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(54) Title: RAPID ACTING AND LONG ACTING INSULIN COMBINATION FORMULATIONS

(57) Abstract: A combined rapid acting-long acting insulin formulation has been developed wherein the pI of the rapid acting insulin is adjusted so that the long acting insulin remains soluble when they are mixed together, in the preferred embodiment, this injectable basal bolus insulin, is administered before breakfast, provides adequate bolus insulin levels to cover the meal, does not produce hypoglycemia alter the meal and provides, adequate basal insulin for 24 hours. Lunch and dinner can be covered by two bolus injections of a fast acting, or a rapid acting or a very rapid acting insulin. As a result, a patient using intensive insulin therapy should only inject three, rather than four, times a day. Experiments have been performed to demonstrate the importance of the addition of specific acids to hexameric insulin to enhance speed and amount of absorption and preserve bioactivity following dissociation into the monomeric form by addition of a chelator such as EDTA. As shown by the examples, the preferred acids are aspartic, malic, succinic, glutamic and citric acid. These are added in addition to a chelator, preferably ethylenediaminetetraacetic acid (EDTA). The results show that the citric acid formulation was more effective at dropping the blood glucose rapidly than the identical rapid acting formulation prepared with ICI in swine. Charge masking by the polyacid appears to be responsible for rapid insulin absorption, EDTA was not effective when used with adipic acid, oxalic acid or ICI at hastening the absorption of insulin. These results confirm the results seen in clinical subjects and patients with diabetes treated with the rapid acting insulin in combination with citric acid and EDTA.

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RAPID ACTING AND LONG ACTING INSULIN COMBINATION FORMULATIONS

Background of the Invention

The present invention generally relates to formulations combining
5 rapid acting and long acting insulin formulations.

This application claims priority to U.S.S.N. 60/744,687 entitled
"Rapid Acting and Long Acting Insulin Combination Formulations" filed
April 12, 2006 by Solomon S. Steiner and Roderike Pohl, U.S.S.N.
11/537,335 entitled "Rapid Acting and Prolonged Acting Insulin
10 Preparations" filed September 29, 2006 by Solomon S. Steiner and Roderike
Pohl, and U.S.S.N. 11/695,562 entitled "Rapid Acting and Long Acting
Insulin Combination Formulations" filed April 2, 2007, by Solomon S.
Steiner and Roderike Pohl.

Intensive insulin therapy for diabetes involves providing a basal
15 insulin, ideally present at a uniform level in the blood over a 24 hour period
and a bolus or meal time (prandial) insulin to cover the added carbohydrate
load from digestion concomitant with each meal.

In 1936, Hans Christian Hagedorn and B. Norman Jensen discovered
that the effects of injected insulin could be prolonged by the addition of
20 protamine obtained from the "milt" or semen of river trout. The insulin was
added to the protamine and the solution was brought to pH 7 for injection. In
1946, Nordisk Company was able to form crystals of protamine and insulin
and marketed it in 1950 as NPH, (Neutral Protamine Hagedorn, "NPH")
insulin. NPH insulin has the advantage that it can be mixed with an insulin
25 that has a faster onset to compliment its longer lasting action. Eventually all
animal insulins were replaced by human recombinant insulin.

Until very recently, and in many places today, basal insulin is usually
provided by the administration of two daily doses of NPH insulin, separated
by 12 hours. A patient eating three meals a day and using NPH insulin as the
30 basal insulin requires five injections per day, one with each of three meals
and two NPH insulin injections, one in the morning and the other at bedtime.
To reduce the number of injections the patient must take, the morning dose

of NPH insulin has been combined with a short acting insulin, (recombinant human insulin) or a rapid acting insulin analog, such as lispro. A typical combination is a 70% NPH to 30% rapid acting insulin analog mixture. As a result, the patient can reduce the number of injections from five per day to four per day. See, for example, Garber, *Drugs* 66(1):31-49 (2006).

More recently insulin glargine, (trade name LANTUS®) a “very long-acting” insulin analog has become available. It starts to lower blood glucose about one hour after injection and keeps working evenly for 24 hours. J. Rosenstock and colleagues found that patients who took insulin glargine had a much lower risk of low blood glucose (hypoglycemia) than the patients who took NPH insulin.

Glargine cannot be mixed with other short or rapid acting insulins because the mixture causes glargine to precipitate prior to injection and administration of a precipitated insulin makes it virtually impossible to administer a known and reliable dose. The manufacturer of glargine warns users against mixing glargine with any other insulin.

It is therefore an object of the present invention to provide insulin formulations that can be used to reduce the number of daily injections to three.

It is another object of the present invention to provide a basal-bolus insulin formulation.

It is still another object of the present invention to provide a stable insulin formulation having immediate and long term release characteristics.

Summary of the Invention

A combined fast or rapid acting-long acting insulin formulation has been developed wherein the pH of the fast or rapid acting insulin is adjusted so that both rapid and long acting insulins remain soluble when they are mixed together. Included in the preferred embodiment are any very rapid, rapid or fast acting insulin formulations combined with any intermediate, long or very long acting insulin at low pH. In another embodiment, any very rapid, rapid, or fast insulin can be combined with any intermediate, long or very long acting insulin at low pH, in the presence of a chelating agent. In

the most preferred embodiment, VIAject™ (a very rapid acting insulin) is mixed with insulin glargine at pH4 to produce a rapid initial spike in blood insulin concentration to cover the carbohydrates being absorbed from digestion of a meal and continue with a sustained basal blood insulin concentration.

When an injectable basal bolus insulin formulation is co-administered with a rapid or very rapid acting insulin before breakfast, it provides adequate bolus insulin levels to cover the meal, does not produce hypoglycemia after the meal and provides adequate basal insulin for 24 hours. Lunch and dinner can be covered by two bolus injections of a fast acting, or a rapid acting or a very rapid acting insulin. As a result, a patient using intensive insulin therapy would only inject three, rather than four, times a day.

