

RAPID-ACTING INSULIN COMPOSITIONS

The present invention is a pharmaceutical insulin composition for parenteral injection to counteract prandial and post-prandial blood glucose excursions. The composition includes an insulin and a polyphosphate compound, and has faster uptake of insulin from injection sites than existing commercial insulin compositions. The composition is useful for rapidly providing insulin activity when insulin is needed, e.g., when food is consumed.

Diabetes mellitus is a chronic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Type 1 diabetes mellitus is characterized by little or no insulin secretory capacity, and patients with type 1 diabetes mellitus require insulin for survival. In type 2 diabetes mellitus, the combined effects of impaired insulin secretion and insulin resistance result in elevated blood glucose levels. In at least one-third of patients with type 2 diabetes mellitus the disease progresses to an absolute requirement for insulin therapy.

The time-action profile of insulin is important for controlling post-prandial blood glucose levels. In healthy individuals, the pancreas secretes a spike of insulin in response to absorbed food, which results in increased blood insulin levels within several minutes. In individuals with type 1 diabetes and in certain individuals with type 2 diabetes, insulin must be administered. However, administered insulin enters the blood more slowly than endogenously secreted insulin, and slow onset may result in hyperglycemia during the early postprandial period. Too long duration of action can result in excessive insulin between meals which results in late postprandial hypoglycemia and/or weight gain.

There have been previous attempts to accelerate the time-action of insulin products. The “rapid-acting” insulin analogs became available in the 1990s and early 2000s. Even with so-called “rapid-acting” insulin analogs, such as insulin lispro (HUMALOG®), insulin aspart (NOVOLOG®) and insulin glulisine (APIDRA®), the maximum circulating insulin concentration is not reached until 50-90 minutes following the injection. This is not rapid enough to match carbohydrate absorption profiles.

Research has been conducted more recently in attempts to develop a product with more rapid time action profile than those described above. For example, US2014/0378383 discloses insulin compositions containing a combination of a

substituted anionic compound consisting of a saccharide backbone formed from between 1 and 8 hexose saccharide units having partially substituted carboxyl functional groups with a polyanionic compound, and states that such a combination makes it possible to accelerate the passage of the insulin into the blood. Similarly, US2015/0231160 discloses
5 insulin compositions containing a combination of an oligosaccharide and a polyanionic compound, and states this combination allows a significant reduction in the time for the start of action of a formulation of rapid-acting insulin analog. These disclosures each describe an array of polyanionic compounds, including polyphosphoric acids such as triphosphate, but no compositions are described which contain triphosphate but do not
10 also contain either a substituted anionic compound, as that term is used in US2014/0378373, or an oligosaccharide, as described in US2015/0231160, and no data are provided on the pharmacokinetics or pharmacodynamics of compositions containing triphosphate or any other polyphosphoric acid. US2014357554 and US2015273022 describe compositions said to have rapid onset of insulin action which contain EDTA,
15 citrate, and magnesium containing compounds, one example of which is magnesium pyrophosphate. The magnesium compound is stated to minimize injection site irritation “but not change the rate of subcutaneous absorption,” and no data on compositions containing magnesium pyrophosphate are described.

There remains a need for compositions of insulin, intended for use at meal-time,
20 that have more rapid uptake of insulin from the injection site and more rapid onsets of action than existing commercial insulin products.

The present invention seeks to meet these needs by providing pharmaceutically-acceptable, formulations of insulin that have more rapid uptake of insulin into the blood and more rapid onset of action than existing commercial insulin products.

25 According to a first aspect of the present invention, there is provided a pharmaceutical composition comprising an insulin and a polyphosphate compound selected from the group consisting of pyrophosphate, trimetaphosphate, triphosphate, and tetrathosphate, provided that the composition does not contain either a saccharide multimer or EDTA.

30 In certain embodiments, the concentration of polyphosphate is from about 5 to about 50 mM. In certain embodiments, the concentration of polyphosphate is about 10 to

about 30 mM. In certain embodiments, the concentration of polyphosphate is about 20 to about 25 mM. In certain embodiments, the concentration of polyphosphate is selected from the group consisting of 5, 10, 15, 20, 25 or 30 mM.

In certain embodiments, the composition further comprises zinc. In certain
5 embodiments, the zinc concentration is from about 0.2 to about 5 mM.

In certain embodiments, the composition further comprises a tonicity agent. In certain embodiments, the tonicity agent is glycerol.

In certain embodiments, the composition further comprises one or more
preservatives. In certain embodiments, the one or more preservatives are selected from
10 the group consisting of phenol, meta-cresol, and benzyl alcohol.

In certain embodiments, the insulin is selected from the group consisting of human insulin, insulin lispro, insulin aspart and insulin glulisine. In certain embodiments, the insulin concentration is from about 40 to about 500 IU/mL. In certain embodiments, the insulin concentration is from about 100 to about 200 IU/mL.

15 According to another aspect of the present invention, there is provided a method of treating diabetes comprising administering to a human in need thereof an effective dose of one of the above-described compositions.

According to another aspect of the present invention, there is provided one of the above-described compositions for use as a medicament.

20 According to another aspect of the present invention, there is provided one of the above-described compositions for use in the treatment of diabetes.

According to another aspect of the present invention, there is provided an article of manufacture comprising one of the above-described compositions. In certain
embodiments, the article of manufacture is a multi-use vial. In certain embodiments, the
25 article of manufacture is a pre-filled, disposable pen. In certain embodiments, the article of manufacture is a re-usable pen. In certain embodiments, the article of manufacture is an autoinjector. In certain embodiments, the article of manufacture is a pump for continuous subcutaneous insulin infusion (CSII).

When used herein, "saccharide multimer" means any compound containing more
30 than one saccharide unit bound together, including for example the substituted anionic

compounds described in US2014/0378383 and the oligosaccharides described in US2015/0231160.

