UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NEW JERSEY

SANOFI-AVENTIS U.S. LLC, SANOFI-AVENTIS DEUTSCHLAND GMBH, and SANOFI WINTHROP INDUSTRIE, Plaintiffs, v. MERCK SHARP & DOHME CORP., Defendant.	C.A. No. _____

COMPLAINT FOR PATENT INFRINGEMENT

Plaintiffs Sanofi-Aventis U.S. LLC ("Sanofi U.S."), Sanofi-Aventis Deutschland GmbH ("Sanofi GmbH"), and Sanofi Winthrop Industrie ("SWIND") (collectively, "Plaintiffs" or "Sanofi"), by and through their attorneys, for their Complaint against Merck Sharp & Dohme Corp. ("Merck"), hereby allege as follows:

THE PARTIES

1. Plaintiff Sanofi U.S. is a Delaware limited liability corporation with its principal place of business located at 55 Corporate Drive, Bridgewater, New Jersey 08807.

2. Plaintiff Sanofi GmbH is a German corporation with its principal place of business located at Industriepark Hoechst, Frankfurt Am Main, Germany D-65926.

3. Plaintiff SWIND is a French corporation with its principal place of business located at 20 avenue Raymond Aron, 92160 Antony, France.

4. Defendant Merck is a New Jersey corporation with its principal place of business located at 2000 Galloping Hill Road Kenilworth, N.J. 07033.

5. Defendant Merck conducts business operations in the state of New Jersey.

JURISDICTION AND VENUE

6. This is an action for patent infringement and arises under the patent laws of the United States, Title 35, United States Code. This Court has jurisdiction over the subject matter of this action under 28 U.S.C. §§ 1331 and 1338(a).

7. Venue is proper in this judicial district pursuant to 28 U.S.C. § 1400(b).

8. Defendant Merck is a New Jersey corporation with its principal place of business located at 2000 Galloping Hill Road, Kenilworth, N.J. 07033, and thus resides within the District of New Jersey for purposes of venue.

9. Defendant Merck is licensed to do business with the New Jersey Department of Health and Senior Services as a "Manufacturer and Wholesale[r]" of pharmaceuticals in the State of New Jersey (Registration No. 5003161).

10. Defendant Merck committed an act of infringement in New Jersey when it submitted New Drug Application ("NDA") No. 209-764 pursuant to § 505(b)(2) of the Federal Food, Drug, and Cosmetic Act ("FFDCA") (codified at 21 U.S.C. § 355(b)(2)) to the Food and Drug Administration ("FDA") from the District of New Jersey, accompanied by a Certification pursuant to 21 U.S.C. § 355(b)(2)(A)(iv) ("Paragraph IV Certification").

11. As part of the foregoing act of infringement, Defendant Merck transmitted a Notice of Certification pursuant to 21 U.S.C. § 355(b)(3) and 21 C.F.R. § 314.52 regarding NDA No. 209-764 to, *inter alia,* Sanofi U.S. located in New Jersey.

12. This Court has personal jurisdiction over Merck because Merck is incorporated in this district. Further, Merck maintains continuous and systematic contacts with this judicial

district. Either directly, or through its subsidiaries, agents, and/or affiliates, Merck has conducted and continues to conduct business in this judicial district, including, upon information and belief, by manufacturing, marketing, and selling drug products throughout the United States and in the District of New Jersey. In addition, on information and belief, Merck intends to market the insulin glargine product that is the subject of NDA 209-764 in this judicial district and elsewhere in the United States upon approval of such product by the FDA.

PATENTS-IN-SUIT

13. On January 13, 2009, United States Patent No. 7,476,652 ("the '652 Patent"), entitled "Acidic Insulin Preparations Having Improved Stability," was duly and legally issued by the United States Patent and Trademark Office ("PTO"). A true and correct copy of the '652 Patent is attached as **Exhibit A** to this Complaint.

14. On May 11, 2010, United States Patent No. 7,713,930 ("the '930 Patent"), entitled "Acidic Insulin Preparations Having Improved Stability," was duly and legally issued by the PTO. A true and correct copy of the '930 Patent is attached as **Exhibit B** to this Complaint.

15. The '652 and '930 Patents are collectively referred to herein as the "Patents-in-Suit." By assignment, Sanofi GmbH owns all right, title, and interest in and to the Patents-in-Suit. Sanofi U.S. and/or SWIND are the exclusive licensees of certain rights in or to the Patents-in-Suit in the United States. Plaintiffs have the right to sue and recover damages and other relief for the infringement of the Patents-in-Suit.

BACKGROUND

16. Sanofi U.S. is the holder of approved NDA No. 21-081 for insulin glargine [rDNA origin] for injection, which is prescribed and sold in the United States under the trademarks Lantus® and Lantus® SoloSTAR®. Currently, there is one follow-on version of

Lantus® SoloSTAR® on the market in the United States, manufactured and distributed by Eli Lilly and Company under license from Plaintiffs.