Experiments have been performed to demonstrate the importance of the addition of specific acids such as aspartic acid, glutamic acid, maleic, fumaric, or succinic acid to hexameric insulin to enhance speed and amount of absorption and preserve bioactivity following dissociation into the dimeric/monomeric form. These are added in addition to a chelator, preferably ethylenediaminetetraacetic acid (EDTA). Polyacids were selected based on their molecular size and structure to optimize association with hydrogen bonding sites on the insulin surface, effectively masking charged amino acid residues (Figure 1), regardless of the source (including native insulin, recombinant insulin, long acting insulin, porcine, bovine, insulin derivatives and analogues thereof). The acids were used at a concentration that provided optimal charge masking effect, (As shown by the examples, the preferred acids are aspartic, glutamic, succinic, maleic, fumaric and citric acid) The combination of both the preferred acid and the chelator together in the insulin formulation appear to be responsible for rapid insulin absorption. EDTA was not effective with all acids. When used with adipic acid, oxalic acid or HCl, there was no apparent increase in the rate of absorption of insulin. These studies establish the importance of an acid and chelator in both *in vitro* (human oral epithelial cells) and *in vivo* (rat, pig and human)

studies. These findings confirm the results seen in patients with diabetes treated with the very rapid acting insulin (in combination with citric acid and EDTA) and the basal insulin glargine.

Brief Description of the Drawings

5 Figure 1 is a three dimensional schematic of insulin showing charges.

Figure 2 is a diagram of the transwell device used to measure insulin absorption through oral epithelial cells.

Figure 3 is a graph of the mean insulin accumulation (μ U) over time (minutes) in the lower chamber of a transwell membrane plate seeded with epithelial cells, comparing the effect of an insulin formulation containing EDTA (\blacklozenge) with one without EDTA (\blacksquare), with a control, no cells (\blacktriangle).

Figure 4 is a graph of cumulative insulin (U) over time in minutes for samples of citric acid with and without EDTA versus aspartic acid with EDTA.

15 Figure 5 is a graph of insulin apparent permeability for insulin with (solid) and without (hatched) EDTA, for samples with citric acid, glutamic, adipic, and oxalic acid, over time in minutes.

Figure 6 is a graph of percent glucose lowering from baseline over time in minutes comparing insulin with aspartic acid and EDTA with insulin with HCl and EDTA in rats.

Figure 7 is a graph of mean glucose levels in miniature swine over time, comparing insulin with EDTA and citric acid versus insulin with EDTA and HCl.

25 Figure 8 is a graph of continuous blood glucose values over time (hours) from a human clinical trial, where insulin glargine (Lantus®) and VIAject were administered separately (two injections) or together, combined in one injection.

Detailed Description of the Invention

I. Compositions

30 A. Insulin

The composition includes a fast, rapid or very rapid acting insulin and an intermediate or long acting insulin. The rapid acting insulin is

provided at a low pH, at which the long acting insulin does not precipitate when mixed together, even over a wide range of ratios of rapid acting to long acting insulin.

5 There are several differing types of commercial insulin available for diabetes patients. These types of insulins vary according to (1) how long they take to reach the bloodstream and start reducing blood glucose levels; (2) how long the insulin operates at maximum strength; and (3) how long the insulin continues to have an effect on blood sugar.

Fast Acting Insulin

10 Fast acting insulins are intended to respond to the glucose derived from ingestion of carbohydrates during a meal. Fast acting insulins start to work within one to 20 minutes, peaking about one hour later and lasting from three to five hours. Fast acting insulin takes about two hours to fully absorb into the systemic circulation. Fast acting insulins include regular
15 recombinant human insulin (such as Humulin®, marketed by Lilly, and Novalin®, marketed by NovoNordisk) which are administered in an isotonic solution at pH 7. Bovine and porcine insulins, which differ in several amino acids to human insulin, but are bioactive in humans, are also fast acting insulins.

20 Rapid Acting Insulin.

Some diabetes patients use rapid-acting insulin at mealtimes, and long-acting insulin for 'background' continuous insulin. This group includes insulins that have been modified or have altered locations of amino acids in order to enhance their rate of absorption.

25 At present there are three types of rapid-acting commercial insulin analogs available: lispro insulin (Lysine-Proline insulin, sold by Eli Lilly as HUMALOG®), glulisine insulin (sold by Sanofi-Aventis as APIDRA®) and aspart insulin (sold by Novo Nordisk as NOVOLOG®).

Very Rapid acting Insulin

30 Bidel has a proprietary insulin formulation of regular human insulin that is even more rapid than the rapid acting insulin analogs, VIAJECT™.

This is a formulation combining regular human insulin with EDTA and citric acid, at a pH of 4.

Intermediate acting insulin

Intermediate-acting insulin has a longer lifespan than short-acting insulin but it is slower to start working and takes longer to reach its maximum strength. Intermediate-acting insulin usually starts working within 2-4 hours after injection, peaks somewhere between 4-14 hours and remains effective up to 24 hours. Types of intermediate-acting insulin include NPH (Neutral Protamine Hagedorn) and LENTE insulin. NPH insulin contains protamine which slows down the speed of absorption so that the insulin takes longer to reach the bloodstream but has a longer peak and lifespan. Intermediate acting insulins may be combined with rapid acting insulins at neutral pH, to reduce the total number of injections per day.

Long Acting Insulin

LANTUS™ (insulin glargine) is a recombinant human insulin analog that can have up to 24 hour duration. It differs from human insulin by having a glycine instead of asparagine at position 21 and two arginines added to the carboxy-terminus of the beta-chain. LANTUS™ consists of insulin glargine dissolved in a clear aqueous fluid (100 IU, 3.6378 mg insulin glargine, 30 micrograms zinc, 2.7 mg m-cresol, 20 mg glycerol 85%, and water to 1 ml). The pH is adjusted with HCl to 4.

The median time between injection and the end of the pharmacological effect is a maximum of 24 hours after the injection. The median time between injection and the end of pharmacological effect was 24 hours for insulin glargine and 14.5 hours for NPH human insulin

The package insert says not to mix LANTUS™ with any other types of insulin, unlike most rapid acting and intermediate acting insulins, due to precipitation of the insulins on mixing.

In the case of insulin glargine, there is no precipitate formed on mixing with Viaject™ which also has a pH of 4, matching that of the insulin glargine. Ultimately, this combination provides very rapid acting insulin to carry the patient through a meal with less bolus insulin, since it is very

rapidly absorbed and eliminated shortly after meal digestion, thereby reducing the chance of hypoglycemia and providing 24 hour long lasting basal insulin. This ultimately reduces the number of injections required per day from four to three.