When used herein, the term “does not contain a saccharide multimer or EDTA” means that the composition contains no saccharide multimers or EDTA, or contains only
5 a *de minimis* quantity of saccharide multimers or EDTA such that the time action profile of the insulin is unaffected.

When used herein, “insulin” means human insulin or a structural variant, mutant, or analog of human insulin that has the functional activity of human insulin. Analogs of human insulin include but are not limited to insulin lispro, insulin aspart, and insulin
10 glulisine, or other “rapid-acting” insulin analogs. Insulin for commercial products may be produced using recombinant DNA methods or by chemical synthesis. Recombinant methods are well-known and are strongly preferred. A molecule of human insulin (CAS No. 11061-68-0) consists of two amino acid chains, A and B, whose sequences are well-known. The chains are joined by two disulfide bonds: CysA7-CysB7 and CysA20-
15 CysB19. The A-chain has an intra-chain disulfide bond at CysA6-CysA11.

The human insulin A-chain has the following sequence of amino acids:
Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Asn Tyr Cys Asn
(SEQ ID NO:1)

The human insulin B-chain has the following sequence of amino acids:
20 Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly
Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr (SEQ ID NO: 2).

Insulin lispro (CAS No. 133107-64-9), the drug substance in HUMALOG[®], has been shown to be equipotent to human insulin on a molar basis but its effect after subcutaneous injection is more rapid and of shorter duration than that of injected soluble
25 human insulin. A consistent pattern of kinetics with a shorter T_{max} and half-life and with a higher C_{max} was observed for insulin lispro when compared to the human insulin. Insulin lispro is biologically equivalent to insulin in several *in vitro* tests including insulin receptor binding in cultured lymphocytes, human placenta and human liver, and glucose transport in adipocytes. HUMALOG[®] contains m-cresol as a preservative and a
30 stabilizer, a tonicity modifier (glycerin), a buffering agent (dibasic sodium phosphate), a stabilizer (zinc oxide) and pH adjustment for the vehicle.

A molecule of insulin lispro consists of the human insulin A-chain cross-linked with the insulin lispro B-chain, whose amino acid sequence is given by SEQ ID NO:3, below:

Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly
5 Glu Arg Gly Phe Phe Tyr Thr Lys Pro Thr (SEQ ID NO: 3).

One unit of insulin lispro is equivalent to 0.0347mg insulin lispro.

Insulin aspart (CAS No. 116094-23-6), the drug substance in NOVOLOG®, is another rapid-onset insulin analog. Its structure consists of the A-chain of human insulin and a B-chain analog as reflected in the following amino acid sequence:

10 Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly
Glu Arg Gly Phe Phe Tyr Thr Asp Lys Thr (SEQ ID NO: 4).

One unit of insulin aspart corresponds to 6 nmol, corresponding with 0.035 mg salt-free anhydrous insulin aspart.

Insulin glulisine (CAS No. 207748-29-6), the drug substance in APIDRA®, is yet
15 another rapid-onset insulin analog. A molecule of insulin glulisine consists of human insulin A-chain and a modified B-chain compared with human insulin, as reflected in the following amino acid sequence:

Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly
Glu Arg Gly Phe Phe Tyr Thr Pro Glu Thr (SEQ ID NO: 5).

20 One unit of insulin glulisine corresponds approximately to 0.0349 mg of insulin glulisine.

The compositions of the present invention have concentrations of insulin between 0.24 and 3 mM (40 - 500 IU/mL; 1.4 mg/mL – 17.5 mg/mL). The compositions of the present invention are likely to have specific concentrations of 40, 100, 200, 300, 400, and 500 IU/mL (1.4, 3.5, 7, 10.5, 14, and 17.5 mg/mL). Preferred concentrations are 100 and
25 200 IU/mL.

Polyphosphates are inorganic, multi-charged, polyvalent anions consisting of 2 or more phosphate groups covalently bonded via P-O-P bonds. They are widely used in detergents, foods, cosmetics, and biomedical applications as chelating agents, buffers, and cross-linking agents, among other uses. A number of polyphosphates are “generally
30 regarded as safe” by the U.S. Food and Drug Administration for use in foods (“GRAS”), including for example those listed in Table 1 below.

GRAS Substance	Formula (m.w.)	CAS No.	21 CFR
Calcium pyrophosphate	$\text{Ca}_2\text{P}_2\text{O}_7$ (254.1)	7790-76-3	182.8223
Potassium pyrophosphate	$\text{K}_4\text{P}_2\text{O}_7$ (330.3)	7320-34-5	None
Potassium tripolyphosphate	$\text{K}_5\text{P}_3\text{O}_{10}$ (453.5)	13845-36-8	None
Sodium acid pyrophosphate	$\text{Na}_2\text{H}_2\text{P}_2\text{O}_7$ (221.9)	7758-16-9	182.1087
Sodium pyrophosphate	$\text{Na}_4\text{P}_2\text{O}_7$ (265.9)	7722-88-5	182.6760
Sodium tetrakisphosphate	$\text{Na}_6\text{P}_4\text{O}_{13}$ (469.8)	14986-84-6	None
Sodium trimetaphosphate	$\text{Na}_3\text{P}_3\text{O}_9$ (305.9)	7785-84-4	None
Sodium tripolyphosphate	$\text{Na}_5\text{P}_3\text{O}_{10}$ (367.9)	7758-29-4	182.1810 182.1610

Table 1. Examples of GRAS polyphosphates.