17. The publication *Approved Drug Products with Therapeutic Equivalence Evaluations* (the "Orange Book") identifies drug products approved on the basis of safety and effectiveness by FDA under the FFDCA. The Patents-In-Suit are listed in the Orange Book as covering Sanofi's Lantus® products.

18. The '652 and '930 Patents are both directed to a modified formulation of insulin glargine that the FDA approved in 2005.

19. Each of the Patents-in-Suit was submitted for listing and listed in the Orange Book for Sanofi's NDA No. 21-081 prior to Merck's submission of NDA No. 209-764.

20. On information and belief, on August 4, 2016, Merck submitted NDA No. 208-722 to the FDA pursuant to 21 U.S.C. § 355(b)(2) and transmitted a Notice of Certification pursuant to 21 U.S.C. § 355(b)(3) and 21 C.F.R. § 314.52 to Sanofi U.S. and Sanofi GmbH, disclosing that Merck's NDA No. 208-722 contained a Paragraph IV Certification to, *inter alia*, the '652 and '930 Patents. By its NDA 208-722, Merck is seeking FDA's approval to manufacture commercially and sell its proposed product—an insulin glargine [rDNA origin] for subcutaneous injection in a prefilled insulin delivery device, 100 units/mL, i.e., a cartridge ("Proposed Cartridge Product"). Merck later filed three amendments to its NDA 208-722 with Paragraph IV Certifications seeking approval of the Proposed Cartridge Product. The filing of Merck's NDA No. 208-722 with a Paragraph IV Certification constituted an act of infringement under 35 U.S.C. § 271(e)(2)(A). As a result, Plaintiffs filed Civil Action No. 16-812-RGA in the

United States District Court for the District of Delaware against Merck.[1] That action involves nine patents covering the components and/or subassemblies of Merck's Proposed Cartridge Product, and the '652 and '930 Patents asserted in the instant Complaint.

21. Recently, on information and belief, Merck submitted NDA No. 209-764 to the FDA pursuant to 21 U.S.C. § 355(b)(2) seeking FDA's approval to manufacture commercially and sell another proposed product—an insulin glargine [rDNA origin] for subcutaneous injection provided in a vial, 100 units/mL ("Proposed Vial Product")—that contains data from bioavailability or bioequivalence studies conducted in connection with Sanofi U.S.'s NDA No. 21-081. The filing of Merck's NDA No. 209-764 with a Paragraph IV Certification constituted an act of infringement under 35 U.S.C. § 271(e)(2)(A). On information and belief, Merck's NDA No. 209-764 and its subject, the Proposed Vial Product, are different from Merck's NDA No. 208-722 and its subject, the Proposed Cartridge Product.

22. On information and belief, on June 27, 2017, as part of the foregoing act of infringement, Merck sent a Notice of Certification pursuant to 21 U.S.C. § 355(b)(3) and 21 C.F.R. § 314.52 to Sanofi U.S. and Sanofi GmbH, which discloses that Merck's NDA No. 209-764 contained Paragraph IV Certifications for the '652 and '930 Patents. In this Notice, Merck stated that its certification to the FDA alleges that, *inter alia*, each of the Patents-In-Suit is invalid, unenforceable, and/or will not be infringed by the commercial manufacture, use, or sale of Merck's Proposed Vial Product before their respective expirations.

[1] On June 5, 2017, non-party Mylan Pharmaceuticals Inc. filed petitions with the PTO seeking *inter partes* review of the '652 and '930 Patents. As of the filing of this Complaint, the PTO has not instituted the requested reviews.

23. Sanofi U.S. received Merck's Notice of Certification on the Proposed Vial Product on June 28, 2017 in the District of New Jersey.

24. Sanofi GmbH received Merck's Notice of Certification on the Proposed Vial Product on June 30, 2017 in Germany.

25. Merck's Notice of Certification was accompanied by an Offer of Confidential Access ("OCA").

26. After conferring with Merck early in the week of July 10 concerning the OCA, Sanofi sent written correspondence to Merck on July 13, 2017 in an effort to reach agreement as to the terms of the OCA. In particular, Sanofi proposed edits to the OCA and sought a copy of Merck's un-redacted 505(b)(2) application and additional information regarding Merck's Proposed Vial Product, including physical samples of Merck's Proposed Vial Product. On July 25, 2017, after Merck failed to respond to Sanofi's proposal concerning the OCA terms and its request for additional information, Sanofi again contacted Merck in writing in a further attempt to reach resolution of the outstanding issues. On July 26, 2017, Merck communicated its general acceptance of Sanofi's proposed terms of the OCA and agreed to provide its un-redacted 505(b)(2) application to Sanofi. Merck did not agree to provide Sanofi any other information regarding its Proposed Vial Product, including physical samples. Sanofi responded to Merck's July 26 communication on July 31, requesting final confirmation of the OCA terms and clarification on how and when Merck would provide the NDA to Sanofi. Merck has not responded.