5 **B. Acid stabilizers and chelators**

Acid Stabilizers

 Acids which effectively solublize hexameric crystalline insulin are selected based on their molecular size and pKa, and are predicted to be most effective in masking the charges on the insulin molecule which are exposed upon dissociation into the monomeric or dimeric form (Figure 1). Specific acids preserve bioactivity, effectively charge mask and increase the rate of absorption following dissociation of hexameric insulin into the monomeric form. The examples establish the importance of the acid in both *in vitro* (human oral epithelial cells) and *in vivo* (rat, pig and human). Regular human insulin (Humulin®R) is solubilized with HCl, formulated at physiological pH. Pharmacokinetic profiles of this insulin show the insulin is not absorbed very rapidly, having a peak insulin concentration at approximately two hours post dose.

 Preferred acids are "polyacidic", i.e., have multiple acidic residues. The preferred polyacids include aspartic, glutamic, succinic, fumaric, maleic and citric acids. The useful concentration range is 0.1 to 3 mg/ml acid, for solutions containing 0.5 to 4 mg insulin/ml. The range of pH is 3.0 to 4.2, while the preferred range is 3.8-4.1. These acids may be used in conjunction with any rapid acting insulin to reduce pH and make them soluble following mixing with Lantus.

Chelators

 In the preferred embodiment, a chelator is mixed with the active agent. The chelator, in addition to its primary role in chelation, is also believed to hydrogen bond with the insulin, thereby masking the charge and facilitating absorption of the insulin. It is believed that the chelator pulls the zinc away from the insulin, thereby favoring the monomeric form of the insulin over the hexameric form following injection into subcutaneous tissue

control. Figure 8 shows data on human clinical trials on the injectable mixture of Viaject and insulin glargine.

Solid Formulations

Solid or semi-solid gels may be formulated for s.c. injection
5 (syringable low viscosity gels) or dried into a film for buccal, sublingual, oral, rectal, or vaginal administration of the dual acting insulin. They may be formed by mixing one or more hydrophilic polymers in solution, which gel or solidify by ionic and/or covalent binding. Suitable materials include, but are not limited to, starch, pregelatinized starch, gelatin, sugars (including
10 sucrose, glucose, dextrose, lactose and sorbitol), chitosans (various forms) dextrin, maltodextrin, polyethylene glycol, waxes, natural and synthetic gums such as acacia, guar gum, tragacanth, alginate, sodium alginate, celluloses, including hydroxypropylmethylcellulose, carboxymethylcellulose sodium, hydroxypropylcellulose,
15 hydroxyethylcellulose, ethylcellulose, methyl cellulose, and veegum, hydrogenated vegetable oil, Type I, magnesium aluminum silicate, and synthetic polymers such as acrylic acid and methacrylic acid copolymers, carbomer, methacrylic acid copolymers, methyl methacrylate copolymers, aminoalkyl methacrylate copolymers, polyacrylic acid/polymethacrylic acid,
20 and polyvinylpyrrolidone. Blending or copolymerization sufficient to provide a certain amount of hydrophilic character can be useful to improve wettability and mucoadhesion of the materials. For example, about 5% to about 20% of monomers may be hydrophilic monomers. Hydrophilic polymers such as hydroxylpropylcellulose (HPC),
25 hydroxypropylmethylcellulose (HPMC), carboxymethylcellulose (CMC) are commonly used for this purpose. Preferably, the polymers are bioerodable, with preferred molecular weights ranging from 1000 to 15,000 Da, and most preferably 2000 to 5000 Da. These can also be nonionic polymers such as ethylene glycol monostearate, propylene glycol myristate, glyceryl
30 monostearate, glyceryl stearate, polyglyceryl-4-oleate, sorbitan acylate, sucrose acylate, PEG-150 laurate, PEG-400 monolaurate, polyoxyethylene monolaurate, polysorbates, polyoxyethylene octylphenylether, PEG-1000

cetyl ether, polyoxyethylene tridecyl ether, polypropylene glycol butyl ether, Poloxamer[®] 401, stearyl monoisopropanolamide, and polyoxyethylene hydrogenated tallow amide. Examples of amphoteric surfactants include sodium N-dodecyl- β -alanine, sodium N-lauryl- β -iminodipropionate, myristoamphoacetate, lauryl betaine and lauryl sulfobetaine.

In one embodiment, the formulation is a sublingual solid formulation that contains excipients, such as poly(vinyl alcohol), glycerin, carboxymethyl cellulose (CMC), chitosan and optionally poly(ethylene glycol) and water.

The composition may be in the form of a clear or opaque, flexible, thin material. Typical thicknesses range from 0.01 to 4mm. The film may have any suitable shape, including round, oval, rectangle, or square. The film may be a monolayer, bilayer or trilayer film. In the preferred embodiment, the film is designed to be suitable for sublingual administration. The monolayer film contains an active agent and one or more excipients. The bilayer film contains one or more excipients, such as a solubilizing agent and/or a metal chelator, in a first layer, and an active agent in the second layer. This configuration allows the active agent to be stored separated from the excipients, and may increase the stability of the active agent, and optionally increases the shelf life of the composition compared to if the excipients and active agent were contained in a single layer. The trilayer film contains three layers of film. Each of the layers may be different, or two of the layers, such as the bottom and top layers, may have substantially the same composition.

In one embodiment, the bottom and top layers surround a core layer containing the active agent. The bottom and top layers may contain one or more excipients, such as a solubilizing agent and a metal chelator.

Preferably the bottom and top layers have the same composition.

Alternatively, the bottom and top layers may contain different excipient(s), or different amounts of the same excipient(s). The core layer typically contains the insulin, optionally with one or more excipients. In one

embodiment, the film is a bilayer film that contains EDTA and citric acid in one layer and insulin in the second layer. Each layer may contain additional excipients, such as glycerin, polyvinyl alcohol, carboxymethyl cellulose, and

optionally PEG (such as PEG 400 or PEG 1600). In one embodiment, a third layer can be located between the insulin layer and the layer containing the other ingredients to further protect the active agent from degradative ingredients located in the other layer during storage. Suitable materials for the protective layer include carboxymethylcellulose sodium, carnauba wax, cellulose acetate phthalate, cetyl alcohol, confectioner's sugar, ethylcellulose, gelatin, hydroxyethyl cellulose, hydroxypropyl methylcellulose, liquid glucose, maltodextrin, methylcellulose, microcrystalline wax, polymethacrylates, polyvinyl alcohol, shellac, sucrose, talc, titanium dioxide, and zein. By altering the composition of the excipients, the film can be designed to dissolve rapidly (less than 30 seconds) or slowly (up to 15 minutes) in order to achieve the desired absorption profile and subsequent effect. The film may dissolve in a time period ranging from 3 to 5 minutes, 5 to 8 minutes, or 8 to 15 minutes. Preferably, the film dissolves in a time period ranging from 2 -10 minutes.