5 Triphosphate has been used as a cross-linking agent in polymer-based nanocarriers, especially chitosan-based nanocarriers, for oral, nasal, parenteral, or transdermal delivery of a large range of medically-important payloads, such as antigens, anti-cancer drugs, genetic materials, and proteins, including insulins. Kouchak, *et al.*, “Effect of different molecular weights of chitosan on preparation and characterization of insulin loaded nanoparticles by ion gelation method,” 4 Internat’l J. Drug Dev. & Res. 10 271 (2012). Unlike the present invention, however, such carriers are not directed towards accelerating the time action profile of administered insulin.

 The polyphosphates shown herein to be useful for increasing the rate of insulin absorption from injection sites are pyrophosphate (diphosphate, $[\text{O}_3\text{-P-O-P-O}_3]^{-4}$, P_2O_7) 15 and triphosphate ($[\text{O}_3\text{-P-O-(PO}_2\text{)-O-P-O}_3]^{-5}$, P_3O_{10}). The effects on insulin absorption are believed to be provided by these polyphosphates as well as trimetaphosphate (P_3O_9)⁻ and tetrakisphosphate (P_4O_{13})⁻⁶. The particular polyphosphate compound used may be the acidic form or various salt forms, especially the alkali (e.g., sodium and potassium) salts. Pyrophosphate, triphosphate, trimetaphosphate and tetrakisphosphate, and their various salts, 20 especially their alkali (e.g., sodium and potassium) and alkaline earth metal (e.g., calcium and magnesium) salts may be used in the present invention. Of these, triphosphate and

salts thereof are preferred. The concentration of polyphosphate in the compositions ranges from 5 mM to 50 mM, particularly 5, 10, 15, 20, 25, 30, 35, 40 or 50 mM. Certain compositions have polyphosphate concentrations in the range of 10 mM to 30 mM. Certain compositions have polyphosphate concentrations in the range of 15 mM to 25 mM.

Commercial insulin compositions have about 2.4 atoms of zinc per six molecules of insulin (HUMULIN® R U-500), and some have about 3.0 atoms of zinc per six molecules of insulin (HUMALOG®, NOVOLOG®). Certain embodiments of the present invention include zinc in a concentration sufficient to provide between about 2-4 zinc atoms per six molecules of insulin. Other embodiments include zinc in a concentration of up to about 5 mM. In certain embodiments, the concentration of zinc ranges from about 0.2 to about 5 mM. In certain embodiments, the concentration of zinc ranges from about 0.5 to about 2 mM. In certain embodiments, the concentration of zinc is selected from the group consisting of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.3 and 2 mM.

The compositions are sterile when first produced. When provided in a multi-use vial or cartridge, an anti-microbial preservative compound or mixture of compounds that is compatible with the other components of the formulation is typically added at sufficient strength to meet regulatory and pharmacopoeial anti-microbial preservative requirements. See U.S. Pharmacopeia Monographs. Insulin lispro injection. USP29-NF24; British Pharmacopeia Monographs 2008 Volume III: Insulin aspart injection; U.S. Pharmacopeia Monographs. Insulin assays; and U.S. Pharmacopeia general chapters. USP29-NF24. Rockville, MD: U.S. Pharmacopeial Convention; 2005. Antimicrobial effectiveness testing; pp. 2499–2500. Preferred preservatives are aryl acids and phenolic compounds, or mixtures of such compounds. Preservatives most commonly used in insulin products are phenol, m-cresol, and benzyl alcohol. Effective concentrations can be ascertained readily using the methods referenced above. Present commercial compositions, for example, contain 3.15 mg/mL m-cresol (HUMALOG® and APIDRA®), 1.72 mg/mL m-cresol and 1.50 mg/mL phenol (NOVOLOG®), and 2.5 mg/mL m-cresol (HUMULIN® R U-500).

The pH of insulin compositions of the present invention is typically 7.0 to 7.8 and it is adjusted using physiologically appropriate acids and bases, typically hydrochloric acid 10% and sodium hydroxide 10%. The pH for commercial insulin formulations is usually in the range 7.2 to 7.6, with 7.4 ± 0.1 as a common target pH.

5 It is desirable to approximately match the tonicity (i.e., osmolality) of body fluids at the injection site as closely as possible when administering the compositions because solutions that are not approximately isotonic with body fluids can produce a painful stinging sensation when administered. Thus, it is desirable that the compositions be approximately isotonic with body fluids at the sites of injection. If the osmolality of a
10 composition in the absence of a tonicity agent is sufficiently less than the osmolality of the tissue (for blood, about 300 mOsm/kg; the European Pharmacopeial requirement for osmolality is > 240 mOsm/kg), then a tonicity agent should generally be added to raise the tonicity of the composition to about 300 mOsm/kg. Typical tonicity agents are glycerol (glycerin) and sodium chloride. The amount of tonicity agent to add is readily
15 determined using standard techniques. Remington: The Science and Practice of Pharmacy, David B. Troy and Paul Beringer, eds., Lippincott Williams & Wilkins, 2006, pp. 257-259; Remington: Essentials of Pharmaceutics, Linda Ed Felton, Pharmaceutical Press, 2013, pp. 277-300.

20 The compositions of the present invention are typically administered subcutaneously, either in multiple daily injections (MDI) from a pre-filled, disposable pen, reusable pen, automatic pen injector, multi-use vial or a pump for CSII.

Additional embodiments of the present invention include those described below:

1. A pharmaceutical composition comprising an insulin and a polyphosphate compound selected from the group consisting of pyrophosphate, triphosphate,
25 trimetaphosphate and tetraphosphate.
2. The pharmaceutical composition of any of the above embodiments wherein the composition does not contain either a saccharide multimer or EDTA.
3. The pharmaceutical composition of any of the above embodiments wherein the polyphosphate compound is triphosphate.
- 30 4. The pharmaceutical composition of any of the above embodiments wherein the polyphosphate compound is pyrophosphate.