27. As a result, as of August 8, 2017, Sanofi and Merck have not yet finalized the OCA and Merck has failed to produce to Sanofi a copy of its un-redacted 505(b)(2) application. Sanofi timely responded to all correspondence with Merck and sought to reach reasonable

compromise with Merck on these issues. In the absence of such a final agreement and production of Merck's un-redacted NDA, Sanofi resorts to the judicial process and the aid of discovery to obtain, under appropriate judicial safeguards, such information as is required to confirm its allegations of infringement and to present to the Court evidence that the Proposed Vial Product falls within the scope of one or more claims of the Patents-in-Suit.

28. Plaintiffs commenced this action within 45 days after receiving Merck's Notice of Certification accompanying its NDA No. 209-764.

29. FDA's approval of Merck's NDA 209-764 may only be made effective upon a date consistent with 21 U.S.C. § 355(c)(3)(C).

30. On information and belief, Merck's manufacture, use, sale and/or offer to sell in the United States, and/or importation into the United States of its Proposed Vial Product would infringe one or more claims of each of the Patents-in-Suit, directly or indirectly.

COUNT I

(Infringement of U.S. Patent No 7,476,652)

31. Plaintiffs repeat and re-allege paragraphs 1-30 above as if fully set forth herein.

32. On information and belief, Merck submitted NDA No. 209-764 to the FDA and sought approval from the FDA under the FFDCA to engage in the commercial manufacture, use, importation, offer to sell and/or sale of its Proposed Vial Product, which is claimed in the '652 Patent, before the expiration of the '652 Patent. Merck's submission of NDA No. 209-764 is an act of infringement of the '652 Patent under 35 U.S.C. § 271(e)(2)(A).

33. On information and belief, Merck was aware of the '652 Patent prior to filing NDA No. 209-764. If Merck's NDA No. 209-764 is approved, Merck's manufacture, use, sale and/or offer to sell in the United States and/or importation into the United States of its Proposed

Vial Product would infringe the ’652 Patent under 35 U.S.C. §§ 271(a), (b), and/or (c), literally and/or under the doctrine of equivalents.

34. The acts of infringement set forth above will cause Plaintiffs irreparable harm for which they have no adequate remedy at law. Such infringement and resulting irreparable harm will continue unless FDA’s approval of NDA No. 209-764 is stayed, and Merck is enjoined by the Court from engaging in further infringing acts, pending a date that is not earlier than the expiration of the ’652 Patent, or any later date of exclusivity to which Plaintiffs and/or the ’652 Patent are, or become, entitled.

COUNT II

(Infringement of U.S. Patent No. 7,713,930)

35. Plaintiffs repeat and re-allege paragraphs 1-34 above as if fully set forth herein.

36. On information and belief, Merck submitted NDA No. 209-764 to the FDA and sought approval from the FDA under the FFDCA to engage in the commercial manufacture, use, importation, offer to sell and/or sale of its Proposed Vial Product, which is claimed in the ’930 Patent, before the expiration of the ’930 Patent. Merck’s submission of NDA No. 209-764 is an act of infringement of the ’930 Patent under 35 U.S.C. § 271(e)(2)(A).

37. On information and belief, Merck was aware of the ’930 Patent prior to filing NDA No. 209-764. If Merck’s NDA No. 209-764 is approved, Merck’s manufacture, use, sale and/or offer to sell in the United States and/or importation into the United States of its Proposed Vial Product would infringe the ’930 Patent under 35 U.S.C. §§ 271(a), (b), and/or (c), literally and/or under the doctrine of equivalents.

38. The act of infringement set forth above will cause Plaintiffs irreparable harm for which they have no adequate remedy at law. Such infringement and resulting irreparable harm will continue unless FDA’s approval of NDA No. 209-764 is stayed, and Merck is enjoined by

the Court from engaging in further infringing acts, pending a date that is not earlier than the expiration of the ’930 Patent, or any later date of exclusivity to which Plaintiffs and/or the ’930 Patent are, or become, entitled.

REQUESTED RELIEF

Plaintiffs respectfully seek the following relief:

a) The entry of judgment holding that Merck has infringed the ’652 and ’930 Patents;

b) The entry of an order pursuant to 35 U.S.C. § 271(e)(4)(A), declaring that the effective date of any approval of Merck’s NDA No. 209-674 shall be a date that is not earlier than the last date of expiration of either of the ’652 and ’930 Patents or any additional period of exclusivity to which Plaintiffs and/or the ’652 and ’930 Patents are, or become, entitled;

c) The entry of a preliminary injunction, enjoining Merck, its officers, agents, attorneys, and employees, and those acting or attempting to act in active concert with them or acting on their behalf, from infringing either of the ’652 and ’930 Patents by engaging in any commercial manufacture, use, offer to sell, or sale within the United States, or importation into the United States of its Proposed Vial Product as claimed by the ’652 and ’930 Patents for the full terms thereof and any additional period of exclusivity to which Plaintiffs and/or the’652 and ’930 Patents are, or become, entitled, and from inducing or contributing to such activities;