There are a number of colorings and flavorings that are commercially available. Flavorings include mint, lemon, cherry, bubblegum, and other standard flavors. Sweeteners can be added, including non-glucose sweeteners, which are particularly advantageous for administration of insulin. Colorings can be red, blue, green, yellow, orange, or any other standard FDC approved color.

Stabilizers are used to inhibit or retard drug decomposition reactions which include, by way of example, oxidative reactions. A number of stabilizers may be used. Suitable stabilizers include polysaccharides, such as cellulose and cellulose derivatives, and simple alcohols, such as glycerol; bacteriostatic agents such as phenol, m-cresol and methylparaben; isotonic agents, such as sodium chloride, glycerol, and glucose; lecithins, such as example natural lecithins (e.g. egg yolk lecithin or soya bean lecithin) and synthetic or semisynthetic lecithins (e.g. dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine or distearoyl-phosphatidylcholine; phosphatidic acids; phosphatidylethanolamines; phosphatidylserines such as distearoyl-phosphatidylserine, dipalmitoylphosphatidylserine and

diarachidoylphosphatidylserine; phosphatidylglycerols; phosphatidylinositols; cardiolipins; sphingomyelins; and synthetic detergents, such as dioctanoylphosphatidyl choline and polyethylene-polypropylene glycol). Other suitable stabilizers include acacia, albumin, 5 alginic acid, bentonite, carboxymethylcellulose calcium, carboxymethylcellulose sodium, cyclodextrins, glyceryl monostearate, hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium aluminum silicate, propylene glycol, propylene glycol alginate, sodium alginate, white wax, xanthan gum, and yellow wax. In the preferred 10 embodiment, the agent is insulin and the stabilizer may be a combination of one or more polysaccharides and glycerol, bacteriostatic agents, isotonic agents, lecithins, or synthetic detergents.

II. Methods of making the formulations

Pharmaceutical compositions may be formulated in a conventional 15 manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Formulation of drugs is discussed in, for example, Hoover, John E., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, 20 Pennsylvania (1975), and Liberman, H.A. and Lachman, L., Eds., Pharmaceutical Dosage Forms, Marcel Decker, New York, N.Y. (1980). Proper formulation is dependent upon the route of administration chosen.

III. Methods of Using Formulations

The formulations may be administered in a variety of manners, 25 including by injection, preferably subcutaneously, or topically to a mucosal surface such as oral, buccal, nasal, sublingual, rectal, vaginal, pulmonary, or ocular administration. Subcutaneous, buccal or sublingual are preferred. Following administration, the dosage form dissolves quickly releasing the drug or forming small particles containing drug, optionally containing one or 30 more excipients. The formulation is designed to be rapidly absorbed and transported to the plasma for systemic delivery.

In the preferred embodiment, the formulation is formed by mixing a powdered active agent with a liquid diluent that contains a pharmaceutically acceptable liquid carrier and one or more solubilizing agents. In the most preferred embodiment, the active agent is insulin, and the diluent contains saline or glycerin, EDTA and citric acid. Prior to administration the powder and diluent are mixed together to form an injectable composition.

The present invention will be further understood by reference to the following non-limiting examples.

10 **Example 1: Effect of EDTA on Insulin Absorption Through an Epithelial Cell Multilayer**

Purpose: To demonstrate *in vitro* the effect of EDTA in the presence of citric acid on absorption of insulin through an epithelial cell multilayer.

Materials and Methods:

15 Two saline solutions were mixed containing 1mg/ml insulin, 2 mg/ml EDTA and 2 mg/ml citric acid ("solution 1") or 1mg/ml insulin and 2mg/ml citric acid ("solution 2"). The control (no cells) contained EDTA, citric acid and insulin. Immortalized human epithelial cell line cultures were seeded on transwell plates (Figure 2). Cells were grown to confluence and tested for membrane integrity using trans-epithelial resistance. At time zero, the fluid in the top chambers of the transwell plates was replaced with 0.5 ml of insulin solution (i.e. solution 1 or solution 2). Two plates with solution 1, two plates with solution 2 and one plate with the control solution (no cells) were tested simultaneously. The lower chamber of each plate contained 20 1.5mL of saline solution. At each time point, 100 μ L of fluid from the lower chamber was removed and analyzed with insulin Enzyme-Linked Immunosorbent Assay (ELISA). 100 μ L of saline was added to the lower chamber to maintain a constant volume of 1.5 mL throughout the study. The amount of insulin removed from the lower chamber at each time point was 25 added to the amount removed in the previous time point(s) to determine the cumulative amount of insulin recovered in the lower chamber. Cells were 30

stained to check for viability before and after the experiment. There was no statistical difference in the cell viability for each of the plates.

Results:

Figure 3 is a graph of the mean insulin accumulation (μU) over time (minutes) in the lower chamber of a transwell plate seeded with epithelial cells, comparing the effect of an insulin formulation containing EDTA (\diamond) with one without EDTA (\blacksquare), with a control, no cells (\blacktriangle).

Solution 1, which contained EDTA, moved through the multilayer of epithelial cells more effectively than solution 2, which did not contain EDTA. Therefore, the effect of combining EDTA with citric acid is to promote the speed and amount of absorption.

Example 2: Effect of Aspartic and Citric Acid on Absorption of Insulin Through an Epithelial Cell Multilayer.

Purpose: Demonstrate that polyacids have different affinities for insulin with EDTA as shown by an increase in absorption through cells.

Methods and Materials:

Oral epithelial cells that have been seeded on transwell plates were used to determine the rate of absorption through the cell multilayer, as described in example 1. Insulin (1mg/ml) was dissolved in either aspartic (0.2 mg/mL) or citric acid (2 mg/ml) and EDTA (2 mg/ml) was added to both. Insulin with citric acid (no EDTA) was used as a control. The pH of the solution was approximately 3.5 to 4, and physiological saline was present to provide an isotonic environment for the cells (0.85% NaCl, sufficient to produce a range of 280-310 mOsm as measured by freezing point depression, Microsmette, Precision systems, Natick, MA). Samples were taken from the receiver chamber and assayed by ELISA (Linco Corp.) for human recombinant insulin ($\mu\text{U}/\text{mL}$).

