pharmaceutical composition comprising 200IU/ml of insulin human, wherein said pharmaceutical composition comprises: (i) a solution of insulin human, (ii) crystals of insulin human with protamine having particle size in range of 8 μ m and 15 μ m and (iii) pharmaceutically acceptable excipient comprising m-cresol, phenol, glycerol and zinc or salts thereof; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml
25 insulin composition comprising (i) a solution of insulin human and (ii) crystals of insulin human with protamine, when administered to a subject at the same dose.

In another embodiment of the invention, there is provided a process of preparing an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin, insulin analogues or
30 derivative thereof, wherein said pharmaceutical composition comprises: (i) a solution of insulin, insulin analogue or derivative thereof and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8 μ m and 20 μ m and optionally, (iii) one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml insulin composition
35 comprising (i) a solution of insulin, insulin analogue or derivative thereof and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine, when administered to a subject at the same dose; wherein said process comprises steps of:

a. preparing a suspension of protamine and insulin by dissolving the insulin, insulin analogue or derivative thereof, protamine, one or more pharmaceutically acceptable excipients and
40 adjusting a pH of the suspension in a range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH;

- 5 b. preparing the solution of insulin by dissolving insulin, insulin analogue or derivative thereof, one or more pharmaceutically acceptable excipients and adjusting the pH of the solution in the range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH; and
- c. mixing about 70% suspension of step (a) with the about 30% solution of step (b) to obtain an aqueous injectable biphasic pharmaceutical composition.

10 In another embodiment of the invention, there is provided a process of preparing an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin human, wherein said pharmaceutical composition comprises: (i) a solution of insulin human; (ii) crystals of insulin human with protamine having particle size in range of 8 μ m and 15 μ m and (iii) pharmaceutically acceptable

15 excipient comprising m-cresol, phenol, glycerol and zinc or salts thereof, wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml insulin composition comprising (i) a solution of insulin human and (ii) crystals of insulin human with protamine, when administered to a subject at the same dose; wherein said process comprises steps of:

- a. preparing a suspension of protamine and insulin human by dissolving insulin human, protamine, zinc or salts thereof, meta-cresol, phenol in water for injection and adjusting a pH
- 20 of the suspension in a range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH;
- b. preparing the solution of insulin human by dissolving insulin human, phenol, m-cresol, glycerol and disodium hydrogen phosphate in water for injection and adjusting the pH of solution in the range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH; and
- 25 c. mixing about 70% of suspension of step (a) with 30% of solution of step (b) to obtain the aqueous injectable biphasic pharmaceutical composition.

 In another embodiment of the invention, there is provided an aqueous injectable pharmaceutical composition in the form of a solution, wherein the pharmaceutical composition comprises 200IU/ml

30 of insulin, insulin analogues or derivative thereof and one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml composition of insulin, insulin analogues or derivative thereof and one or more pharmaceutically acceptable excipients, when administered to a subject at the same dose.

 In another embodiment of the invention, there is provided an aqueous injectable pharmaceutical composition in the form of a solution, wherein the pharmaceutical composition comprises 200IU/ml

 of insulin, insulin analogues or derivative thereof and one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer

40 duration of action as compared to 100IU/ml composition of insulin, insulin analogues or derivative thereof and one or more pharmaceutically acceptable excipients, when administered to a subject at the

5 same dose, and wherein the pharmaceutical composition has a pH in the range of 6.0 and 8.5, and wherein the insulin, insulin analogue or derivative thereof are selected from the group comprising insulin human, insulin isophane (NPH), insulin lispro, insulin glulisine, insulin aspart, Gly(A21) human insulin, Gly(A21) Lys(B28) human insulin, Gly(A21) Lys(B28) Pro(B29) human insulin, Gly(A21) Asp(B28), and wherein the insulin, insulin analogues or derivative thereof is insulin
10 human, and wherein the one or more pharmaceutically acceptable excipients comprise isotonic agent, complexing agent, buffer, preservative, pH modifying agent, stabilizing agent or mixture thereof, and wherein the pharmaceutical composition is administered through subcutaneous, intravenous or intramuscular route to a subject, and wherein the pharmaceutical composition is for use in the treatment of type I and type II diabetes mellitus.