5. The pharmaceutical composition of any of the above embodiments wherein the concentration of the polyphosphate is from about 5 to about 50 mM.

6. The pharmaceutical composition of any of the above embodiments wherein the concentration of the polyphosphate is from about 10 to about 30 mM.

5 7. The pharmaceutical composition of any of the above embodiments wherein the concentration of the polyphosphate is selected from the group consisting of 5, 10, 15, 20, 25, 30, 35, 40 and 50 mM.

8. The pharmaceutical composition of any of the above embodiments further comprising zinc.

10 9. The pharmaceutical composition of any of the above embodiments wherein the zinc concentration is from about 0.2 to about 5 mM.

10. The pharmaceutical composition of any of the above embodiments wherein the concentration of zinc ranges from about 0.5 to about 2 mM.

11. The pharmaceutical composition of any of the above embodiments wherein
15 the concentration of zinc is selected from the group consisting of 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.3, 2 and 5 mM.

12. The pharmaceutical composition of any of the above embodiments further comprising a tonicity agent.

13. The pharmaceutical composition of any of the above embodiments further
20 comprising a tonicity agent which is glycerol.

14. The pharmaceutical composition of any of the above embodiments further comprising one or more preservatives.

15. The pharmaceutical composition of any of the above embodiments wherein
25 the one or more preservatives are selected from the group consisting of phenol, meta-cresol, and benzyl alcohol.

16. The pharmaceutical composition of any of the above embodiments wherein the insulin is selected from the group consisting of human insulin, insulin lispro, insulin aspart and insulin glulisine.

17. The pharmaceutical composition of any of the above embodiments wherein
30 the insulin concentration is from about 40 to about 500 IU/mL.

18. The pharmaceutical composition of any of the above embodiments wherein the insulin concentration is from about 100 to about 200 IU/mL.

19. A method of treating diabetes comprising administering to a human in need thereof an effective dose of the pharmaceutical composition of any of the above
5 embodiments.

20. The pharmaceutical composition of any of the above embodiments for use as a medicament.

21. The pharmaceutical composition of any of the above embodiments for use in the treatment of diabetes.

10 22. The pharmaceutical composition of any of the above embodiments comprising a mixture of two or more polyphosphate compounds selected from the group consisting of pyrophosphate, triphosphate, trimetaphosphate and tetrphosphate.

23. An article of manufacture comprising any one of the above-described pharmaceutical compositions.

15 24. A multi-use vial containing any one of the above-described pharmaceutical compositions.

25. A re-usable pen injector containing any one of the above-described pharmaceutical compositions.

20 26. A pre-filled, disposable pen injector containing any one of the above-described pharmaceutical compositions.

27. An automatic pen injector containing any one of the above-described pharmaceutical compositions.

28. A pump for CSII containing any one of the above-described pharmaceutical compositions.

25 **Pharmacokinetic (PK) and Pharmacodynamic (PD) Studies**

Study 1. 25 mM Pyrophosphate or 25 mM Triphosphate

 Fifteen diabetic (alloxan induced), castrated, male Yucatan miniature swine (average age 17 months old and average body weight 40 kgs) with previously fitted vascular access ports are used. The diabetic animals are housed individually and have ad
30 lib access to fresh water at all times. They are fed two meals per day of house diet S-9

and receive appropriate maintenance basal and prandial insulin twice per day to manage their diabetic condition.

Test articles (Formulation A and B) are formulated and shipped overnight on cold packs to the study site. They are stored refrigerated until time of dosing and then returned to the refrigerator after dosing of all animals was complete. During the dosing period the test articles remain in an insulated box when not being withdrawn from. HUMALOG® insulin control is from a commercial vial.

Formulation Name	Formulation Composition
HUMALOG	3.5 mg/mL insulin lispro 7 mM sodium phosphate 0.3 mM 3.15 mg/mL m-cresol 16 mg/mL glycerin pH 7.4
insulin lispro + 25 mM Pyrophosphate (Formulation A)	92 U/mL insulin lispro (3.2 mg/mL) 7 mM sodium phosphate 0.3 mM zinc 3.15 mg/mL m-cresol 6.33 mg/mL glycerin 25 mM sodium pyrophosphate pH 7.4
insulin lispro + 25 mM Triphosphate (Formulation B)	94 U/mL insulin lispro (3.3 mg/mL) 7 mM sodium phosphate 0.3 mM zinc 3.15 mg/mL m-cresol 4.49 mg/mL glycerin 25 mM sodium triphosphate pH 7.4

Table 2. Compositions of test and control articles.

The study is designed as a three-way cross-over design. This design allows for each individual animal to receive each of the three test articles by dosing one test article each study date (3 dates each 7 days apart). The day prior to study, animals are fed half their daily ration and received 0.2 U/kg Humalog Mix 75/25 Insulin at their morning maintenance administration. All study animals are food-fasted overnight and do not receive their evening insulin or meal prior to drug administration on study day.

On the morning of study, all animals are placed into slings for restraint and have their vascular access ports accessed (equipped for blood sampling) and checked for

patency. The animals are randomly placed into treatment groups (3 groups n=5 per group yields n=15 per treatment).

After two baseline blood samples are collected (-30 and -20 min), the animals are returned to their pens and fed ~300 g S-9 diet. Twenty minutes after the presentation of the fully consumed meal, the animals are injected with test article subcutaneously in the flank (0 min) with a Terumo insulin syringe (0.5 ml 1/2" needle). Dosing involves a single injection of 0.2 U/kg of insulin activity. All study animals have ad libitum access to clean, fresh water throughout the remaining blood collection period.

Serial blood samples (2.0 mL each) are collected from each animal at the following time points: -30, -20 (then immediately Fed), 0 (just before dose), 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 240, and 360 minutes following the SC dosing. Blood samples (anticoagulant: none [serum]) are maintained at ambient temperature for at least 30 minutes but no more than 2 hours to allow for clotting. Serum is then separated by centrifugation and divided into two aliquots and stored frozen at approximately -70 °C.