Results: Insulin/citric acid absorption through the cell layers was enhanced by the addition of EDTA, as shown by Example 2. However, aspartic acid was even more potent at enhancing insulin transport in the presence of EDTA, as shown by Figure 4.

Conclusion: Different polyacids in the presence of EDTA have varying effects on insulin absorption, possibly due to varying degrees of charge masking.

Example 3: Comparison of Effect of Citric Acid, Glutamic Acid, Adipic Acid and Oxalic Acid on Insulin Absorption Through an Epithelial Cell Multilayer

Materials and Methods:

Transwell plates seeded with oral epithelial cells were used for these experiments. The effect of EDTA was monitored by the amount of insulin that came through the lower chamber of the transwell plate.

Oral epithelial cells were grown on transwell inserts for 2 weeks until multiple (4-5) cell layers had formed. Transport studies were conducted by adding the appropriate solution (all contained 1 mg/ml human insulin) to the donor well and removing samples from the receiver well after 10 minutes. Insulin amounts in the receiver wells were assayed using ELISA. Apparent Permeability was calculated using the formula: $\text{Apparent Permeability} = \frac{Q}{A(C)t}$ where Q = total amount permeated during incubation time in μg , A = area of insert in cm^2 , C = initial concentration in donor well in $\mu\text{g}/\text{cm}^3$ and t = total time of experiment in sec.

EDTA concentration is 0.45 mg/mL in all cases and the acid concentrations are as follows: Citric acid 0.57mg/ml, Glutamic acid 0.74 mg/mL, Adipic acid 0.47 mg/mL, Oxalic acid 0.32 mg/mL. The pH of the solutions was 3.6 in all cases.

Results:

Figure 5 shows the change in apparent permeability resulting from different organic polyacids that have been tested, with and without EDTA. The results show that there is an increase in the cumulative amount of insulin apparent permeability when EDTA is added to the acid/insulin in the case of citric and glutamic acids. This did not hold true for all organic polyacids. Adipic and oxalic acids did not show such a response.

Example 4: Effect of Acid on Absorption of Insulin from Polymeric Gel Administered Rectally to Rats.

Purpose: To observe effect of acids and EDTA in an *in vivo* model.

Materials and Methods:

5 **Samples**

Insulin was incorporated into a gel consisting of PVA (0.5%), Carbopol (2.7%), CMC (0.005%) and PEG 400 (0.14%), glycerin (0.14%), and EDTA (0.005%) by blending with insulin/aspartic acid or insulin/HCl. The final concentration of insulin in insulin/aspartic acid gel was 0.7 and
10 insulin concentration in insulin/HCl gel was 1.7mg/g.

Rat rectal study:

Rats were fasted overnight and were cleared of all fecal matter with a warm water enema. Then the gel formulation was inserted into the rectum and the rat's blood glucose was monitored over an 8 hour time period.

15 **Results:**

The results are shown in Figure 6 as a percent glucose lowering from baseline comparing insulin with aspartic acid and EDTA to insulin with HCl and EDTA. The results show significantly better lowering of glucose for the insulin containing aspartic acid as compared to insulin containing HCl.

20 **Example 5: Comparison of Effect of HCl and Citric Acid on Absorption of Insulin with EDTA in miniature diabetic swine.**

Purpose: To look at timing of glucose response when insulin is injected with a polyacid or organic acid in conjunction with EDTA.

Materials and Methods

25 To further demonstrate that the type of acid is important to the rapid action of the dissociated insulin, a comparison of citric acid to HCl, was performed in miniature diabetic swine. Insulin (0.9 mg/mL) was prepared as a clear isotonic solution containing citric acid (1.8 mg/mL), EDTA (1.8 mg/mL), and m-cresol as a preservative, pH ~4. The comparator was
30 prepared in the same manner, substituting HCl (0.001N) for citric acid and adjusting the pH with NaOH to approximately 4,

Diabetic mini pigs were not fed on the day of the study and were dosed with 0.08 U/kg on three occasions with the HCl formulation. For comparison, the citric acid formulation was used on two occasions with this dose, and four other occasions at a higher dose of 0.125 U/kg. Blood was drawn for insulin and glucose determination over the 8 hour study period.

Results:

The results shown in Figure 7 compare the time to reach the lowest glucose level (nadir) following insulin administration to diabetic mini-pigs. The citric acid formulation was consistently faster at reaching the nadir than an identical formulation made with HCl.

Example 6: Insulin Glargine and Viaject™ administered together and separately to patients with diabetes:

Methods and Materials:

Blood glucose ("BG") of 9 patients (5 males and 4 females; age 40 ± 10 yrs, body mass index ("BMI") 24.0 ± 2.0 kg/m²) were stabilized by glucose clamps (target BG 120 mg/dl). Prior to dosing, the glucose infusion was turned off. Using a cross over design with random treatment order, the same patient specific dose of VIAject™ and Lantus was injected s.c. immediately before the meal. On one occasion, the doses were together in the same injection. On another occasion the identical dose of each insulin was administered separately in two injections. Blood glucose was continuously monitored for 8 hours and glucose infusion was re-initiated if BG was less than 60 mg/dl. Plasma insulin levels were determined throughout the study.

Results:

The mean blood glucose data over the first three hours is shown in Figure 8. The first three hours have a very similar profile, which is typical to the rapid action of Viaject™ after a meal.

We claim:

1. A composition comprising a fast, rapid or very rapid insulin in combination with an intermediate acting or long acting insulin, wherein the pH of the composition is adjusted to solubilize the long acting insulin.
2. The composition of claim 1, comprising glargine insulin.
3. The composition of claim 1, further comprising rapid acting insulin.
4. The composition of claim 1, further comprising intermediate acting insulin.
5. The composition of claim 1, having a pH of 3.0-4.2.
6. The composition of claim 1, comprising a chelator selected from the group consisting of ethylenediaminetetraacetic acid (EDTA), dimercaprol (BAL), penicillamine, alginic acid, Chlorella, Cilantro, Alpha Lipoic Acid, Dimercaptosuccinic Acid (DMSA), dimercaptopropane sulfonate (DMPS), and oxalic acid.
7. The composition of claim 6, wherein the chelator is ethylenediaminetetraacetic acid (EDTA).
8. The composition of claim 1, comprising an acid selected from the group consisting of aspartic acid, glutamic acid, maleic, fumaric, succinic and citric acid.
9. An insulin formulation comprising an insulin selected from the group consisting of rapid acting insulin, long acting insulin and combinations thereof, an acid selected from the group consisting of aspartic, glutamic, succinic, maleic, fumaric or citric acid, insulin, and a chelator.
10. The formulation of claim 9 comprising EDTA.
11. The formulation of claim 9 comprising citric acid.
12. The formulation of claim 9 comprising a rapid acting insulin in a ratio of 1:2:2.
13. The formulation of claim 9 wherein the insulin is native human insulin, recombinant human insulin, porcine insulin, bovine insulin a derivative of human insulin, or an analog of human insulin suitable for administration to a human.