d) The entry of a permanent injunction enjoining Merck, its officers, agents, attorneys, and employees, and those acting or attempting to act in active concert with them or acting on their behalf, from infringing any of the ’652 and ’930 Patents by engaging in any commercial manufacture, use, offer to sell, or sale within the United States, or importation into the United States, of its Proposed Vial Product as claimed by any of the ’652 and ’930 Patents

for the full terms thereof and any additional period of exclusivity to which Plaintiffs and/or ’652 and ’930 Patents are, or become, entitled, and from inducing or contributing to such activities;

e) The entry of an order declaring that this is an exceptional case and awarding Plaintiffs their costs, expenses, and reasonable attorneys’ fees under 35 U.S.C. § 285 and all other applicable statutes, rules, and common law;

f) The taxation of all allowable costs against Merck; and

g) The award to Plaintiffs of any other relief that the Court deems just and proper under the circumstances.

Dated: August 8, 2017

By: */s/ Liza M. Walsh*

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RULE 11.2 CERTIFICATION

We hereby certify that, to the best of our knowledge, the matter in controversy is not the subject of any action pending in any court or of any arbitration or administrative proceeding, but it is related to the following actions:

- *Sanofi-Aventis U.S. LLC et al v. Merck Sharp & Dohme Corp.*, Civil Action No. 1:16-cv-00812-RGA, pending in the United States District Court, District of Delaware before the Honorable Richard G. Andrews, U.S.D.J.;
- *Mylan Pharmaceuticals Inc. v. Sanofi-Aventis Deutschland GMBH*, Petition for *Inter Partes* Review as to Patent No. 7,476,652 filed on June 5, 2017 with the United States Patent and Trademark Office, Patent Trial and Appeal Board (IPR2017-01526); and
- *Mylan Pharmaceuticals Inc. v. Sanofi-Aventis Deutschland GMBH*, Petition for *Inter Partes* Review as to Patent No. 7,713,930 filed on June 5, 2017 with the United States Patent and Trademark Office, Patent Trial and Appeal Board (IPR2017-01528).

Dated: August 8, 2017

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RULE 201.1 CERTIFICATION

We hereby certify that the above-captioned matter is not subject to compulsory arbitration in that the Plaintiffs seek, *inter alia*, injunctive relief.

Dated: August 8, 2017

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EXHIBIT A

US007476652B2

(12) **United States Patent**
Brunner-Schwarz et al.

(10) **Patent No.: US 7,476,652 B2**
(45) **Date of Patent: Jan. 13, 2009**

(54) **ACIDIC INSULIN PREPARATIONS HAVING IMPROVED STABILITY**

(75) Inventors: **Anette Brunner-Schwarz**, Frankfurt (DE); **Norbert Lill**, Kronberg (DE)

(73) Assignee: **Sanofi-Aventis Deutschland GmbH**, Frankfurt (DE)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 40 days.

(21) Appl. No.: **11/089,777**

(22) Filed: **Mar. 25, 2005**

(65) **Prior Publication Data**

US 2005/0171009 A1 Aug. 4, 2005

Related U.S. Application Data

(63) Continuation of application No. 10/461,740, filed on Jun. 13, 2003, now abandoned.

(60) Provisional application No. 60/409,338, filed on Sep. 9, 2002.

(30) **Foreign Application Priority Data**

Jun. 18, 2002 (DE) 102 27 232

(51) **Int. Cl.**
A61K 38/28 (2006.01)

(52) **U.S. Cl.** **514/3**; 514/4

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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Primary Examiner—Jeffrey E Russel
(74) *Attorney, Agent, or Firm*—Balaram Gupta

(57) **ABSTRACT**

The invention relates to a pharmaceutical formulation comprising a polypeptide selected from the group consisting of insulin, an insulin metabolite, an insulin analog, an insulin derivative and combinations thereof; a surfactant or combinations of two or more surfactants; optionally a preservative or combinations of two or more preservatives; and optionally an isotonicizing agent, buffers or further excipients or combinations thereof, the pharmaceutical formulation having a pH in the acidic range.

25 Claims, No Drawings

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ACIDIC INSULIN PREPARATIONS HAVING IMPROVED STABILITY

This application is entitled to the benefit of U.S. Provisional Application 60/409,338, filed Sep. 9, 2002, and Federal Republic of Germany Application 10227232.8-41, filed Jun. 18, 2002.

SUMMARY OF THE INVENTION

The invention relates to a pharmaceutical formulation comprising a polypeptide selected from the group consisting of insulin, an insulin metabolite, an insulin analog, an insulin derivative or combinations thereof; a surfactant or combinations of two or more surfactants; optionally a preservative or combinations of two or more preservatives; and optionally an isotonicizing agent, buffers or further excipients or combinations thereof, the pharmaceutical formulation having a pH in the acidic range. These formulations can be employed for the treatment of diabetes, and are particularly suitable for preparations in which a high stability to thermal and/or physico-mechanical stress is necessary. The invention likewise relates to parenteral preparations which contain such formulations and can be used in diabetes and to methods for producing the preparations and for improving the stability of insulin preparations.