Serum glucose concentrations are determined using an automated Cobas c311 Clinical Chemistry Analyzer (Roche Diagnostics, Indianapolis). Two animals are excluded from the Humalog treatment group yielding n=13 for that test article. One animal is excluded due to not meeting the criteria for baseline glucose of >200 mg/dL and another animal did not participate due to port patency issues. Serum glucose concentrations (mg/dL) after treatment with insulin lispro-containing formulations (0.2 U/kg at time 0) are provided in Table 3 below.

Time (min)	Humalog (n=13)		Formulation A (n=15) 25 mM pyrophosphate		Formulation B (n=15) 25 mM triphosphate	
	Average	St. dev.	Average	St. dev.	Average	St. dev.
-30	293	46	270	77	292	43
-20	297	47	293	40	299	46
0	322	52	304	48	306	45
5	325	45	312	52	309	48
10	313	51	245	48	238	50
15	285	60	201	47	189	53
30	216	80	133	56	108	56

	Humalog (n=13)		Formulation A (n=15) 25 mM pyrophosphate		Formulation B (n=15) 25 mM triphosphate	
Time (min)	Average	St. dev.	Average	St. dev.	Average	St. dev.
45	165	80	99	63	70	51
60	131	87	70	45	52	54
75	104	80	57	39	44	52
90	83	70	44	35	35	42
105	68	62	40	33	34	36
120	58	52	37	34	35	38
150	49	43	38	38	38	27
180	48	40	47	48	44	33
240	69	44	70	73	74	59
360	143	73	113	87	151	106

Table 3. Serum glucose concentrations (mg/dL).

Serum insulin concentrations are determined using a competitive radioimmunoassay (RIA). In the RIA, which measures both endogenous pig insulin and exogenous insulin, serum insulin displaced ¹²⁵I-insulin for binding to guinea pig anti-rat insulin. The antibody complex is precipitated with a goat anti-guinea pig IgG serum reagent. The upper and lower limits of quantitation of the RIA are 5000 and 20 pM, respectively, in heat-treated charcoal-stripped serum. Non-compartmental pharmacokinetic analysis is performed using Phoenix WinNonLin 6.3. Values below the lower limit of quantitation are assigned a value of 20 pM for calculations. Samples above the upper limit of quantitation are either diluted and reanalyzed or were ignored. As noted above, one animal from the HUMALOG treatment did not participate in the study due to port patency issues, yielding n=14 for that test article.

Formulation		Tmax (min)	Cmax (nM)	AUC _∞ (min*nM)	CL/F (mL/min/kg)
Humalog (n=14)	Mean±SE	61.1±11.1	1.30±0.25	141±21.2	12.1±1.97
	Median	45.0	1.08	142	8.43
Formulation A (n=15) 25 mM Pyrophosphate	Mean±SE	32.3±7.6	1.29±0.12	113±10.7	10.6±1.12
	Median	30.0	1.30	107	11.1
Formulation B (n=15) 25 mM Triphosphate	Mean±SE	14.3±1.88	2.93±0.34	220±31.4	6.88±0.764
	Median	15.0	2.53	159	7.56

Table 4. PK data. Abbreviations = Tmax – time at maximal concentration, Cmax – maximal concentration, AUCINF – area under the curve from 0 to infinity, CL/F – clearance/bioavailability.

The PK/PD data demonstrate that pyrophosphate or triphosphate accelerated time-
5 action and reduced Tmax as compared with HUMALOG. Formulation A with
pyrophosphate had Tmax that was ~47% faster than HUMALOG (~33% faster by median
Tmax) and had a comparable mean Cmax to HUMALOG. Formulation B with
triphosphate had ~77% faster mean Tmax than HUMALOG (~67% faster in median
Tmax) and ~125% higher Cmax than Humalog. Formulations containing 25 mM
10 pyrophosphate or 25 mM triphosphate caused faster Tmax and higher Cmax as compared
with HUMALOG.

Study 2. Effect of Triphosphate Concentration on PK/PD

Fourteen diabetic (Alloxan induced), castrated, male Yucatan miniature swine
(average age 14 mos old and average body weight 35 kgs) with previously fitted vascular
15 access ports are used. The diabetic animals are housed individually and have ad lib
access to fresh water at all times. They are fed two meals per day of house diet S-9 and
receive appropriate maintenance basal and prandial insulin twice per day to manage their
diabetic condition.

Test articles are formulated and shipped overnight on cold packs. They are stored
20 refrigerated until time of dosing and then returned to the refrigerator after dosing of all
animals was complete. During the dosing period the test articles remained in an insulated
box when not being withdrawn from. HUMALOG control is a commercial vial.

Formulation Name	Formulation Composition
HUMALOG	3.5 mg/mL insulin lispro 7 mM sodium phosphate, pH 7.4 0.3 mM Zn molecules 16 mg/mL glycerin 3.15 mg/mL m-cresol

Formulation Name	Formulation Composition
Formulation C: insulin lispro + 5 mM triphosphate	3.5 mg/mL insulin lispro 7 mM sodium phosphate 0.3 mM zinc 13.70 mg/mL glycerin 3.15 mg/mL m-cresol 5 mM sodium triphosphate, pH 7.4
Formulation D: insulin lispro + 10 mM triphosphate	3.5 mg/mL insulin lispro 7 mM sodium phosphate 0.3 mM zinc 11.40 mg/mL glycerin 3.15 mg/mL m-cresol 10 mM sodium triphosphate, pH 7.4
Formulation E: insulin lispro + 20 mM triphosphate	3.5 mg/mL insulin lispro 7 mM sodium phosphate 0.3 mM zinc 6.79 mg/mL glycerin 3.15 mg/mL m-cresol 20 mM sodium triphosphate, pH 7.4

Table 5. Compositions of test and control articles.