14. A method of treating a patient with insulin comprising administering to an individual the composition of any of claims 1-13.

15. The method of claim 14 wherein the insulin is administered by injection.

16. The method of claim 14 wherein the insulin is in a formulation for administration sublingually, oral, basal, buccal, rectal or vaginally.

Insulin Charge Distribution

- Cationic residues on insulin are clustered
- Multivalent anions may neutralize

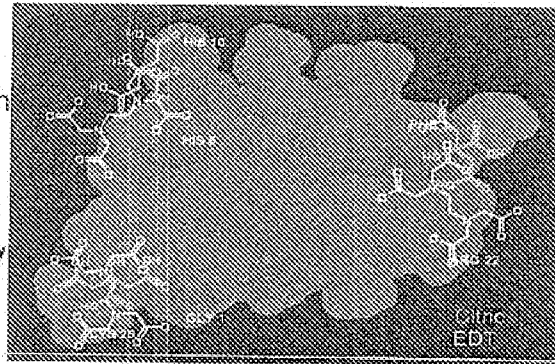


FIG. 1

Epithelial Cells: Transwell setup

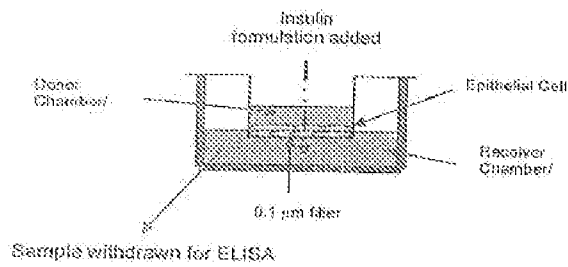


FIG. 2

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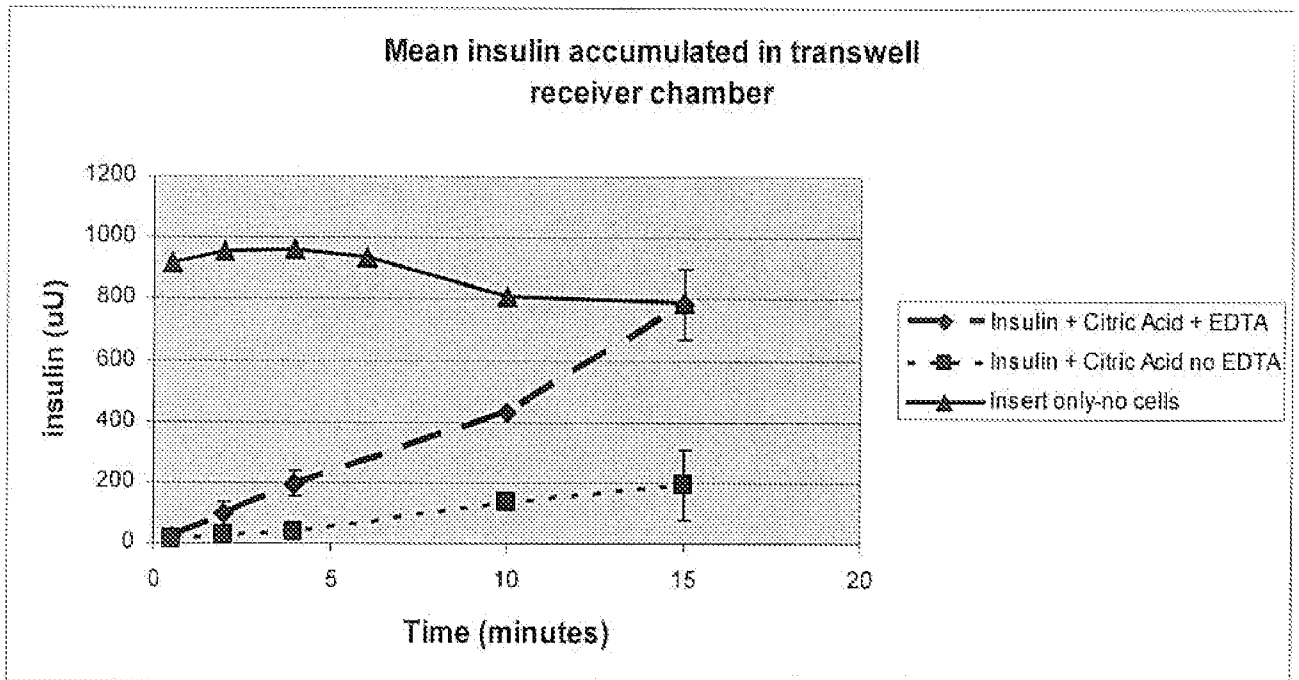