BACKGROAND OF THE INVENTION

Worldwide, approximately 120 million people suffer from diabetes mellitus. Among these, approximately 12 million are type I diabetics, for whom the substitution of the lacking endocrine insulin secretion is the only currently possible therapy. The affected persons are dependent lifelong on insulin injections, as a rule a number of times daily. In contrast to type I diabetes, there is not basically a deficiency of insulin in type II diabetes, but in a large number of cases, especially in the advanced stage, treatment with insulin, optionally in combination with an oral antidiabetic, is regarded as the most favorable form of therapy.

In the healthy person, the release of insulin by the pancreas is strictly coupled to the concentration of blood glucose. Elevated blood glucose levels, such as occur after meals, are rapidly compensated by a corresponding increase in insulin secretion. In the fasting state, the plasma insulin level falls to a basal value which is adequate to guarantee a continuous supply of insulin-sensitive organs and tissue with glucose and to keep hepatic glucose production low at night. The replacement of endogenous insulin secretion by exogenous, mostly subcutaneous administration of insulin, as a rule does not approximate the quality of the physiological regulation of the blood glucose described above. Often, deviations of blood glucose upward or downward occur, which in their severest forms can be life-threatening. In addition, however, blood glucose levels which are increased for years without initial symptoms are a considerable health risk. The large-scale DCCT study in the USA (The Diabetes Control and Complications Trial Research Group (1993) N. Engl. J. Med. 329, 977-986) demonstrated clearly that chronically elevated blood glucose levels are essentially responsible for the development of diabetic late damage. Diabetic late damage is microvascular and macrovascular damage which is manifested, ander certain circumstances, as retinopathy, nephropathy or neuropathy and leads to loss of sight, kidney failure and the loss of extremities and is moreover accompanied by an increased risk of cardiovascular diseases. In view of this, an improved therapy of diabetes should be aimed at keeping

the blood glucose as closely as possible in the physiological range. According to the concept of intensified insulin therapy, this should be achieved by repeated daily injections of rapid- and slow-acting insulin preparations. Rapid-acting formulations are given at meals in order to level out the postprandial increase in the blood glucose. Slow-acting basal insulins should ensure the basic supply with insulin, in particular during the night, without leading to hypoglycemia.

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 2 disulfide bridges. Insulin preparations have been employed for diabetes therapy for many years. Not only are naturally occurring insulins used, but recently also insulin derivatives and analogs.

Insulin analogs are analogs of naturally occurring insulins, namely human insulin or animal insulins, which differ by substitution of at least one naturally occurring amino acid residue with other amino acids and/or addition/removal of at least one amino acid residue from the corresponding, otherwise identical, naturally occurring insulin. The amino acids can in this case also be those which do not occur naturally.

Insulin derivatives are derivatives of naturally occurring insulin or an insulin analog which are obtained by chemical modification. This chemical modification can consist, for example, of the addition of one or more specific chemical groups to one or more amino acids. As a rule, insulin derivatives and insulin analogs have a somewhat modified action compared with human insulin.

Insulin analogs having an accelerated onset of action are described in EP 0 214 826, EP 0 375 437 and EP 0 678 522. EP 0 124 826 relates, inter alia, to substitutions of B27 and B28. EP 0 678 522 describes insulin analogs which in position B29 have various amino acids, preferably proline, but not glutamic acid. EP 0 375 437 includes insulin analogs with lysine or arginine in B28, which can optionally be additionally modified in B3 and/or A21.

In EP 0 419 504, 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 of the positions B1-B6 is replaced by lysine or arginine. According to WO 92/00321, insulins of this type have a prolonged action. The insulin analogs described in EP-A 0 368 187 also have a delayed action.

The insulin preparations of naturally occurring insulins on the market for insulin substitution differ in the origin of the insulin (e.g. bovine, porcine, human insulin), and also the composition, whereby the profile of action (onset of action and duration of action) can be influenced. By combination of various insulin preparations, very different profiles of action can be obtained and blood sugar values which are as physiological as possible can be established. Recombinant DNA technology today makes possible the preparation of such modified insulins. These include insulin glargine (Gly(A21)-Arg(B31)-Arg(B32)-human insulin) with a prolonged duration of action. Insulin glargine is injected as an acidic, clear solution and precipitates on account of its solution properties in the physiological pH range of the subcutaneous tissue as a stable hexamer associate. Insulin glargine 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-Zsilavecz et al., 2: 125-130(2001)).

The specific preparation of insulin glargine, which leads to the prolonged duration of action, is characterized, in contrast

to previously described preparations, by a clear solution having an acidic pH. Especially at acidic pH, insulins, however, show a decreased stability and an increased proneness to aggregation on thermal and physicomechanical stress, which can make itself felt in the form of turbidity and precipitation (particle formation) (Brange et al., J. Ph. Sci 86:517-525 (1997)).

