



(51) International Patent Classification:

A61K 9/00 (2006.01) *A61K 47/26* (2006.01)
A61K 47/02 (2006.01)

(21) International Application Number:

PCT/IB2015/054974

(22) International Filing Date:

2 July 2015 (02.07.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

2179/MUM/2014 4 July 2014 (04.07.2014) IN
2180/MUM/2014 4 July 2014 (04.07.2014) IN

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(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, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
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, 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).

Published:

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

(54) Title: EXTENDED RELEASE FORMULATIONS OF INSULINS

(57) Abstract: The present invention provides a pharmaceutical composition comprising an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 9h. The present invention also provides a process for preparing the same.



EXTENDED RELEASE FORMULATIONS OF INSULINS**Preamble of Invention**

The present invention provides a pharmaceutical composition comprising an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 9h. The present invention also provides a process for preparing the same.

Background of Invention

Glucose is a simple sugar used by all the cells of the body to produce energy and support life. Humans need a minimum level of glucose in their blood at all times to stay alive. The primary manner in which the body produces blood glucose is through the digestion of food. When a person is not getting sufficient glucose from food digestion, glucose is produced from stores in the tissue and released by the liver. The body's glucose levels are primarily regulated by insulin. Insulin is a peptide hormone that is naturally secreted by the pancreas. Insulin helps glucose enter the body's cells to provide a vital source of energy.

Diabetes is a disease characterized by abnormally high levels of blood glucose and inadequate levels of insulin. There are two types of diabetes – Type 1 and Type 2. In Type 1 diabetes, also known as Diabetes Insipidus, body produces no insulin. In Type 2 diabetes, also known as diabetes mellitus, pancreas produces insulin but either the body does not produce insulin at right time or the body's cell ignore the insulin, a condition known as Insulin resistance. Diabetes mellitus (DM) 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.

Insulin injections are prescribed to the patients suffering from diabetes. Insulin is a natural hormone, which controls the level of the glucose in the blood. In healthy people, insulin is released in blood by the pancreas as the concentration of blood glucose rises. Increased blood glucose levels, occur after meals and are rapidly compensated by a corresponding increase in insulin secretion. Insulin plays major role in converting the excess blood glucose into glycogen and storing it in liver.

Since the introduction of insulin in the 1920's, continuous efforts have been made to improve the treatment of diabetes mellitus. To help avoid extreme glycaemia levels, diabetic patients often practice multiple injection therapy, whereby insulin is administered with each meal.

Insulin is a polypeptide of 51 amino acids, which are divided into 2 amino acid chains: the A chain having 21 amino acids and the B chain having 30 amino acids. The chains are connected to one another by means of two disulfide bridges. Insulin preparations have been employed for diabetes therapy for many years.

Traditionally short acting regular insulin formulations or intermediate acting Insulin Protamine formulations were used for treating patients with diabetes mellitus. With time, new insulin analogues and derivatives have been developed. Insulin analogues and derivatives differ from human insulin at one or more than one amino acid positions and/or amino acid chain length.

A number of insulin, insulin analogs and derivatives are available in the market. The commonly used types of insulin, insulin analogs or insulin derivatives are categorized as:

Rapid-acting Insulin analogs (Bolus): For example insulin aspart (Novolog[®]); Insulin lispro (Humalog[®]), Insulin Glulisine (Apidra[®]), Rapid acting human insulin (Viaject[®]). These analogs begin to work within 5 to 15 minutes of administration and are active for 3 to 4 hours.

Short-acting insulin (Bolus): For example Regular insulin (Humulin[®] or Novolin[®]). Regular insulin starts working within 30 minutes after administration and duration of action lasts from about 5 to 8 hours.

Intermediate-acting insulin: For example as Isophane insulin, Aspart protamine, Lispro protamine. It starts working in 1 to 3 hours after administration. Its duration of action varies between 16 to 24 hours.

Long-acting Insulin (Basal): For example Insulin glargine, Insulin degludec and Insulin detemir. These analogs starts working within 1 to 2 hours and their duration of action varies from about 12 to about 24 hours.

Mixed Insulins: For example mixture of NPH and regular insulin. There are several variations with different proportions of the mixed insulins. The onset of action of these mixed preparations is about 30 minutes. The mixed insulins comprise same type of insulin. Two different types of insulins cannot be mixed i.e. insulin lispro cannot be mixed with insulin

detemir, insulin aspart or insulin glargine. The mixed formulation of insulin Lispro can only comprise insulin lispro regular and insulin lispro protamine – two forms of insulin lispro.

Insulin analogs having an accelerated onset of action are described in EP0214826, EP0375437 and EP0678522. EP0124826 relates, inter alia, to substitutions of B27 and B28. EP0678522 describes insulin analogs, which have various amino acids, preferably proline, in position B29, but not glutamic acid.

EP0375437 includes insulin analogs with lysine or arginine in B28, which can optionally additionally be modified in B3 and/or A21. In EP0419504, insulin analogs are disclosed which are protected against chemical modifications, in which asparagine in B3 and at least one further amino acid in the positions A5, A15, A18 or A21 are modified. In WO 92/00321, insulin analogs are described in which at least one amino acid from the positions B1-B6 is replaced by lysine or arginine. According to WO92/00321, insulins of this type have a prolonged action.

The fast acting insulins include Insulin Lispro, Insulin aspart and Insulin Glulisine. Fast acting insulin begins to work very quickly i.e. with 30min of administration. Thus, they are used to control post-prandial increase in the sugar levels efficiently. These analogs are used to treat type 1 (insulin-dependent) diabetes and type 2 (non-insulin-dependent) diabetes. Insulin aspart is usually given together with another long-acting insulin.

The marketed composition of NovoLog® comprises 100IU insulin aspart, glycerin, phenol, metacresol, zinc, disodium hydrogen phosphate dihydrate, sodium chloride and water for injection. The pH of the composition is adjusted to pH 7.2 to 7.6.

The marketed composition of Humalog® comprises 100IU Insulin Lispro, glycerin, dibasic sodium phosphate, metacresol, zinc oxide, phenol and water for injection. The pH of the composition is adjusted between pH 7.0-8.0.

The marketed composition of Aprida® comprises 100 IU insulin glulisine, metacresol, tromethamine, sodium chloride, polysorbate 20, and Water for injection. The pH of the composition is adjusted to pH 7.3.

Novo Nordisk has formulated a soluble co-formulation of long acting basal insulin, insulin degludec and the rapid acting insulin analogue, insulin aspart (B28Asp human insulin). Ryzodeg is the first fully soluble ready to use insulin product for subcutaneous (s.c.) injection.