FIGURE 3

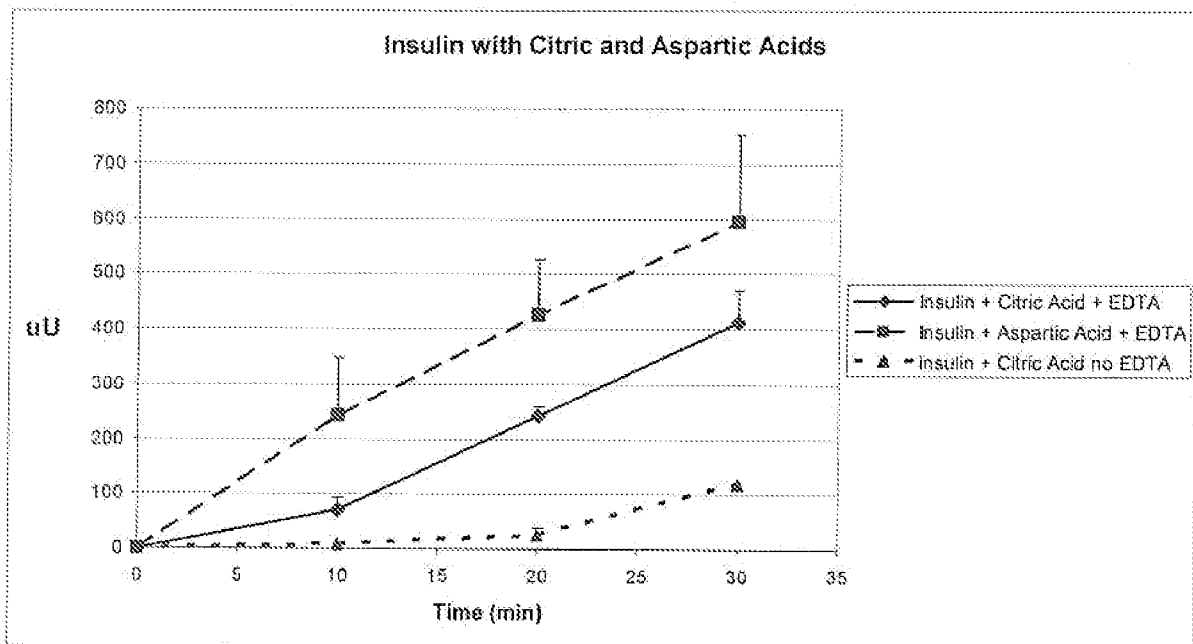


FIGURE 4

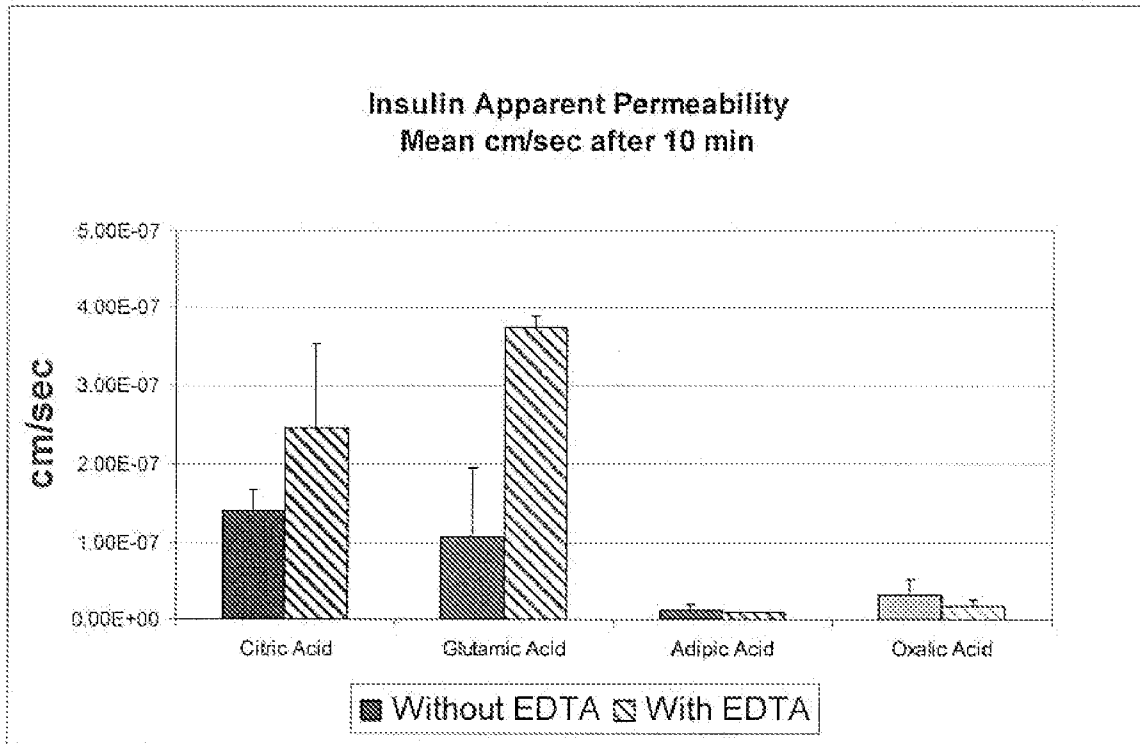


FIGURE 5

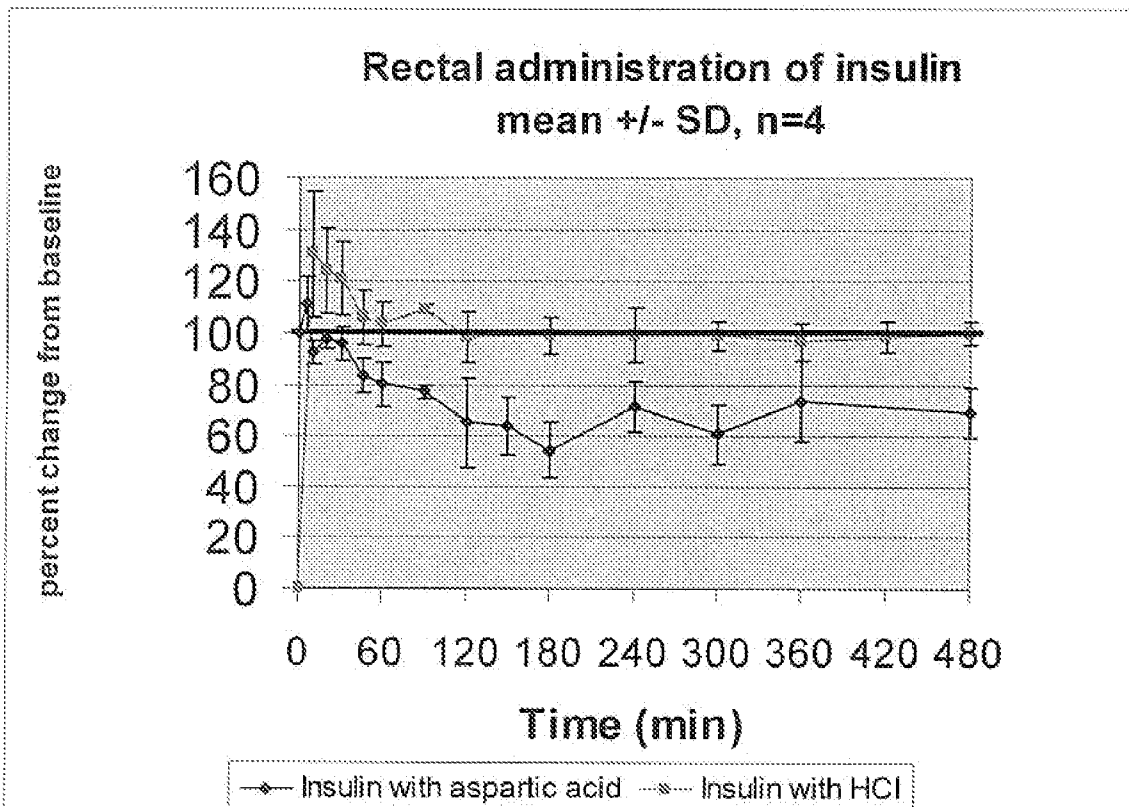