The proneness to aggregation can additionally be promoted by hydrophobic surfaces which are in contact with the solution (Sluzky et al., Proc. Natl. Acad. Sci. 88:9377-9381 (1991). Surfaces which can be considered as hydrophobic are the glass vessels of the preparations, the stopper material of the sealing caps or the boundary surface of the solution with the air supernatant. In addition, very fine silicone oil droplets can function as additional hydrophobic aggregation nuclei in the taking of the daily insulin dose by means of customary, siliconized insulin syringes and accelerate the process.

WO 01/43762 describes aqueous, parenteral pharmaceutical preparations comprising a polypeptide and glycerol, in which the stabilization of the preparation is to be achieved by purifying off destabilizing constituents of the glycerol.

WO 00/23098 describes insulin preparations stabilized using polysorbate 20 or poloxamer 188 for pulmonary administration, but does not describe the stabilization in an acidic solution against aggregation nuclei.

Published International patent application WO 02/076495 describes zinc-free and low-zinc insulin preparations having improved stability at room and body temperature and to mechanical stress by the addition of surfactants, but does not describe the stabilization of acidic insulin preparations against hydrophobic aggregation nuclei.

The present invention was thus based on the object of finding preparations for acid-soluble insulins containing surfactants, which are distinguished by a high long-term stability to stress due to temperature or physicomechanical stressing and tolerate a high stress with hydrophobic aggregation nuclei.

DETAILED DESCRIPTION OF THE INVENTION

It has now surprisingly been found that the addition of surfactants can greatly increase the stability of acidic insulin preparations and thus preparations can be produced which guarantee superior stability to hydrophobic aggregation nuclei for several months ander temperature stress.

The pharmaceutical preparations of the present invention contain 60-6000 nmol/ml, preferably 240-3000 nmol/ml, of an insulin, an insulin metabolite, an insulin analog or an insulin derivative.

The surfactants which can be used are, inter alia, nonionic surfactants. In particular, pharmaceutically customary surfactants are preferred, such as, for example: partial and fatty acid esters and ethers of polyhydric alcohols such as of glycerol, sorbitol and the like (Span®, Tween®, in particular Tween® 20 and Tween® 80, Myrj®, Brij®), Cremophor® or poloxamers. The surfactants are present in the pharmaceutical composition in a concentration of 5-200 μg/ml, preferably of 5-120 μg/ml and particularly preferably of 20-75 μg/ml.

The preparation can additionally optionally contain preservatives (e.g. phenol, cresol, parabens), isotonicizing agents (e.g. mannitol, sorbitol, lactose, dextrose, trehalose, sodium chloride, glycerol), buffer substances, salts, acids and alkalis and also further excipients. These substances can in each case be present individually or alternatively as mixtures.

Glycerol, dextrose, lactose, sorbitol and mannitol are customarily present in the pharmaceutical preparation in a concentration of 100-250 mM, NaCl in a concentration of up to 150 mM. Buffer substances, such as, for example, phosphate, acetate, citrate, arginine, glycylglycine or TRIS (i.e. 2-amino-2-hydroxymethyl-1,3-propanediol) buffer and corresponding salts, are present in a concentration of 5-250 mM, preferably 10-100 mM. Further excipients can be, inter alia, salts or arginine.

The invention therefore relates to a pharmaceutical formulation comprising a polypeptide selected from the group consisting of insulin, an insulin analog, an insulin derivative, an active insulin metabolite and combinations thereof; a surfactant or combinations of two or more surfactants; optionally a preservative or combinations of two or more preservatives; and optionally an isotonicizing agent, buffer substances and/or further excipients or combinations thereof, the pharmaceutical formulation being a clear solution which has a pH in the acidic range (pH 1-6.8), preferably pH 3.5-6.8, very particularly preferably 3.5-4.5.

Preferred pharmaceutical formulations of the present invention are those wherein the surfactant is selected from the group consisting of partial and fatty acid esters and ethers of polyhydric alcohols such as of glycerol and sorbitol, and polyols; the partial and fatty acid esters and ethers of glycerol and sorbitol being selected from the group consisting of Span®, Tween®, Myrj®, Brij®, Cremophor®; the polyols being selected from the group consisting of polypropylene glycols, polyethylene glycols, poloxamers, Pluronics®, and Tetronics®; the preservative being selected from the group consisting of phenol, cresol, and parabens; the isotonicizing agent being selected from the group consisting of mannitol, sorbitol, sodium chloride, and glycerol; the excipients being selected from the group consisting of buffer substances, acids, and alkalis; the insulin analog being selected from the group consisting of Gly(A21)-Arg(B31)-Arg(B32)-human insulin; Lys(B3)-Glu(B29)-human insulin; $Lys^{B28}Pro^{B29}$ human insulin, B28 Asp-human insulin, human insulin in which proline in position B28 has been substituted by Asp, Lys, Leu, Val or Ala and where in position B29 Lys can be substituted by Pro; AlaB26-human insulin; des(B28-B30)-human insulin; des(B27)-human insulin and des(B30)-human insulin; the insulin derivative being selected from the group consisting of B29-N-myristoyl-des(B30) human insulin, B29-N-palmitoyl-des(B30) human insulin, B29-N-myristoyl human insulin, B29-N-palmitoyl human insulin, B28-N-myristoyl $Lys^{B28}Pro^{B29}$ human insulin, B28-N-palmitoyl-$Ly^{B28}Pro^{B29}$ human insulin, B30-N-myristoyl-$Thr^{B29}Lys^{B30}$ human insulin, B30-N-palmitoyl-$Thr^{B29}Lys^{B30}$ human insulin, B29-N-(N-palmitoyl-γ-glutamyl)-des(B30) human insulin, B29-N-(N-lithocholyl-γ-glutamyl)-des(B30) human insulin, B29-N-(ω-carboxyheptadecanoyl)-des(B30) human insulin and B29-N-(ω-carboxyheptadecanoyl) human insulin.