15 In another embodiment of the invention, there is provided an aqueous injectable pharmaceutical composition in the form of a solution, wherein the pharmaceutical composition comprises 200IU/ml of insulin, insulin analogues or derivative thereof and one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer
20 duration of action as compared to 100IU/ml composition of insulin, insulin analogues or derivative thereof and one or more pharmaceutically acceptable excipients, when administered to a subject at the same dose, and wherein the pharmaceutical composition has a pH in the range of 6.0 and 8.5, or 6.5 and 8, or 7 and 7.5, and wherein the insulin, insulin analogues or derivative thereof is insulin human, and wherein the one or more pharmaceutically acceptable excipients comprise isotonic agent,
25 complexing agent, buffer, preservative, pH modifying agent, stabilizing agent or mixture thereof, and wherein the pharmaceutical composition is administered through subcutaneous, intravenous or intramuscular route to a subject, and wherein the pharmaceutical composition is for use in the treatment of type I and type II diabetes mellitus.

30 In another embodiment of the invention, there is provided an aqueous injectable pharmaceutical composition in the form of a suspension, wherein the pharmaceutical composition comprises 200IU/ml of crystals of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8 μ m and 20 μ m and one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as
35 compared to 100IU/ml composition of crystals of insulin, insulin analogue or derivative thereof with protamine and one or more pharmaceutically acceptable excipients, when administered to a subject at the same dose.

40 In another embodiment of the invention, there is provided an aqueous injectable pharmaceutical composition in the form of a suspension, wherein the pharmaceutical composition comprises 200IU/ml of crystals of insulin, insulin analogue or derivative thereof with protamine having particle

- 5 size in range of 8 μ m and 20 μ m and one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml composition of crystals of insulin, insulin analogue or derivative thereof with protamine and one or more pharmaceutically acceptable excipients, when administered to a subject at the same dose, and wherein the pharmaceutical composition has a pH in the range of 6.0 and 8.5, and
- 10 wherein the insulin, insulin analogue or derivative thereof are selected from the group comprising insulin human, insulin isophane (NPH), insulin lispro, insulin glulisine, insulin aspart, Gly(A21) human insulin, Gly(A21) Lys(B28) human insulin, Gly(A21) Lys(B28) Pro(B29) human insulin, Gly(A21) Asp(B28), and wherein the insulin, insulin analogues or derivative thereof is insulin human, and wherein the one or more pharmaceutically acceptable excipients comprise isotonic agent,
- 15 complexing agent, buffer, preservative, pH modifying agent, stabilizing agent or mixture thereof, and wherein the pharmaceutical composition is administered through subcutaneous, intravenous or intramuscular route to a subject, and wherein the pharmaceutical composition is for use in the treatment of type I and type II diabetes mellitus.
- 20 In another embodiment of the invention, there is provided an aqueous injectable pharmaceutical composition in the form of a suspension, wherein the pharmaceutical composition comprises 200IU/ml of crystals of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8 μ m and 15 μ m and one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as
- 25 compared to 100IU/ml composition of crystals of insulin, insulin analogue or derivative thereof with protamine and one or more pharmaceutically acceptable excipients, when administered to a subject at the same dose, and wherein the pharmaceutical composition has a pH in the range of 6.0 and 8.5 or 6.5 and 8 or 7 and 7.5, and wherein the insulin, insulin analogues or derivative thereof is insulin human, and wherein the one or more pharmaceutically acceptable excipients comprise isotonic agent,
- 30 complexing agent, buffer, preservative, pH modifying agent, stabilizing agent or mixture thereof, and wherein the pharmaceutical composition is administered through subcutaneous, intravenous or intramuscular route to a subject, and wherein the pharmaceutical composition is for use in the treatment of type I and type II diabetes mellitus.

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EXAMPLES

EXAMPLE 1: An aqueous injectable biphasic pharmaceutical composition containing 200 IU/mL insulin human.