Since the first use of insulin over 80 years ago, a major aim of research has been the ever closer imitation of physiological insulin delivery. Basal insulin secretion is essential for the maintenance of fasting glucose levels, especially through inhibition of excessive glucose output from the liver. Basal Insulin provides optimal levels of insulin over a period of 12 to 24 hours after administration to manage the patient's normal daily glucose fluctuations in absence of meal. There are three basal insulins available in market – Insulin glargine, Insulin detemir, Insulin degludec, Insulins complexed with protamine and hexamer-stabilizing agent zinc (lente and ultralente insulins). The insulins complexed with protamine and the lente and ultralente insulins formulations fall short of providing an appropriate basal supply of insulin because of variable absorption, undesirable peaks in hypoglycemic action, and an insufficient duration of action. These limitations have led to attempts to develop improved basal insulins with no pronounced peaks in insulin levels, reproducible antihyperglycemic efficacy, and once-daily administration.

Insulin glargine Gly(A21)-Arg(B31)-Arg(B32)-human insulin, a basal insulin, has a prolonged duration of action. Insulin glargine has a substitution of glycine for asparagine at A21 (Asn21) and two arginines added to the carboxy terminal of B chain. The arginine amino acids shift the isoelectric point from a pH of 5.4 to 6.7, while glycine substitution makes the molecule more stable at an acidic pH, allowing for the subcutaneous injection of a clear solution. The asparagine substitution prevents deamidization of the acid-sensitive glycine at acidic pH. In the neutral subcutaneous space, higher-order aggregates are formed, resulting in a slow, nearly peakless dissolution and absorption of insulin from the site of injection.

Insulin glargine is marketed under the trade name of Lantus® by Sanofi Aventis. Lantus® is injected as an acidic, clear solution having pH 4.0 and precipitates on account of its solution properties in the physiological pH range of the subcutaneous tissue as a stable hexamer associate. It is injected once daily and is distinguished compared with other long-acting insulins by its flat serum profile and the reduction of the danger of nightly hypoglycemia associated therewith (Schubert-Zsilavec et al., 2: 125-130(2001)).

Two Formulations of Lantus[®] are available in market

- 3ml Cartridge Composition
- 10ml Vial Composition

The 3ml Cartridge composition comprises 100IU (3.6378 mg) Insulin glargine, zinc, m-cresol, glycerol, and water for injection. The pH of the composition is adjusted to pH 4.0. The 10ml vial composition comprises 100 IU Insulin glargine, zinc, m-cresol, glycerol, and water for injection. The pH of the composition is adjusted to pH 4.0 by addition of aqueous solutions of hydrochloric acid and sodium hydroxide.

Basal insulin like Insulin glargine is a human insulin analogue designed to have a low solubility at neutral pH. Insulin glargine is formulated at an acidic pH 4, where it is completely water soluble. After subcutaneous injection of the acidic solute (which can cause discomfort and a stinging sensation), when a physiologic pH (approximately 7.4) is achieved the increase in pH leads to formation of microprecipitates of insulin hexamers. The higher order aggregation slows the dissociation of the hexamers into insulin monomers, the functional and physiologically active unit of insulin. This gradual process ensures that small amounts of insulin glargine are released into the body continuously, giving an almost peakless profile. Lantus[®] exhibits a relatively constant glucose-lowering profile over 24 hours that permits once-daily dosing.

The longer duration of action (up to 24 hours) of insulin glargine is directly related to size of microprecipitate particle formed at the site injection. Slow rate of dissociation of microprecipitate into monomer unites of insulin glargine and its metabolites leads to slower rate of absorption of insulin glargine from subcutaneous tissue. However, in many patients, Insulin glargine does not last for 24 hours. A major disadvantage with administration of insulin glargine formulation in vivo is that there is no control on the particle size of microprecipitates formed inside the body after the administration of formulation. This leads to lot of variability in the pattern of release of the insulin glargine and sometimes leads to nocturnal hypoglycemia in patients taking Insulin glargine. Thus, there is a need to develop newer analogues or newer formulation which exhibits a flatter P/K and P/D profile than Insulin glargine and can precisely control the release of the glargine from the site of injection. There is a need to develop a new formulation of insulin glargine which provides better

control on the release of the drug in-vivo and whose release can be predicted using the in-vitro parameters.

A further disadvantage of insulin glargine is that, unlike isophane insulin, it cannot be mixed with soluble insulins as this results in precipitation. Thus, many patients with biphasic (mixture) insulins will be required to increase their number of daily injections and/or change to a basal - bolus injection regimen.

Further, patients are used to using one type of device for injecting the insulin. Thus, there is a need to develop such a formulation which is amendable to presently existing devices available in the market for precise dose delivery and does not require a new device or specialized mechanism to control the accuracy of the dose.

In order to reduce the number of injections to be administered to a patient, attempts have been made to develop newer insulin analogues with flatter PK profile or slow releasing basal insulins having once-daily dosing or even more than 24h release profile.

US Patent No. 8637458 B2 discloses a basal insulin formulation composed of insulin, preferably insulin glargine, injectable zinc and injectable iron compounds as precipitating and/or stabilizing agents has been developed for subcutaneous, intradermal or intramuscular administration. The formulation is designed to form a precipitate of insulin following injection, creating slow releasing "basal insulin" over a period of 12 to 24 hours.

US Patent No. 7713929 B2 discloses a composition comprising a rapid or intermediate acting insulin in combination with a long acting insulin, wherein the pH of the composition is adjusted to a pH between 3.8 and 4.2 to solubilize the long acting insulin. The invention further discloses that the pH of the rapid acting insulin is decreased through the use of an acid such as aspartic, glutamic or citric acid, so that the long acting glargine remains soluble when they are mixed together.

PCT Patent Publication No. 2013082116 A1 discloses the pharmaceutical compositions comprising an insulin amino acid sequence and an amino acid sequence that provide slow

absorption from an injection site, such as, an amino acid sequence that has a substantially repeating pattern of proline residues.

PCT Patent Publication No. 2011156476 A2 discloses a basal insulin formulation composed of insulin (preferably human recombinant insulin), buffering agents, precipitating agents, and/or stabilizing agents for subcutaneous, intradermal or intramuscular administration. The suspension contains precipitated insulin particles prior to injection and provides a sustained release basal insulin profile following injection.