A further subject of the invention is a pharmaceutical formulation such as described above, in which the insulin, the insulin analog, the active insulin metabolite and/or the insulin derivative is present in a concentration of 60-6000 nmol/ml, preferably in a concentration of 240-3000 nmol/ml (this corresponds approximately to a concentration of 1.4-35 mg/ml or 40-500 units/ml);

in which the surfactant is present in a concentration of 5-200 μg/ml, preferably of 5-120 μg/ml and particularly preferably of 20-75 μg/ml.

A further subject of the invention is a pharmaceutical formulation such as mentioned above, in which glycerol and/or mannitol is present in a concentration of 100-250 mM, and/or NaCl is preferably present in a concentration of up to 150 mM.

A further subject of the invention is a pharmaceutical formulation such as mentioned above, in which a buffer substance is present in a concentration of 5-250 mM.

A further subject of the invention is a pharmaceutical insulin formulation which contains further additives such as, for example, salts which delay the release of insulin. Mixtures of such delayed-release insulins with formulations described above are included therein.

A further subject of the invention is a method for the production of such pharmaceutical formulations. Likewise, a further subject of the invention is the use of such formulations for the treatment of diabetes mellitus.

A further subject of the invention is the use or the addition of surfactants as stabilizer during the process for the production of insulin, insulin analogs or insulin derivatives or their preparations.

EXAMPLES

The following examples illustrate, but by no means limit, the present invention.

Comparison investigations: Different preparations containing the insulin analog insulin glargine (Gly(A21),Arg(B31),Arg(B32)-human insulin) are prepared. To this end, insulin glargine is suspended in one part of water for injection, dissolved at pH 3-4, the other constituents are added, the pH is adjusted to 4.0+/−0.2 using hydrochloric acid/NaOH and the mixture is made up to the final volume. The concentration of insulin glargine in each of the experiments described below is 3.6378 mg/ml (corresponds to 100 units/ml). A second preparation is produced identically, but a specific amount of a surfactant is additionally added. The solutions are filled into 10 ml glass vessels (vials) and fitted with crimp caps. These vessels are now exposed to simulated in use or physicomechanical stress conditions:

1. In use test: The vessels are sorted into boxes with turned-up lids and stored during the investigation period of 28 days at +25° C. and controlled room humidity with exclusion of light. To simulate taking by the patient, once daily about 5 IU of the solutions are withdrawn using a customary insulin syringe and discarded. At the beginning and end of the working week this procedure is carried out twice in order to to simulate taking at the weekend. Before each withdrawal, visual assessment of the solution in the vessels for turbidity and/or particle formation is carried out.
2. Shaking test: The vessels are placed in a box with a turned-up lid lying on a laboratory shaker having an incubator and thermostat and shaken at 25° C. with 90 movements/min parallel to the horizontal movement for a period of time of 10 days. After defined times, the turbidity value of the samples is determined by means of a laboratory turbidity photometer (nephelometer) in formaldazine nephelometric units (formaldazine nephelometric unit=FNU). The turbidity value corresponds to the intensity of the scattered radiation of the light incident on suspended particles in the sample.

Example 1

Stabilization of the in Use Period of Insulin Glargine Using Polysorbate 20 (Tween® 20)

a) The solution is sterile-filtered through a combination of 0.2 μm and 0.1 μm filters. It is then poured into 10 ml injection vials and sealed using crimp caps having an inserted sealing disk.

b) A comparison solution is prepared identically, but first a suitable amount of surfactant (10-30 ppm of polysorbate 20) is suspended in water for injection. The samples are stored at +5° C., 25° C. and 37° C. for a fixed period of time. 10 samples in each case are then subjected to an in use test. The results are shown in the table below.

Storage for 3 Months at 5° C.

	Number of vials with particle formation after			
Test sample	7 days	14 days	21 days	28 days
Insulin glargine	7	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	1
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0

storage for 6 months at 5° C.