Table 1:

Sr. No.	Ingredients	Quantity/mL
1	Insulin Human (r-DNA)	200.0 IU
2	<i>m</i> -Cresol	1.60 mg
3	Phenol	0.65 mg
4	Glycerol, 98%	16.32 mg
5	Zinc as Zinc Oxide	0.050 mg
6	Sodium Hydroxide	q.s. to pH
7	Hydrochloric Acid	q.s.to pH
8	Disodium Hydrogen o-Phosphate Anhydrous	2.08 mg
9	Protamine Sulphate	0.518 mg
10	Water for Injection	q.s.to 1.0 mL

10 Manufacturing Procedure:

Step 1: Preparing of protamine insulin human suspension

Half of the quantity of *m*-cresol, phenol, glycerol and dihydrogen phosphate were dissolved in water for injection and pH of the mixture was adjusted in between 7.6 to 7.9 by 1N HCl or 1N NaOH. Further remaining quantity of insulin human, zinc, *m*-cresol, phenol and glycerol were dissolved with protamine sulphate in water for injection and the pH of solution was adjusted to 3.2±0.2 by 1N HCl or 1N NaOH. Both the mixture were mixed in 1:1 ratio to get final insulin human protamine suspension and the pH of final mixture was adjusted to 7.2 to 7.45 by 1N HCl or 1N NaOH. Particle size of crystals of insulin, insulin analogue or derivative thereof with protamine was determined using Malvern morphologi G3 particle characterisation system instrument and particle size was found to be in range of 8 to 12 µm.

Step 2: Preparation of insulin human solution

Human insulin was suspended in water for injection and dissolved by small portion of 1N HCl. Further phenol, *m*-cresol, disodium hydrogen phosphate and glycerol were dissolved in small portion of water for injection. Both the solutions were mixed and the pH was adjusted to 7.3.

25 Step 3: Mixing of protamine insulin human suspension and insulin human solution

The 70% by volume of protamine insulin human suspension obtained in step 1 was mixed with the 30% by volume of insulin human solution obtained in step 2 to get the final composition and pH of the final composition was adjusted to 7.2 to 7.45 by 1N HCl or 1N NaOH.

5 EXAMPLE 2: Comparison of hypoglycemic effect of EXAMPLE 1 composition with commercial huminsulin 30/70 (100IU/ml) composition.

Male beagle dogs weighing 7 kg to 16 kg were received from the Dog House Facility, Wockhardt Research Centre, Aurangabad. Dogs were housed under controlled kennel condition with a 12h light and 12h dark cycle and given access to food once in day with water ad-libitum. Selected animals were
 10 fasted for 14 hours before the study starts and throughout the experiment with free access to tap water. Following fasting, the blood was withdrawn from forearm vein (Vena cephalica) before drug administration and whole blood glucose was measured using glucometer (ACCU-CHEK Active, Roche) which is considered as basal blood glucose levels. Dogs were randomized into two treatment arms (n = 6) with comparable average fasting blood glucose levels. At time point 0, animals were
 15 treated with single subcutaneous (s.c.) injection of the example 1 composition and huminsulin 30/70 composition at the dose of 0.35 IU/kg body weight in a loose skin over neck region. Blood glucose levels were measured at 0.25, 0.5, 1, 2, 3, 4, 5 and 6h post-dose.

It is evident from Figure 1, that composition of Example 1 showed similar onset and longer duration of action as compared with commercial huminsulin 30/70 (100IU/mL) composition marketed by Eli
 20 Lilly. It is evident from table 2, that the AUC for example 1 composition is 117 and AUC for commercial huminsulin 30/70 (100IU/ml) composition is 75. Also the blood glucose level of example 1 composition at 4hr, 5hr and 6hr is 50, 55 and 60 mg/dL respectively and blood glucose level of commercial huminsulin 30/70 (100IU/ml) composition at 4hr, 5hr and 6hr is 70, 75 and 80 mg/dL respectively. This data confirms that the better glucose lowering effect of composition of example 1
 25 as compared to commercial huminsulin 30/70 (100IU/ml) composition.

Table 2:

Parameter	AUC ^{0-6h}	Blood Glucose level mg/dL		
		at 4Hr	at 5Hr	at 6Hr
Example 1 Composition (200 IU/mL)	117	50	55	60
Commercial huminsulin 30/70 (100IU/mL) composition.	75	70	75	80

EXAMPLE 3: Comparison of particle size of example 1 composition with commercial huminsulin 30/70 (100IU/ml) composition.