US Patent Application No. 20120232002 A1 discloses an aqueous pharmaceutical composition and method of preparation of that formulation that comprises an insulin analog, along with 0.001 to 0.2 mg/ml of zinc, 0.1 to 5.0 mg/ml of a preservative, 5.0 to 100 mg/ml isotonicity agent, pH 5 or less.

US Patent Application No. 20070185033 A1 discloses sustained delivery formulation comprising a water-insoluble complex of a peptidic compound and a carrier macromolecule.

US Patent Application No. 20110301081 A1 discloses an aqueous pharmaceutical formulation comprising 200-1000 U/mL [equimolar to 200-1000 IU human insulin] of insulin glargine. The invention further discloses method of extending the duration of exposure of long acting insulin in the treatment of Type I and Type II Diabetes Mellitus in a patient comprising administering to said patient an aqueous pharmaceutical composition comprising insulin glargine in a concentration of 300 U/mL.

US Patent Application No. 20100069292 A1 discloses a clear basal insulin formulation composed of insulin (preferably human recombinant insulin), buffering agents, precipitating agents, and/or stabilizing agents for subcutaneous, intradermal or intramuscular administration. The formulation is designed to form a precipitate of insulin following injection, creating slow releasing "basal insulin" over a period of 12 to 24 hours, which can be varied by compositional changes to tailor the release profile to the needs of the individual diabetic patient.

US Patent Application No. 20090175840 A1 discloses injectable insulin formulations that are capable of modifying the amount of insulin released based on the patient's tissue glucose

levels, methods for making and using these formulations. The formulation is designed to release insulin into the systemic circulation over time with a basal release profile following injection in a patient.

Sanofi Aventis has developed a new formulation U300 comprising 300IU of insulin glargine formulation per ml. US Patent Application No. 20120122774 A1 discloses that the 300IU formulation has pH between 3.4 to 4.6. The applicant conducted precipitation studies at pH 7.4 simulating in-vivo conditions of the formulations having concentration of 100U/ml, 300U/ml, 500U/ml, 700U/ml, and 1000U/ml. It was observed that the particle size of the precipitates increases with increase in the concentration of Insulin Glargine (See Fig. 8A to 8E of US 20120122774A1). U300 exhibits a flatter P/K and PD profile and prolonged release than insulin glargine U100. The new product U300 contains 03 (three) times the concentration of marketed U100 Insulin glargine (Lantus[®]). Thus, in case of medication errors, three times more dose will be delivered to a patient. Further, as the Insulin glargine precipitates in-vivo, there is no control over the particle size of the precipitate which may result in increased inter-individual variability.

None of the listed prior arts discloses a biphasic pharmaceutical composition comprising an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent, preservative optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 9h, more specifically a release of more than 24h.

Further, there is need of formulations wherein the insulin does not undergo any chemical transformation or complexation and the release of the insulin from the formulation can be controlled and predicted from the in-vitro parameters such as particle size of the drug crystal or excipients. The formulation should involve use of already GRAS listed excipients.

Summary of the Invention

The term “insulin(s)” used herein includes mammalian insulin, insulin analogues or derivatives.

The term “Insulin analogs or derivatives” used in the present invention includes analogs or derivatives of naturally occurring insulins, namely human insulin or animal insulins, brought

about by modifying the structure of human insulin molecule, which results in altered physicochemical, pharmacokinetic and pharmacodynamic properties.

One aspect of the present invention provides a biphasic pharmaceutical composition comprising an insulin analogue, derivative, metabolite or its salts thereof having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 9h.

Another aspect of the present invention provides a biphasic pharmaceutical composition comprising an insulin analogue, derivative, metabolite or its salts thereof having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 24h.

Another aspect of the present invention provides a biphasic pharmaceutical composition consisting an insulin analogue, derivative, metabolite or its salts thereof having isoelectric point between 5.8 to 8.5, zinc or salts thereof, one or more isotonic agent, pH modifying agent and preservative, wherein the formulation exhibits an extended release profile for a period of more than 9h.

Another aspect of the present invention provides a pharmaceutical formulation comprising from about 40IU to 200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 24h.

Another aspect of the present invention provides a biphasic pharmaceutical composition comprising about 40IU-1000IU/ml of insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 24h; wherein the particle size of the insulin glargine in the composition varies between 3 μ m to 60 μ m; wherein the pharmaceutical composition is devoid of any polymer and precipitating agent.

Another aspect of the present invention provides a pharmaceutical formulation comprising from an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 in a concentration range of 40IU-200IU/ml, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, pH modifying agent and preservative, wherein the formulation exhibits an extended release profile for a period of more than 24h.

Another aspect of the present invention provides a method of treating Type I and Type II Diabetes Mellitus in a patient comprising administering to said patient a pharmaceutical formulation comprising from about 40IU to 200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 24h.

Another aspect of the present invention provides a method of reducing the nocturnal hypoglycemia, wherein nocturnal hypoglycemia in patients suffering from Type I or Type II diabetes, wherein the method comprise administering to the said patient comprising an extended release composition comprising 40-200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 having pH between 6.0-8.5, wherein the composition comprises crystals of an insulin, insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 optionally along with one or more excipients, wherein the said crystal have an average particle size between about 2 μm to about 60 μm ; wherein the pharmaceutical composition is devoid of any polymer and precipitating agent.

Another aspect of the present invention provides a process for preparing an extended release biphasic pharmaceutical composition comprising a biphasic pharmaceutical composition comprising an insulin analogue, derivative, metabolite or its salts thereof having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the process comprises:

- a) preparing a concentrated solution of insulin glargine by dissolving an accurately weighed zinc-containing crystals of insulin glargine in water for injection using hydrochloric acid solution and adjusting the pH of the said solution between 6.0-8.0 with alkali.

- b) dissolving zinc chloride and isotonic agent in a buffer solution, wherein the pH of the buffer solution is between 6.0-8.0.
- c) diluting insulin glargine suspension of step a) with the buffer solution of step b).

Detailed description of the invention

While working on present invention, inventors of the present invention have surprisingly found that there is no need to increase the amount or the IU units of insulin glargine in the formulation for producing bigger precipitates or use of “special polymers” or “special excipients” for prolonging the release of insulin glargine or for exhibiting a flatter P/K profile that is similar to insulin glargine U300 (Toujeo). During the development of newer insulin glargine formulation having flatter P/K profile than Insulin analogues or derivatives, applicants have surprisingly found that the release of insulin glargine and its pharmacokinetic profile from the formulation can be controlled by formulating a biphasic formulation, modulating pH, precipitation or crystal morphology of the Insulin glargine *in-vitro*.