The study is designed a four-way cross over design allowing for each individual animal to receive each of the three test articles and the control by dosing one test article on each study date (4 dates each, 7 days apart).

- 5 The day prior to study, animals are fed half their daily ration and received 0.2 U/kg Humalog Mix 75/25 Insulin at their morning maintenance administration. All study animals are food-fasted overnight and do not receive their evening insulin or meal prior to drug administration on study day.

- 10 On the morning of study, all animals are placed into slings for restraint and have their vascular access ports accessed (equipped for blood sampling) and checked for patency. The animals are randomly placed into treatment groups (4 groups n=3-4 per group yields n=14 per treatment). Two pigs are never on study due to being on vet observation which caused a total n=14 to be reduced to n=12 prior to any other exclusions. One animal is excluded from the HUMALOG group and two animals are
15 excluded from the insulin lispro + Triphosphate 10 mM group for not meeting inclusion criteria, thereby yielding n=11 and n=10 respectively for those groups.

After two baseline blood samples are collected (-30 and -20 min), the animals are returned to their pens and fed ~300 g S-9 diet. Twenty minutes after the presentation of the fully consumed meal, the animals are injected with test article subcutaneously in the flank (0 min) with a Terumo insulin syringe (0.5 mL, 1/2" needle). All study animals have ad libitum access to clean, fresh water throughout the remaining blood collection period.

Serial blood samples (2.0 mL each) are collected from each animal at the following time points: -30, -20 (then immediately Fed), 0 (just before dose), 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 240, and 360 minutes following the SC dosing. Blood samples (anticoagulant: none [serum]) were maintained at ambient temperature for at least 30 minutes but no more than 2 hours to allow for clotting. Serum is then separated by centrifugation and divided into two aliquots and stored frozen at approximately -70 °C. Aliquots are shipped on dry ice by a next day shipping service.

Serum insulin concentrations are determined using a competitive radioimmunoassay (RIA), as described above. Data are analyzed utilizing non-compartmental pharmacokinetic analysis using Phoenix WinNonLin 6.3, as described above. Serum glucose concentrations are determined using an automated Cobas c311 Clinical Chemistry Analyzer (Roche Diagnostics, Indianapolis, Indiana).

Serum glucose results (mg/dL) are given in Table 6 below.

	HUMALOG		Formulation C insulin lispro + 5 mM triphosphate		Formulation D insulin lispro + 10 mM triphosphate		Formulation E insulin lispro + 20 mM triphosphate	
Time (min)	Average	St. dev.	Average	St. dev.	Average	St. dev.	Average	St. dev.
-30	287	63	266	49	283	43	285	34
-20	299	65	275	52	295	40	292	37
0	306	65	280	54	294	43	299	43
5	314	64	281	53	294	43	300	49
10	320	61	252	47	266	39	256	52
15	314	59	221	45	232	40	215	56
30	305	65	161	50	171	44	146	59
45	277	73	141	57	141	47	114	59
60	246	74	126	64	126	60	99	61
75	219	73	113	63	113	60	96	63

	HUMALOG		Formulation C insulin lispro + 5 mM triphosphate		Formulation D insulin lispro + 10 mM triphosphate		Formulation E insulin lispro + 20 mM triphosphate	
Time (min)	Average	St. dev.	Average	St. dev.	Average	St. dev.	Average	St. dev.
90	183	76	106	73	102	57	92	63
105	160	71	95	63	99	57	96	63
120	140	72	82	60	94	55	93	60
150	117	72	77	52	81	51	96	58
180	105	80	77	59	72	48	106	71
240	133	93	115	83	99	67	163	101
360	219	93	164	94	163	94	197	92

Table 6. Serum glucose results (mg/dL).

PK parameters as a function of triphosphate concentration are given in Table 7 below.

Formulation		Tmax (min)	Cmax (nM)	AUC _{INF} (min*nM)	CL/F (mL/min/kg)
Humalog N=12	Mean ± SE	58.8 ± 6.0	0.89 ± 0.098	127 ± 12	10.3 ± 0.89
	Median	60	1.03	119	10.2
Formulation C: insulin lispro + 5 mM triphosphate N=12	Mean ± SE	52.5 ± 13.0	1.05 ± 0.093	132 ± 25	11.6 ± 1.5
	Median	37.5	1.12	120	9.97
Formulation D: insulin lispro + 10 mM triphosphate N=10	Mean ± SE	28.0 ± 10.6	1.23 ± 0.15	127 ± 29	12.0 ± 1.5
	Median	15.0	1.21	98.6	12.2
Formulation E: insulin lispro + 20 mM triphosphate N=12	Mean ± SE	12.9 ± 2.5	1.67 ± 0.14	116 ± 13	11.8 ± 1.2
	Median	10.0	1.84	109	11.0

Table 7. PK data. Abbreviations: Tmax – time at maximal insulin concentration, Cmax –
5 maximal insulin concentration, AUC_{INF} – area under the curve from 0 to infinity, CL/F –
clearance/bioavailability

Triphosphate at 5, 10, or 20 mM in formulations similar to HUMALOG
accelerated time action and reduced Tmax and increased Cmax as compared with
HUMALOG® and did so in a dose-dependent manner.

10 Study 3. Effect on Different Commercial Insulins

Fifteen diabetic (Alloxan induced), castrated, male Yucatan miniature swine with previously fitted vascular access ports are used to study the effect of triphosphate on the serum glucose and serum insulin time action profiles of different commercial insulins.

Housing and nutrition of the animals and shipment and storage of the test and control

5 articles are as described above in Studies 1 and 2.