30

The pharmaceutical composition of present invention containing 200IU/mL of insulin human was compared with the commercial huminsulin 30/70 (100IU/mL) composition for particle size determination using Malvern morphologi G3 particle characterisation system instrument. Results of the comparison study are reproduced below in table 3.

35

5 Table 3:

Sr. No.	D90 (Diameter of 90% particles) value of crystals of insulin human with protamine in μm .	
	Commercial huminsulin 30/70 (100IU/mL) composition	Example 1 Composition (200 IU/mL)
1	6.7	11.5
2	6.3	9.4
3	5.9	8.1
4	6.5	9.7
5	6.1	10.09
6	5.8	8.9

It is evident from the table 3 that the pharmaceutical composition of example 1 of present invention (200IU/mL insulin human) has larger particle size (8-12 μm) of crystals of insulin human with protamine as compared to the commercial huminsulin 30/70 (100IU/mL) composition.

10

EXAMPLE 4: Pharmaceutical compositions containing 200 IU/mL insulin human

Table 4

	Composition	4A	4B
Sr No.	Ingredients	Quantity/mL for suspension	Quantity/mL for solution
1	Insulin Human (r-DNA)	200.0 IU	200.0 IU
2	<i>m</i> -Cresol	1.60 mg	2.50 mg
3	Phenol	0.65 mg	-
4	Citric Acid Monohydrate	-	0.0021 mg
5	Glycerol, 98%	16.32 mg	16.32 mg
6	Zinc as Zinc Oxide	0.050 mg	0.050 mg
7	Sodium Hydroxide	q.s.to pH	q.s to pH
8	Hydrochloric Acid	q.s.to pH	q.s.to pH
9	Disodium Hydrogen o-Phosphate Anhydrous	2.08 mg	-
10	Tri-Sodium Citrate Dihydrate	-	0.19 mg
11	Protamine Sulphate	0.74 mg	-
12	Water for Injection	q.s.to 1.0 mL	q.s to 1.0 mL

A. Manufacturing procedure for suspension composition 4A containing 200 IU/mL insulin human:

- 15 Half of the quantity of *m*-cresol, phenol, glycerol and dihydrogen phosphate were dissolved in water for injection and pH of the mixture was adjusted in between 7.6 to 7.9 by 1N HCl or 1N NaOH. Further remaining quantity of zinc, *m*-cresol, phenol and glycerol were dissolved with insulin human, Zinc Oxide and protamine sulphate in water for injection and the pH of solution was adjusted to 3.2 ± 0.2 by 1N HCl or 1N NaOH. Both the mixture were mixed in 1:1 ratio to get final insulin human
- 20 protamine suspension and the pH of final mixture was adjusted to 7.2 to 7.45 by 1N HCl or 1N

- 5 NaOH. This mixture was allowed to crystallize for 20-24h at room temperature without stirring. Particle size of crystals of insulin, insulin analogue or derivative thereof with protamine was determined using Malvern morphologi G3 particle characterisation system instrument and particle size was found to be in range of about 8 to 12 μ m.

B. Manufacturing procedure for solution composition 4B containing 200 IU/mL insulin human:

- 10 Insulin human and zinc oxide was suspended in water for injection and dissolved by small portion of 1N HCl. Further phenol, m-cresol, and glycerol were dissolved in small portion of water for injection. Both the solutions were mixed and the pH was adjusted to 2.9-3.3. In small portion of water for injection citric acid monohydrate and Tri-Sodium Citrate Dihydrate was dissolved and added to above solution, pH of the final composition was adjusted to 7.2 to 7.45 by 1N HCl or 1N NaOH.
- 15 EXAMPLE 5: An aqueous injectable biphasic pharmaceutical composition containing 200 IU/mL insulin human.