The present biphasic pharmaceutical compositions are formed at pH near isoelectric point of insulin glargine so the insulin glargine precipitates *in-vitro* resulting in formation of biphasic formulation. The release profile of the insulin glargine from the biphasic formulation can be controlled *in-vitro* by modulating the process parameters and crystal morphology. The present formulation contains insulin glargine microcrystal's having uniform size of insulin glargine crystals. The present formulation exhibits a prolonged duration of action for more than 9h or even for more than 24h and yet exhibits a flatter P/K profile than the presently marketed formulation.

The present formulation offers various advantages over prior art pharmaceutical composition, e.g. no increase in the concentration/amount of insulin to be delivered, release profile can be controlled by *in-vitro* parameters such as pH, crystal size or particle size, lesser chance of nocturnal hypoglycemia after dose dumping or accidental release of insulin glargine from pharmaceutical composition. The present formulations can be used with the presently marketed device used for delivery of 100IU/ml formulation. There is no increase in the volume of the injection.

Specifically, the insulin glargine formulations of the present invention exhibit a flatter PK (exposure) and flatter PD (activity) profile and maintain the blood glucose level at basal level

equivalent to U300 insulin glargine. Therefore, blood glucose level is controlled for extended duration of action for the treatment of Type I and Type II diabetes from insulin glargine formulations of the present invention.

One aspect of the invention provides a biphasic pharmaceutical composition comprising insulin glargine, its salts or metabolite thereof, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 9h.

Another aspect of the present invention provides a pharmaceutical formulation comprising from about 40IU to 200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 24h and the pH of the composition is adjusted to a pH of between 5.8 and 8.5.

Another aspect of the present invention provides a pharmaceutical formulation comprising from about 40IU to 200IU of Insulin glargine, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 24h.

Another aspect of the present invention provides a pharmaceutical formulation comprising from an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 in a concentration range of 40IU-200IU/ml, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, pH modifying agent and preservative, wherein the formulation exhibits an extended release profile for a period of more than 24h. The concentration of insulin analogue, derivative or metabolite is 100IU.

Another aspect of the present invention provides a pharmaceutical formulation comprising 100IU of Insulin glargine, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 24h.

Another aspect of the present invention provides a biphasic pharmaceutical composition comprising an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 is present in concentration range of 40IU-1000IU/ml and the formulation exhibits an extended release profile for a period of more than 9h.

Another aspect of the present invention provides a biphasic pharmaceutical composition comprising an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 is present in concentration range of 40IU-1000IU/ml and the formulation exhibits an extended release profile for a period of more than 24h.

Another aspect of the present invention provides a biphasic pharmaceutical composition comprising 40IU – 200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the pH of the composition is adjusted to a pH of between 5.8 and 8.5 and the formulation exhibits an extended release profile for a period of more than 9h.

Another aspect of the present invention provides a biphasic pharmaceutical composition consisting essentially of 100IU of insulin glargine, zinc or salts thereof, isotonic agent, preservative, pH modifying agent and water for injection, wherein the pH of the composition is adjusted to a pH of between 5.8 and 8.5 and the formulation exhibits an extended release profile for a period of more than 9h.

Another aspect of the present invention provides a biphasic pharmaceutical composition comprising crystals of insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, and zinc or salts thereof, wherein the crystals comprises 80-100IU of insulin glargine and 10-20 µg/mL zinc.

Another aspect of the present invention provides a method of treating Type I and Type II Diabetes Mellitus in a patient comprising administering to said patient a biphasic pharmaceutical composition comprising insulin glargine, its salts or metabolite thereof, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 9h.

Another aspect of present invention provides a method for treating Type I and Type II Diabetes Mellitus in a patient comprising administering a biphasic pharmaceutical composition consisting of 40-1000IU of insulin glargine, zinc or salts thereof, isotonic agent, preservative, pH modifying agent and water, wherein the composition exhibits an extended release profile for more than 9h. The concentration of insulin glargine is 100IU.

Another aspect of present invention provides a method for treating Type I and Type II Diabetes Mellitus in a patient comprising administering a biphasic pharmaceutical composition consisting of 100IU of insulin glargine, zinc or salts thereof, isotonic agent, preservative, pH modifying agent and water, wherein the composition exhibits an extended release profile for more than 24h.

Another aspect of present invention provides a method of extending the duration of action of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 for more than 9h in the treatment of Type I and Type II Diabetes Mellitus in a patient comprising administering to said patient a biphasic composition comprising crystals of an insulin, insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 optionally along with one or more excipients, wherein the said crystal have an average particle size between about 2 μm to about 60 μm .

Another aspect of present invention provides a method of extending the duration of action of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 for more than 24h in the treatment of Type I and Type II Diabetes Mellitus in a patient comprising administering to said patient a biphasic composition having pH between 6.0-8.5, wherein the biphasic composition comprises crystals of an insulin, insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 optionally along with one

or more excipients, wherein the said crystal have an average particle size between about 2 μm to about 60 μm .

Another aspect of present invention provides a method of extending the duration of action of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 for more than 24h in the treatment of Type I and Type II Diabetes Mellitus in a patient comprising administering to said patient a composition comprising crystals of an insulin, insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 comprising 40IU-1000IU of insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 optionally along with one or more excipients, wherein the said crystal have an average particle size between about 2 μm to about 60 μm .

Another aspect of present invention provides a method of extending the duration of action of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 for more than 24h in the treatment of Type I and Type II Diabetes Mellitus in a patient comprising administering to said patient an extended release composition comprising 40-200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 having pH between 6.0-8.5, wherein the composition comprises crystals of an insulin, insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 optionally along with one or more excipients, wherein the said crystal have an average particle size between about 2 μm to about 60 μm .

Another aspect of the present invention provides a pharmaceutical formulation comprising from about 40IU to 200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the particle size of the an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 in the composition varies between 3 μm to 60 μm and the composition exhibits an extended release profile for a period of more than 24h.

Another aspect of the present invention provides a pharmaceutical formulation comprising from about 40IU to 200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended

release profile for a period of more than 24h; wherein the pharmaceutical composition is in the form of biphasic composition.

Another aspect of the present invention provides a pharmaceutical formulation comprising from about 40IU to 200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 24h; wherein the pharmaceutical composition is in the form of biphasic composition; wherein the pharmaceutical composition is in the form of suspension.

Another aspect of present invention provides a method for treating Type I and Type II Diabetes Mellitus in a patient comprising administering an extended release pharmaceutical composition consisting of 100IU of insulin glargine, zinc or salts thereof optionally along with one or more pharmaceutically acceptable excipients, wherein the composition exhibits the extended release profile for more than 24h.