Test articles (Formulations F, G and H in the table below) are formulated by adding sufficient triphosphate to commercial vials of HUMULIN-R®, NOVOLOG® and APIDRA® to reach a concentration of 20 mM triphosphate. Note that the concentrations of other ingredients listed in the table below reflect the concentrations of those

10 ingredients in the commercial vials of those products; the concentrations have not been adjusted to account for the slight dilution resulting from the addition of triphosphate.

Formulation Name	Formulation Composition
HUMALOG	3.5 mg/mL insulin lispro 7 mM sodium phosphate, pH 7.4 0.3 mM Zn 16 mg/mL glycerin 3.15 mg/mL m-cresol
Formulation F: NOVOLOG + 20 mM triphosphate	100 Units/mL insulin aspart 19.6 mcg/mL zinc 16 mg/mL glycerin 1.50 mg/mL phenol 1.72 mg/mL m-cresol 1.25 mg/mL disodium hydrogen phosphate dehydrate 0.58 mg/mL sodium chloride 20 mM sodium triphosphate
Formulation G: APIDRA + 20 mM triphosphate	100 units/mL insulin glulisine 3.15 mg/mL m-cresol 6 mg/mL tromethamine, 5 mg/mL sodium chloride 0.01 mg/mL polysorbate 20 20 mM sodium triphosphate
Formulation H: HUMULIN + 20 mM triphosphate	100 units/mL insulin 16 mg/mL glycerin 2.6 mg/mL m-cresol 0.23 mM Zn 20 mM sodium triphosphate

Table 8. Compositions of test and control articles.

The study is a four-way cross over design allowing for each individual animal to receive each of the three test articles and the control by dosing one test article on each study date (4 dates each, 7 days apart). Animals are prepared for the study as described above with respect to Studies 1 and 2.

5 The animals are randomly placed into treatment groups (4 groups n=3-4 per group yields n=15 per treatment). One animal is excluded from the HUMULIN R+ 20mM triphosphate and NOVOLOG+20 mM triphosphate groups due to port failure, yielding n=14 for those treatment groups. One animal is excluded from the HUMALOG group due to port failure and one animal is excluded from the HUMALOG group for not
10 meeting inclusion criteria, yielding n=13 for that group.

Collection of baseline blood samples, injection with test articles, collection of blood samples and preparation, shipment and measurement of blood and serum samples are performed as described above with respect to Studies 1 and 2.

Serum glucose concentrations are determined using an automated AU480 Clinical
15 Chemistry Analyzer (Beckman Coulter). Serum glucose results (mg/dL) are given in Table 9 below.

	HUMALOG		Formulation F: HUMULIN + 20 mM triphosphate		Formulation G: APIDRA + 20 mM triphosphate		Formulation H: NOVOLOG + 20 mM triphosphate	
Time (min)	Average	St. dev.	Average	St. dev.	Average	St. dev.	Average	St. dev.
-30	343	39	335	62	336	45	343	56
-20	360	38	356	64	350	45	355	58
0	372	43	368	61	366	42	367	48
5	386	37	371	63	378	39	373	51
10	362	39	315	64	307	37	296	50
15	348	39	274	65	276	45	255	50
30	274	65	209	73	201	63	177	56
45	239	82	187	85	169	76	139	59
60	190	90	184	101	143	76	118	67
75	173	82	185	110	130	79	113	76
90	150	77	175	115	114	67	105	82
105	136	67	164	118	104	64	103	84
120	129	68	164	124	102	68	102	82
150	116	67	145	123	94	68	94	78
180	111	73	134	122	91	65	92	71
240	131	80	120	108	107	65	103	74
360	174	101	116	111	149	86	149	88

Table 9. Serum glucose results (mg/dL).

Serum insulin concentrations are determined using a competitive radioimmunoassay (RIA), as described above with respect to Studies 1 and 2. Data are analyzed utilizing non-compartmental pharmacokinetic analysis using Phoenix

- 5 WinNonLin. Pharmacokinetic parameters as a function of triphosphate concentration are given in Table 10 below.

Formulation		Tmax (min)	Cmax (nM)	AUC _{INF} (min*nM)	CL/F (mL/min/kg)
HUMALOG N=13	Mean ± SE	58.8 ± 7.49	1.12 ± 0.176	129 ± 9.27	9.88 ± 0.669
	Median	60	1.00	120	9.99
Formulation F: HUMULIN + 20 mM triphosphate N=14	Mean ± SE	22.5 ± 9.55	1.45 ± 0.230	173 ± 14.6	7.48 ± 0.527
	Median	7.50	1.12	159	7.54
Formulation G: APIDRA + 20 mM triphosphate N=15	Mean ± SE	17.7 ± 4.02	1.65 ± 0.196	166 ± 11.7	7.75 ± 0.527
	Median	10.0	1.52	153	7.85
Formulation H: NOVOLOG + 20 mM triphosphate N=14	Mean ± SE	27.9 ± 10.8	1.10 ± 0.111	121 ± 12.7	10.9 ± 0.784
	Median	12.5	0.980	108	11.1

Table 10. PK data. Abbreviations: Tmax – time at maximal insulin concentration, Cmax – maximal insulin concentration, AUC_{INF} – area under the curve from 0 to infinity, CL/F – clearance/bioavailability.

The PK/PD data demonstrate that the use of triphosphate in formulations of various commercial insulins accelerated time-action and reduced Tmax as compared with HUMALOG with no triphosphate. All formulations containing 20 mM triphosphate caused faster Tmax as compared with HUMALOG alone. Formulations containing APIDRA and HUMULIN with 20 mM triphosphate caused higher Cmax as compared with HUMALOG alone.