Table 5:

Sr. No.	Ingredients	Quantity/mL				
		5A	5B	5C	5D	5E
1	Insulin Human (r-DNA)	200.0 IU	200.0 IU	200.0 IU	200.0 IU	200.0 IU
2	<i>m</i> -Cresol	1.25mg	1.35 mg	1.45 mg	1.50 mg	1.75 mg
3	Phenol	0.50 mg	0.60mg	0.70 mg	0.75 mg	0.80 mg
4	Glycerol, 98%	12.55 mg	14.30mg	11.30 mg	15.22 mg	18.30 mg
5	Zinc as Zinc Oxide	0.040 mg	0.050 mg	0.060 mg	0.070 mg	0.080 mg
6	Sodium Hydroxide	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH
7	Hydrochloric Acid	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH	q.s. to pH
8	Disodium Hydrogen o-Phosphate Anhydrous	2.08 mg	2.08 mg	2.08 mg	2.08 mg	2.08 mg
9	Protamine Sulphate	0.518 mg	0.450 mg	0.610 mg	0.350 mg	0.250 mg
10	Water for Injection	q.s. to 1.0 mL	q.s. to 1.0 mL	q.s. to 1.0 mL	q.s. to 1.0 mL	q.s. to 1.0 mL

Manufacturing Procedure:

- 20 Manufacturing procedure of example 5 composition 5A, 5B, 5C, 5D and 5E is same as mentioned above in Example 1.

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CLAIMS

We claim:

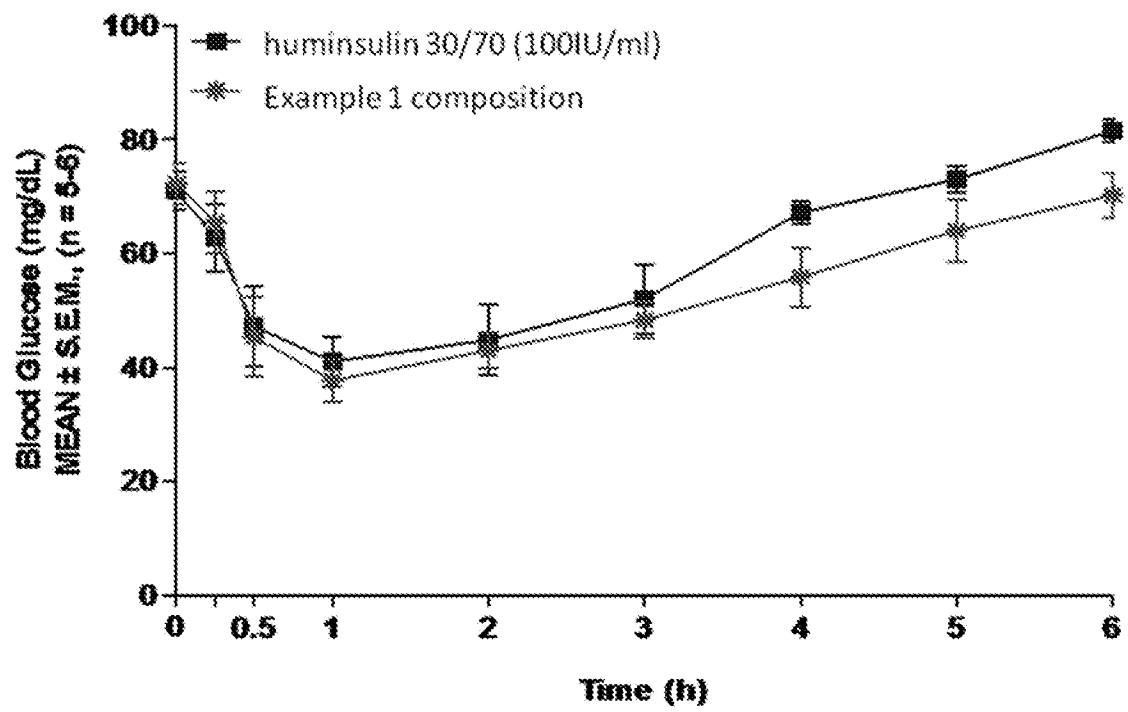
1. An aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin, insulin analogues or derivative thereof, wherein said composition comprises: (i) a solution of
10 insulin, insulin analogue or derivative thereof and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine having particle size in range of 8 μ m and 20 μ m and optionally, (iii) one or more pharmaceutically acceptable excipients; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to
15 100IU/ml insulin composition comprising (i) a solution of insulin, insulin analogue or derivative thereof and (ii) crystals of insulin, insulin analogue or derivative thereof with protamine, when administered to a subject at the same dose.
2. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition has a pH in the range of 6.0 and 8.5.
- 20 3. The pharmaceutical composition of claim 1, wherein the solution of insulin, insulin analogue or derivative thereof and the crystals of insulin, insulin analogue or derivative thereof with protamine are present in a volume proportion ranging from about 99:1 to 1:99.
- 25 4. The pharmaceutical composition of claim 3, wherein the solution of insulin, insulin analogue or derivative thereof and the crystals of insulin, insulin analogue or derivative thereof with protamine are present in the volume proportion of about 30:70.
- 30 5. The pharmaceutical composition of claim 1, wherein the insulin, insulin analogue or derivative thereof are selected from the group comprising insulin human, insulin isophane (NPH), insulin lispro, insulin glulisine, insulin aspart, Gly(A21) human insulin, Gly(A21) Lys(B28) human insulin, Gly(A21) Lys(B28) Pro(B29) human insulin, Gly(A21) Asp(B28).
- 35 6. The pharmaceutical composition of claim 3, wherein the insulin, insulin analogues or derivative thereof is insulin human.
7. The pharmaceutical composition of claim 1, wherein the one or more pharmaceutically acceptable excipients comprise isotonic agent, complexing agent, buffer, preservative, pH modifying agent, stabilizing agent or mixture thereof.