Another aspect of the present invention provides a biphasic pharmaceutical composition comprising an insulin analogue, derivative, metabolite or its salts thereof having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients and an additional therapeutic agent, wherein the formulation exhibits an extended release profile for a period of more than 24h.

Another aspect of the present invention provides a pharmaceutical formulation comprising from about 40IU to 200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients and an additional therapeutic agent, wherein the formulation exhibits an extended release profile for a period of more than 24h.

The composition of the present invention additionally comprises one or more pharmaceutical acceptable therapeutic agents selected from the group consisting of Insulin, Insulin aspart, Insulin aspart protamine, Insulin lispro, Insulin lispro protamine, Insulin glulisine, Viaject®, exenatide, Liraglutide, lixisenatide, semaglutide, dulaglutide, albiglutide, and ITCA 650.

The additional therapeutic agent may be selected from the group consisting of one or more insulin, insulin analogues or derivatives having isoelectric point between 4.0 – 7.0, GLP-1 receptor agonist and GLP-2 receptor agonist. The insulin, insulin analogues or derivatives having isoelectric point between 4.0 – 7.0 include but are not limited to recombinant human insulin, Insulin NPH, Insulin Lispro, Insulin Lispro Protamine, Insulin Glulisine and Insulin Aspart, Insulin Aspart Protamine, A21 Gly Insulin, A21Gly B28Lys insulin, A21Gly B28Lys B29Pro, A21Gly B28Asp or Viaject (rapid acting insulin).

Another aspect of the present invention provides a pharmaceutical composition wherein 1ml of insulin glargine formulation contains 3.67 mg 21A-Gly-30Ba-L-Arg-30Bb-L-Arg human insulin [equimolar to 100 IU human insulin], 30 µg zinc, 2.7 mg m-cresol, 20 mg glycerol 85%, HCl and NaOH ad pH 7.0±0.5.

In another embodiment, the particle size of insulin glargine in the composition varies between 3µm to 60µm.

In another embodiment, the pharmaceutical composition of the present invention is devoid of any polymer and precipitating agent.

Another aspect of the present invention provides a process for preparing an extended release pharmaceutical composition a pharmaceutical formulation comprising from about 40IU to 200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the process comprises:

- a) preparing a concentrated solution of insulin glargine by dissolving an accurately weighed zinc-containing crystals of insulin glargine in water for injection using hydrochloric acid solution and adjusting the pH of the said solution between 6.0-8.0 with alkali.
- b) dissolving zinc chloride and isotonic agent in a buffer solution, wherein the pH of the buffer solution is between 6.0-8.0.
- c) diluting insulin glargine suspension of step a) with the buffer solution of step b).

Another aspect of the present invention provides a biphasic pharmaceutical composition comprising about 40IU-1000IU/ml of insulin analogue, derivative or metabolite having

isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 24h; wherein the particle size of the insulin glargine in the composition varies between 3 μ m to 60 μ m; wherein the pharmaceutical composition is devoid of any polymer and precipitating agent; wherein the pharmaceutical composition comprises 100IU of Insulin glargine.

Another aspect of the present invention provides a method of reducing the nocturnal hypoglycemia, wherein nocturnal hypoglycemia in patients suffering from Type I or Type II diabetes, wherein the method comprise administering to the said patient comprising a biphasic composition comprising crystals of an insulin, insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 optionally along with one or more excipients, wherein the said crystal have an average particle size between about 2 μ m to about 60 μ m.

Another aspect of the present invention provides a method of reducing the nocturnal hypoglycemia, wherein nocturnal hypoglycemia in patients suffering from Type I or Type II diabetes, wherein the method comprise administering to the said patient comprising a biphasic composition having pH between 6.0-8.5, wherein the biphasic composition comprises crystals of an insulin, insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 optionally along with one or more excipients, wherein the said crystal have an average particle size between about 2 μ m to about 60 μ m.

Another aspect of the present invention provides a method of reducing the nocturnal hypoglycemia, wherein nocturnal hypoglycemia in patients suffering from Type I or Type II diabetes, wherein the method comprise administering to the said patient comprising a composition comprising crystals of an insulin, insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 comprising 40IU-1000IU of insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 optionally along with one or more excipients, wherein the said crystal have an average particle size between about 2 μ m to about 60 μ m.

The excipients include, but are not limited to, isotonic agent, surfactant, buffer, zinc or salt thereof, preservatives, pH modifying agents, buffering agent, stabilizing agent and solubilizing agents.

An “isotonic agent” is a compound, such as glycerin, are commonly used for such purposes at known concentrations. Other isotonicity agents include but not limited to sodium chloride, dextrose, mannitol or lactose.

Precipitating agents are added to enhance the formation of the precipitate of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 by either hastening the precipitate formation, and/or stabilizing the precipitate by reducing its solubility. These may be solubility modifying agents, precipitation seeding agents, or precipitation enhancing agents. Precipitating agent include but are not limited to protamine, protamine sulphates, calcium, magnesium, manganese, iron, copper, and chelating agents.

Suitable surfactants are those known to ordinary skilled in the art and may include one or more of amphoteric, non-ionic, cationic or anionic surfactants. Suitable surfactants comprises one or more of sodium lauryl sulfate, monooleate, monolaurate, monopalmitate, monostearate or another ester of polyoxyethylene sorbitane, sodium dioctylsulfosuccinate (DOSS), lecithin, stearyl alcohol, cetostearyl alcohol, cholesterol, polyoxyethylene ricin oil, polyoxyethylene fatty acid glycerides, poloxamer, cremophore RH 40 and partial and fatty acid esters and ethers of polyhydric alcohols including Span®, Tween®, in particular Tween® 20 and Tween®80, Myrj®, Brij®, Cremophore® or poloxamers, Pluronic® and Tetronics®.

An “buffering agent” as used herein include, but are not limited to, phosphate, acetate, citrate, arginine, glycylglycine, sodium hydroxide, hydrochloric acid or TRIS (i.e. 2-amino-2-hydroxymethyl-1,3-propanediol) buffer and corresponding salts.

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, metacresol or combinations thereof.

An “pH modifying agents” as used herein can be selected from the group comprising of o-phosphoric acid, citric acid, acetic acid, succinic acid, lactic acid, gluconic acid, hydrochloric 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 hydroxide, calcium carbonate, magnesium carbonate, magnesium aluminum silicates, diethanolamine, monoethanolamine, sodium carbonate, sodium bicarbonate or triethanolamine.