10 Clinical Study

A clinical study is conducted to study the pharmacokinetic and pharmacodynamic effects of compositions of the present invention. The study is designed as a 5-period crossover study to compare the effects following subcutaneous (SC) doses of 4 formulations containing different concentrations of triphosphate with insulin lispro, as compared to a formulation of insulin lispro containing no triphosphate. Test articles are formulated by adding sufficient amounts of triphosphate and magnesium chloride to the U200 commercial formulation of insulin lispro to reach the concentrations indicated in Table 11 below:

Formulation Name	Formulation Composition
HUMALOG	100 units/mL insulin lispro 7 mM sodium phosphate 0.3 mM Zn 16 mg/mL glycerin 3.15 mg/mL m-cresol
Formulation I	+ 10 mM triphosphate
Formulation J	+ 20 mM triphosphate
Formulation K	+ 30 mM triphosphate
Formulation L	+ 30 mM triphosphate + 7.5 mM MgCl ₂

Table 11. Compositions of test and control articles. In addition to insulin lispro, the U-200 HUMALOG formulation to which triphosphate and MgCl₂ are added to create the test articles also contains 5 mg/mL tromethamine, 16 mg/mL glycerin, 3.15 mg/mL m-cresol and 0.046 mg/mL Zn²⁺.

5 Healthy subjects are enrolled and each subject is randomized to a treatment sequence, comprising single 15 unit SC doses of insulin lispro alone and a single 15 insulin unit SC dose of each of the test formulations. A minimum of 3 days is required between dosing occasions for an individual subject.

10 Blood samples are collected at multiple time points to determine the serum concentrations of insulin lispro over time. Serum concentrations of insulin lispro are measured using a validated enzyme-linked immunosorbent assay method specific for insulin lispro. Pharmacokinetic analyses are conducted using standard noncompartmental methods of analysis using Phoenix® version 7.0 (or higher) and S-PLUS® software (version 8.2). Free serum insulin lispro concentrations are used to calculate
15 pharmacokinetic parameters. Results are provided in Table 12 below.

	Treatment	LS Mean	Ratio Test:Reference (95% CI)
T _{onset} (min)	Humalog	1.78	
	Formulation I	0.12	0.07 (0.04, 0.10)
	Formulation J	0.10	0.05 (0.04, 0.08)
	Formulation K	0.09	0.05 (0.03, 0.07)
	Formulation L	0.08	0.04 (0.03, 0.07)
Early 50% T _{max} (min)	Humalog	19.06	
	Formulation I	6.04	0.32 (0.26, 0.38)
	Formulation J	5.04	0.26 (0.23, 0.31)
	Formulation K	4.73	0.25 (0.21, 0.30)
	Formulation L	4.58	0.24 (0.20, 0.29)
AUC _(0-15min) (pmol*h/L)	Humalog	19.87	
	Formulation I	136.98	6.89 (5.44, 8.74)
	Formulation J	155.78	7.84 (6.19, 9.93)
	Formulation K	156.21	7.86 (6.21, 9.96)
	Formulation L	157.25	7.92 (6.27, 10.00)
AUC _(0-30min) (pmol*h/L)	Humalog	120.07	
	Formulation I	371.42	3.09 (2.63, 3.64)
	Formulation J	393.84	3.28 (2.79, 3.86)
	Formulation K	397.06	3.31 (2.81, 3.89)
	Formulation L	393.79	3.28 (2.79, 3.85)
Late 50% t _{max} (min)	Humalog	168.28	
	Formulation I	86.10	0.51 (0.45, 0.58)
	Formulation J	82.10	0.49 (0.42, 0.57)
	Formulation K	80.80	0.48 (0.44, 0.52)
	Formulation L	84.52	0.50 (0.45, 0.56)

Table 12. PK data. Abbreviations: LS – least squares, CI – confidence interval, T_{onset} – time to onset of insulin appearance, early 50% T_{max} – time to early half-maximal drug concentration, AUC_(0-15min) – area under the curve from time zero to 15 minutes, AUC_(0-30min) – area under the curve from time zero to 30 minutes, late 50% t_{max} – time to late half-maximal drug concentration. P-value for all test articles compared to Humalog control <.0001.

The results show triphosphate-containing formulations have accelerated pharmacokinetic parameters as compared to the non-triphosphate-containing control.

In addition, a 5-hour euglycemic glucose clamp is conducted in each period to allow an assessment of glucodynamic response to each treatment. In this assessment the glucose infusion rate (GIR) over time is used as a measure of insulin effect. A locally weighted scatterplot smoothing (LOESS) function is applied to all individual GIR versus
5 time profiles in each treatment group and/or period using S-PLUS software version 8.2. The fitted data for each subject are used to calculate glucodynamic parameters.

Analyses of the data show triphosphate-containing formulations have improved pharmacodynamic parameters as compared to the non-triphosphate-containing control. The studies described above demonstrate that addition of small quantities of certain
10 polyphosphates such as pyrophosphate or triphosphate to insulin formulations can cause earlier Tmax and higher Cmax in the insulin pharmacokinetic profile.

Sequences

Human insulin A-chain

Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn Tyr Cys Asn
(SEQ ID NO:1)

5

Human insulin B-chain

Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly
Glu Arg Gly Phe Phe Tyr Thr Pro Lys Thr (SEQ ID NO: 2).

10 Insulin lispro B-chain

Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly
Glu Arg Gly Phe Phe Tyr Thr Lys Pro Thr (SEQ ID NO: 3).

Insulin aspart B-chain

15 Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly
Glu Arg Gly Phe Phe Tyr Thr Asp Lys Thr (SEQ ID NO: 4).

Insulin glulisine B-chain

20 Phe Val Lys Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly
Glu Arg Gly Phe Phe Tyr Thr Pro Glu Thr (SEQ ID NO: 5).

25