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- 5 8. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is administered through subcutaneous, intravenous or intramuscular route to a subject.
9. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition is for use in the treatment of type I and type II diabetes mellitus.
- 10 10. A process of preparing the pharmaceutical composition of claim 1, wherein said process comprises steps of:
- 15 a. preparing a suspension of protamine and insulin by dissolving the insulin, insulin analogue or derivative thereof, protamine, one or more pharmaceutically acceptable excipients and adjusting a pH of the suspension in a range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH;
- b. preparing the solution of insulin by dissolving insulin, insulin analogue or derivative thereof, one or more pharmaceutically acceptable excipients and adjusting the pH of the solution in the range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH; and
- 20 c. mixing about 70% suspension of step (a) with the about 30% solution of step (b) to obtain an aqueous injectable biphasic pharmaceutical composition.
11. An aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin human, wherein said pharmaceutical composition comprises: (i) a solution of insulin human; (ii)
- 25 crystals of insulin human with protamine having particle size in range of 8 μ m and 15 μ m; and (iii) pharmaceutically acceptable excipient comprising m-cresol, phenol, glycerol and zinc or salts thereof; wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml insulin composition comprising (i) a solution of insulin human and (ii) crystals of insulin human with protamine, when administered to a
- 30 subject at the same dose.
12. A process of preparing an aqueous injectable biphasic pharmaceutical composition comprising 200IU/ml of insulin human, wherein said pharmaceutical composition comprises: (i) a solution of insulin human; (ii) crystals of insulin human with protamine having particle size in range of 8 μ m
- 35 and 15 μ m; and (iii) pharmaceutically acceptable excipient comprising m-cresol, phenol, glycerol and zinc or salts thereof, wherein said pharmaceutical composition provides similar onset of action and longer duration of action as compared to 100IU/ml insulin composition comprising (i) a solution of insulin human and (ii) crystals of insulin human with protamine, when administered to a subject at the same dose; wherein said process comprises steps of:

- 5 a. preparing a suspension of protamine and insulin human by dissolving insulin human, protamine, zinc or salts thereof, meta-cresol, phenol in water for injection and adjusting a pH of the suspension in a range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH;
- 10 b. preparing the solution of insulin human by dissolving insulin human, phenol, m-cresol, glycerol and disodium hydrogen phosphate in water for injection and adjusting the pH of solution in the range of 7.2 to 7.5 by addition of 1N HCl or 1N NaOH; and
- c. mixing about 70% of suspension of step (a) with 30% of solution of step (b) to obtain the aqueous injectable biphasic pharmaceutical composition.
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- 20
- 25
- 30
- 35

Figure1



INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2017/051584

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/28 A61K9/00 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, EMBASE, FSTA, WPI Data

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Further documents are listed in the continuation of Box C.



See patent family annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No

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