The pH of the aqueous pharmaceutical formulation is between 6.0 and 8.5.

The zinc concentration of the formulation of the present invention is in the range of the concentration which is reached by the presence of 10-400 µg/mL zinc. However, the zinc may be present in form of zinc chloride, but the salt is not limited to be zinc chloride.

An “solubilizing agents” as used herein include, but are not limited to, include wetting agents such as polysorbates and poloxamers, non-ionic and ionic surfactants, food acids and bases (e.g. sodium bicarbonate), polyhydric alcohols and alcohols.

The “stabilizing agents” as used herein include, but are not limited to esterase inhibitors such as pancreatic secretory inhibitors, protease inhibitors, and serine esterase inhibitors such as aprotinin.

While the invention has been described in term of its specific embodiments, certain modification and equivalents will be apparent to those skilled in the art and are intended to be included within the scope of the invention.

Examples

Example 1: Unit Composition Formula

Table 1: Unit composition of Insulin Glargine

S. No.	Ingredients	Quantity per mL
1	Insulin Glargine (r-DNA Origin)	100 IU
2	<i>m</i> -Cresol	1.5-4.5 mg/mL
3	Glycerol, 98%	17.34 mg/mL

4	Zinc as Zinc chloride	0.01-0.30 mg/mL
5	Sodium Hydroxide	Q.S. to adjust pH 7.4
6	Hydrochloric Acid	Q.S. to adjust pH 7.4
7	Water for Injection	Q.S. to 1.0 mL

Procedure: The pharmaceutical composition described below was prepared according to the procedure detailed below:

Step 1: Preparation of Insulin Glargine Suspension

Insulin glargine concentrated solution was prepared by dissolving accurately weighed zinc-containing crystals of insulin glargine in water for injection with the help of few μL of 1M HCl. This concentrated solution was prepared as 10X. The pH of this solution was further shifted to 7.3 ± 0.1 with 1M NaOH.

Step 2: Preparation of Buffer Solution

Buffer solution was prepared 2X by dissolving *m*-cresol and glycerol to get final concentration as per unit composition formula. The endogenous zinc level was supplemented by adding appropriate volume of zinc chloride solution (1% w/v) to achieve final concentration of $30\mu\text{g}/100\text{ IU}$. The pH of this solution was adjusted to 7.3 ± 0.1 with 1M NaOH.

Step 3: Preparation of Final Suspensions of Desired Concentrations

Insulin glargine suspension (10X) was diluted to desired concentrations with buffer solutions (2X) under constant stirring and pH was ensured to 7.3 ± 0.1 .

Microscopic Observations:

The pharmaceutical composition of present invention containing 100IU/ml of insulin glargine was observed microscopically after micro-precipitation under the optical microscope (Olympus BX40) at 100X in oil immersion. The particle size of micro-precipitates was compared with the micro-precipitates obtained from the marketed formulation of insulin glargine containing 300IU/ml of glargine. The results have been reproduced in Figure 1.

As evident from Figure 1, at higher strength i.e. marketed formulation containing 300IU/ml of insulin glargine, the size of micro-precipitates ranged from 02-60 μ m with variable shapes and closely bound clusters. The pharmaceutical composition of present invention containing 100IU/ml of insulin glargine, the size of micro-precipitates was maintained equivalent to that of 300IU/ml of insulin glargine formulation even after dilution to desired concentrations with placebo containing appropriate levels of zinc. Thus, the pharmaceutical composition of present invention comprising 100IU/ml of insulin glargine will give equivalent release profile as that of 300IU/ml of insulin glargine.

Comparative analysis of glucose level in blood plasma after injection of Control, Lantus and 100IU Insulin Glargine suspension in rat:

To compare the reduction in blood plasma glucose level by the pharmaceutical composition of present invention (100IU/ml of insulin glargine) and the marketed formulation Lantus (300IU/ml of insulin glargine), STZ induced diabetes (Wistar Rat) model was selected.

Animals were kept on fasting initially for 2 hr. Both the insulin glargine formulations were subcutaneously injected at 1.5 IU/kg dose and the formulations were compared based on their ability to reduce the blood glucose levels using Bayer glucometer.

Inventors of the present invention compared the decline in blood plasma glucose level by the pharmaceutical composition of present invention (100 IU/ml of Insulin Glargine) and marketed formulation Lantus® (300IU/ml of insulin glargine). The results have been reproduced in Figure 2.

The marketed formulation of insulin glargine (300IU/ml) decreases the blood plasma glucose level from about 100mg/dL to about 50 mg/dL within 1 hr and to 55 mg/dL within 5 hrs. However, after 5 hrs of administration of 300IU/ml of insulin glargine formulation, blood plasma glucose level starts increasing, which is reverse of what is observed in 100IU/ml of insulin glargine formulation in the present invention. In case of 100IU/ml of insulin glargine formulation, the blood plasma glucose level after 9 hrs of administration was found to be about 50 mg/dL which was lower from that of 300 IU/ml of insulin glargine formulation which was found to be 80 mg/dL.

Thus, the results depict that the composition of present invention comprising 100IU/ml of Insulin Glargine suspension can control the blood plasma glucose level for much more extended duration in comparison to marketed insulin glargine formulation Lantus®.

Example 2: Unit Composition Formula

Table 2: Unit composition of Insulin Glargine

S. No.	Ingredients	Quantity per mL
1	Insulin Glargine (r-DNA Origin)	200 IU
2	<i>m</i> -Cresol	1.5-4.5 mg/mL
3	Glycerol, 98%	17.34 mg/mL
4	Zinc as Zinc chloride	0.01-0.30 mg/mL
5	Sodium Hydroxide	Q.S. to adjust pH 7.4
6	Hydrochloric Acid	Q.S. to adjust pH 7.4
7	Water for Injection	Q.S. to 1.0 mL

Procedure: The pharmaceutical composition described below was prepared according to the procedure detailed below:

Step 1: Preparation of Insulin Glargine Suspension

Insulin glargine concentrated solution was prepared by dissolving accurately weighed zinc-containing crystals of insulin glargine in water for injection with the help of few μL of 1M HCl. This concentrated solution is prepared as 10X. The pH of this solution is further shifted to 7.3 ± 0.1 with 1M NaOH.

Step 2: Preparation of Buffer Solution

Buffer solution was prepared 2X by dissolving *m*-cresol and glycerol to get final concentration as per unit composition formula. The endogenous zinc level was supplemented by adding appropriate volume of zinc chloride solution (1% w/v) to achieve final concentration of $30\mu\text{g}/100\text{ IU}$. The pH of this solution is adjusted to 7.3 ± 0.1 with 1M NaOH.