FIGURE 6

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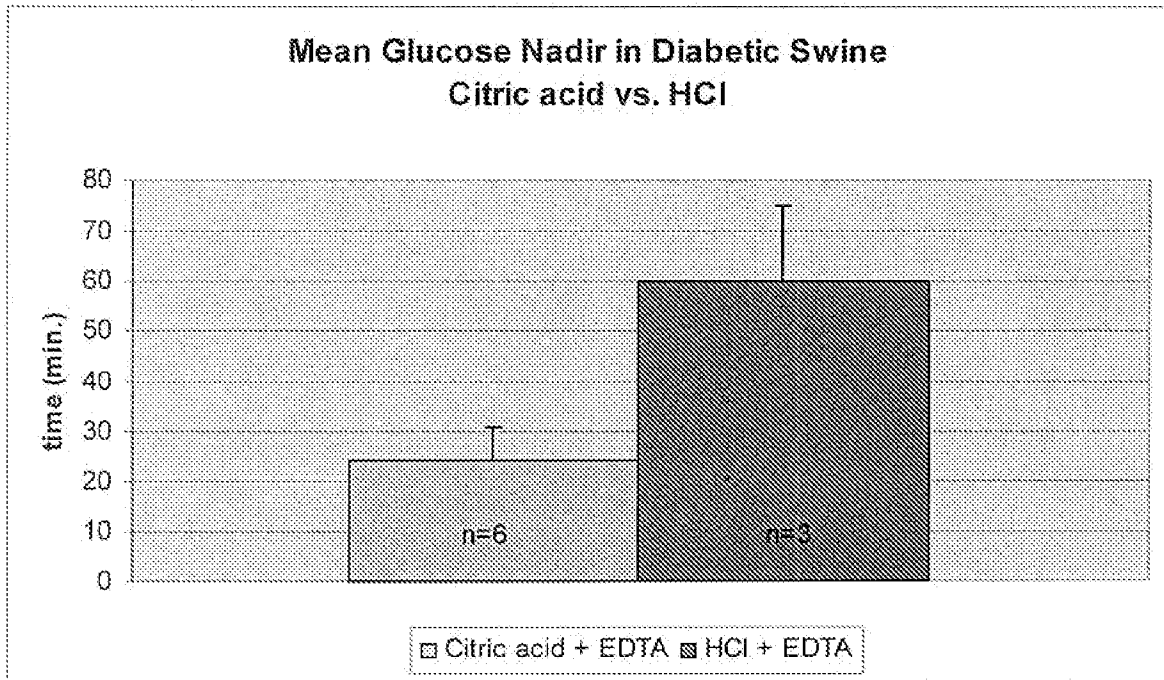


FIGURE 7

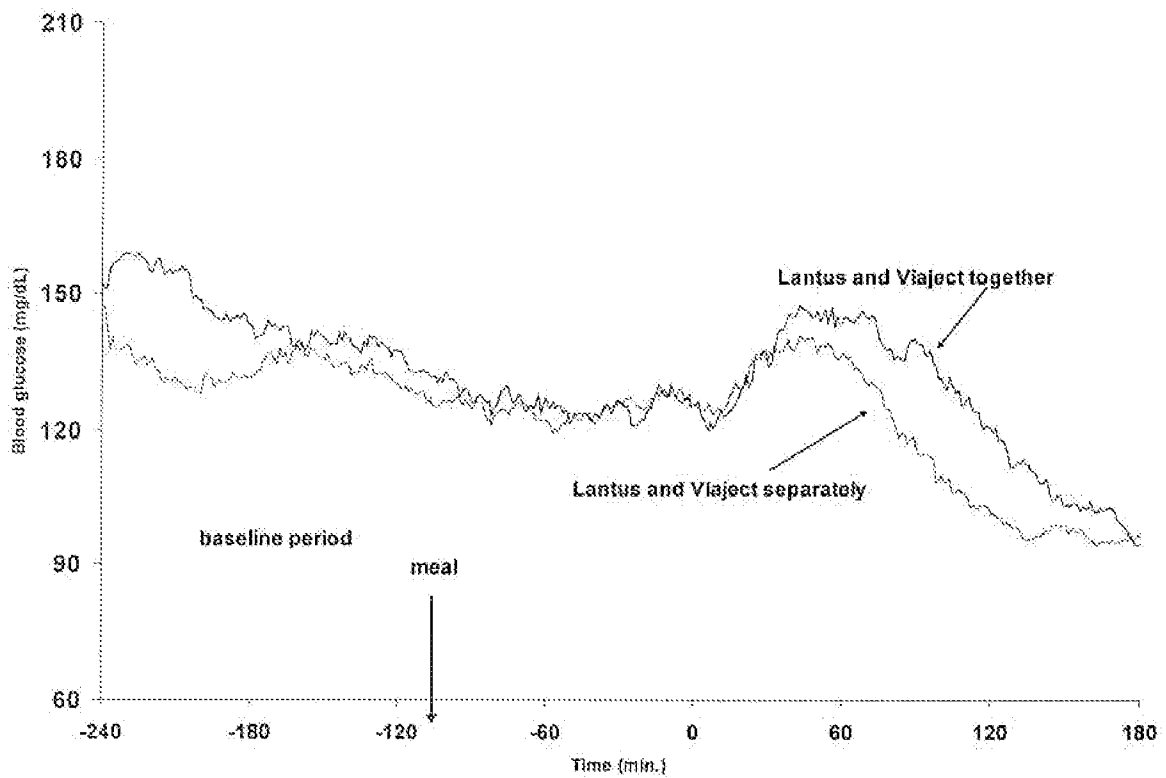


Figure 8