Step 3: Preparation of Final Suspensions of Desired Concentrations

Insulin glargine suspension (10X) was diluted to desired concentrations with buffer solutions (2X) under constant stirring and pH was ensured to 7.3 ± 0.1 .

Example 3: Unit Composition Formula

Table 3: Unit composition of Insulin Glargine

S. No.	Ingredients	Quantity per mL
1	Insulin Glargine (r-DNA Origin)	500 IU
2	<i>m</i> -Cresol	1.5-4.5 mg/mL
3	Glycerol, 98%	17.34 mg/mL
4	Zinc as Zinc chloride	0.01-0.30 mg/mL
5	Sodium Hydroxide	Q.S. to adjust pH 7.4
6	Hydrochloric Acid	Q.S. to adjust pH 7.4
7	Water for Injection	Q.S. to 1.0 mL

Procedure: The pharmaceutical composition described below was prepared according to the procedure detailed below:

Step 1: Preparation of Insulin Glargine Suspension

Insulin glargine concentrated solution was prepared by dissolving accurately weighed zinc-containing crystals of insulin glargine in water for injection with the help of few μL of 1M HCl. This concentrated solution is prepared as 10X. The pH of this solution is further shifted to 7.3 ± 0.1 with 1M NaOH.

Step 2: Preparation of Buffer Solution

Buffer solution was prepared 2X by dissolving *m*-cresol and glycerol to get final concentration as per unit composition formula. The endogenous zinc level was supplemented by adding appropriate volume of zinc chloride solution (1% w/v) to achieve final concentration of $30\mu\text{g}/100\text{ IU}$. The pH of this solution is adjusted to 7.3 ± 0.1 with 1M NaOH.

Step 3: Preparation of Final Suspensions of Desired Concentrations

Insulin glargine suspension (10X) was diluted to desired concentrations with buffer solutions (2X) under constant stirring and pH was ensured to 7.3 ± 0.1 .

Figure 1: The two formulations of Insulin Glargine 300IU/mL and 100 IU/mL after micro-precipitation were observed under the optical microscope (Olympus BX40) at 100X in oil immersion.

Figure 2: Comparative analysis of glucose level in blood plasma after injection of Control, Lantus and 100IU Insulin Glargine suspension in rat.

We Claim:

1. A biphasic pharmaceutical composition comprising an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 9h.
2. The pharmaceutical composition of claim 1, wherein the insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 is present in concentration range of 40IU-1000IU/ml.
3. The pharmaceutical composition of claim 1, the pH of the composition is adjusted to a pH of between 5.8 and 8.5.
4. The pharmaceutical composition of claim 1, wherein the insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 is insulin glargine.
5. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition further comprises surfactant, buffer, preservatives, pH modifying agents or solubilizing agents.
6. The pharmaceutical composition of claim 4, wherein the pharmaceutical composition comprises 100IU of Insulin glargine.
7. The pharmaceutical composition of claim 4, wherein the particle size of the insulin glargine in the composition varies between 3 μ m to 60 μ m.
8. The pharmaceutical composition of claim 1, wherein the composition additionally comprises one or more pharmaceutical acceptable therapeutic agents selected from the group consisting of Insulin, Insulin aspart, Insulin aspart protamine, Insulin lispro, Insulin lispro protamine, Insulin glulisine, Viaject®, exenatide, Liraglutide, lixisenatide, semaglutide, dulaglutide, albiglutide, and ITCA 650.

9. A method for treating Type I and Type II Diabetes Mellitus in a patient comprising administering a pharmaceutical composition of claim 1 to a patient in need thereof.
10. A process for preparing an extended release biphasic pharmaceutical composition comprising a biphasic pharmaceutical composition comprising an insulin analogue, derivative, metabolite or its salts thereof having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the process comprises:
 - a) preparing a concentrated solution of insulin glargine by dissolving an accurately weighed zinc-containing crystals of insulin glargine in water for injection using hydrochloric acid solution and adjusting the pH of the said solution between 6.0-8.0 with alkali.
 - b) dissolving zinc chloride and isotonic agent in a buffer solution, wherein the pH of the buffer solution is between 6.0-8.0.
 - c) diluting insulin glargine suspension of step a) with the buffer solution of step b).
11. A pharmaceutical formulation comprising from about 40IU to 200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 24h.
12. The pharmaceutical composition of claim 11, wherein the insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 is present in concentration range of 40IU-200IU/ml.
13. The pharmaceutical composition of claim 11, the pH of the composition is adjusted to a pH of between 5.8 and 8.5.
14. The pharmaceutical composition of claim 11, wherein the insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 is insulin glargine.

15. The pharmaceutical composition of claim 11, wherein the isotonic agents are selected from the group consisting of glycerol, mannitol, lactose, dextrose and sodium chloride.
16. The pharmaceutical composition of claim 11, wherein the pharmaceutical composition further comprises surfactant, buffer, preservatives, pH modifying agents or solubilizing agents.
17. The pharmaceutical composition of claim 14, wherein the pharmaceutical composition comprises 100IU of Insulin glargine.
18. The pharmaceutical composition of claim 14, wherein the particle size of the insulin glargine in the composition varies between 3 μ m to 60 μ m.
19. The pharmaceutical composition of claim 11, wherein the composition comprises one or more pharmaceutical acceptable therapeutic agents selected from the group consisting of Insulin, Insulin aspart, Insulin aspart protamine, Insulin lispro, Insulin lispro protamine, Insulin glulisine, Viaject®, exenatide, Liraglutide, lixisenatide, semaglutide, dulaglutide, albiglutide, and ITCA 650.
20. A method for treating Type I and Type II Diabetes Mellitus in a patient comprising administering a pharmaceutical composition of claim 11 to a patient in need thereof.
21. A biphasic pharmaceutical composition comprising about 40IU-1000IU/ml of insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5, zinc or salts thereof, isotonic agent optionally along with one or more pharmaceutically acceptable excipients, wherein the formulation exhibits an extended release profile for a period of more than 24h; wherein the particle size of the insulin glargine in the composition varies between 3 μ m to 60 μ m; wherein the pharmaceutical composition is devoid of any polymer and precipitating agent.
22. The pharmaceutical composition of claim 21, the pH of the composition is adjusted to a pH of between 5.8 and 8.5.

23. The pharmaceutical composition of claim 21, wherein the insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 is insulin glargine.
24. The pharmaceutical composition of claim 21, wherein the isotonic agents are selected from the group consisting of glycerol, mannitol, lactose, dextrose and sodium chloride.
25. The pharmaceutical composition of claim 21, wherein the pharmaceutical composition further comprises surfactant, buffer, preservatives, pH modifying agents or solubilizing agents.
26. The pharmaceutical composition of claim 23, wherein the pharmaceutical composition comprises 100IU of Insulin glargine.
27. The pharmaceutical composition of claim 21, wherein the composition comprises one or more pharmaceutical acceptable therapeutic agents selected from the group consisting of Insulin, Insulin aspart, Insulin aspart protamine, Insulin lispro, Insulin lispro protamine, Insulin glulisine, Viaject®, exenatide, Liraglutide, lixisenatide, semaglutide, dulaglutide, albiglutide, and ITCA 650.
28. A method for treating Type I and Type II Diabetes Mellitus in a patient comprising administering a pharmaceutical composition of claim 21 to a patient in need thereof.
29. A method of reducing the nocturnal hypoglycemia, wherein nocturnal hypoglycemia in patients suffering from Type I or Type II diabetes, wherein the method comprise administering to the said patient comprising an extended release composition comprising 40-200IU of an insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 having pH between 6.0-8.5, wherein the composition comprises crystals of an insulin, insulin analogue, derivative or metabolite having isoelectric point between 5.8 to 8.5 optionally along with one or more excipients, wherein the said crystal have an average particle size between about 2 μm to about 60 μm ; wherein the pharmaceutical composition is devoid of any polymer and precipitating agent.

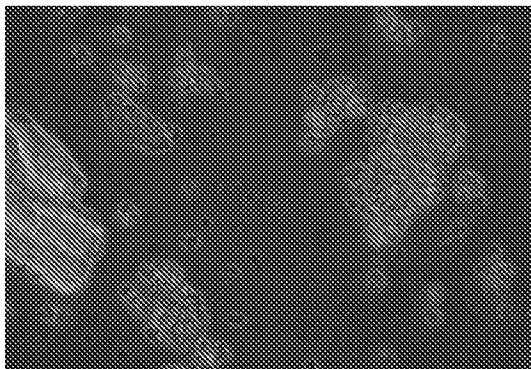
Wockhardt Limited

No. of sheets = 02

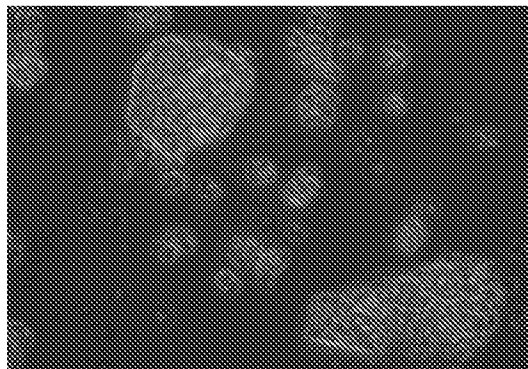
Application No.

Sheet 01 of 02

Figure 1: The two formulations of Insulin Glargine 300IU/mL and 100 IU/mL after micro-precipitation were observed under the optical microscope (Olympus BX40) at 100X in oil immersion



(a) Particle size of 300IU Insulin glargine



(b) Particle size of 100IU insulin glargine

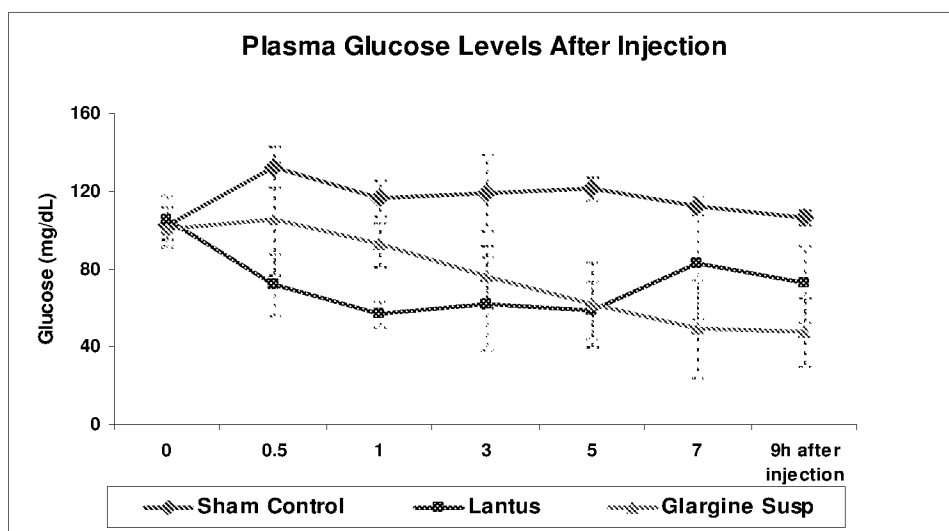
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No. of sheets = 02

Application No.

Sheet 02 of 02

Figure 2: Comparative analysis of glucose level in blood plasma after injection of Control, Lantus and 100IU Insulin Glargine suspension in rat



INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2015/054974

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K9/00 A61K47/02 A61K47/26
ADD.

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

B. FIELDS SEARCHED

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

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, 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	US 2013/178415 A1 (SOULA OLIVIER [FR]) 11 July 2013 (2013-07-11) claims; figure 1; examples paragraphs [0036] - [0045], [0266] -----	1-29
X	US 2011/021423 A1 (OLSEN HELLE BIRK [DK] ET AL) 27 January 2011 (2011-01-27) paragraphs [0072] - [0087], [0136] - [0142], [0151] - [0153] claims; examples -----	1-29
X	US 2013/085101 A1 (WEISS MICHAEL [US]) 4 April 2013 (2013-04-04) paragraphs [0060] - [0072] claims; figures; examples ----- -/-	1-6,8-10



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"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

7 October 2015

Date of mailing of the international search report

14/10/2015

Name and mailing address of the ISA/

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Ceyte, Mathilde

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2015/054974

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FR 2 978 918 A1 (ADOCIA [FR]) 15 February 2013 (2013-02-15) the whole document	1-5
A	----- US 2011/281790 A1 (POHL RODERIKE [US] ET AL) 17 November 2011 (2011-11-17) cited in the application the whole document	1-29
A	----- US 2013/331320 A1 (HAVE Lund SVEND [DK] ET AL) 12 December 2013 (2013-12-12) the whole document	1-29

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2015/054974

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