	Number of vials with particle formation after			
Test sample	7 days	14 days	21 days	28 days
Insulin glargine	1	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	1
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	1
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	1	0

Storage for 3 Months at 25° C.

	Number of vials with particle formation after			
Test sample	7 days	14 days	21 days	28 days
Insulin glargine	9	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	2	2	2	2
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	1
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0

Storage for 6 Months at 25° C.

	Number of vials with particle formation after			
Test sample	7 days	14 days	21 days	28 days
Insulin glargine	10	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	1

-continued

Test sample	Number of vials with particle formation after 7 days	14 days	21 days	28 days
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	1	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0

Storage for 1 Month at 37° C.

Test sample	Number of vials with particle formation after 7 days	14 days	21 days	28 days
Insulin glargine	0	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	3	3	5
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0

Storage for 3 Months at 37° C.

Test sample	Number of vials with particle formation after 7 days	14 days	21 days	28 days
Insulin glargine	5	9	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	1	1	1	1
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0

Storage for 6 Months at 37° C.

Test sample	Number of vials with particle formation after 7 days	14 days	21 days	28 days
Insulin glargine	10	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	1	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	1	1	1	1

Without addition of polysorbate 20, particle formation can occur in the solution even after 7 days in use. By addition of polysorbate 20, the particle formation can be markedly suppressed during the in use period.

The stabilizing action of polysorbate 20 is retained even on storage at elevated temperatures for a period of 3 months.

A decline in the stabilizing action due to possible hydrolysis of the polysorbate in the acidic medium of the solution cannot be determined in comparison with the data after storage for 1 month.

Example 2

Stabilization of Insulin Glargine Using Polysorbate 20 Ander Physico-Mechanical Stress Loading

a) The solution is sterile-filtered through a combination of 0.2 μm and 0.1 μm filters. It is then poured into 10 ml injection vials and sealed using crimp caps having an inserted sealing disk.

b) A comparison solution is prepared identically, but first a suitable amount of surfactant (0.010-0.030 mg/ml of polysorbate 20) is suspended in water for injection.

The samples are stored at +5° C., 25° C. and 37° C. for a fixed period of time. 5 samples in each case are then subjected to a shaking test. The results are shown in the table below, the limit 15 FNU corresponds to turbidities which are discernible in daylight.

Storage for 1 Month at 5° C.

Test sample	Number of vials > 15 FNU 0 days	0.5 days	1 day	2 days	3 days	4 days	6 days	8 days	10 days
Insulin glargine	0	0	0	2	3	3	4	4	4
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	0	0	1	3	4	5
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0

Storage for 1 Month at 25° C.

Test sample	Number of vials > 15 FNU								
	0 days	0.5 days	1 day	2 days	3 days	4 days	6 days	8 days	10 days
Insulin glargine	0	0	0	1	1	1	1	2	3
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	0	0	0	1	2	3
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0

Storage for 1 Month at 37° C.

Test sample	Number of vials > 15 FNU								
	0 days	0.5 days	1 day	2 days	3 days	4 days	6 days	8 days	10 days
Insulin glargine	0	0	0	2	5	5	5	5	5
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0

Without addition of polysorbate 20, even after 2 days of severe physicomechanical stress, a visible turbidity can occur in the solution. By addition of polysorbate 20, the formation of turbidity during physicomechanical stressing can be markedly delayed. The stabilizing action of polysorbate 20 is retained even on storage at elevated temperatures.

A decline in the stabilizing action due to possible hydrolysis of the polysorbate in the acidic medium of the solution cannot be detected.

Example 3

Comparison of the Stabilization of the in Use Period of Insulin Glargine Using Polysorbate 20 (Tween® 20) and Using Polysorbate 80 (Tween® 20)

Open 10 vials in each case to give 5 ml of insulin glargine injection solution and

a) addition of 0.001 mg/ml of polysorbate 20
b) addition of 0.01 mg/ml of polysorbate 20
c) addition of 0.001 mg/ml of polysorbate 80
d) addition of 0.01 mg/ml of polysorbate 80

in the form of a concentrated stock solution.

The samples are then subjected to an in use test.

The results are shown in the table below.

Test sample	Vials with particle formation after			
	7 days	14 days	21 days	28 days
Insulin glargine + 0.001 mg/ml of polysorbate 20	no	yes	Yes, particles increasingly occur	Yes, particles increasingly occur
Insulin glargine + 0.010 mg/ml of polysorbate 20	no	no	no	no
Insulin glargine + 0.001 mg/ml of polysorbate 80	no	yes	Yes, particles increasingly occur	Yes, particles increasingly occur
Insulin glargine + 0.010 mg/ml of polysorbate 80	no	no	no	no

An addition of polysorbate 20 or of polysorbate 80 in a concentration of 0.001 mg/ml are equally able to stabilize the solution against particle formation during the in use period.

What is claimed is:

1. A pharmaceutical formulation comprising Gly(A21), Arg(B31), Arg(B32)-human insulin;

at least one chemical entity chosen from polysorbate 20 and polysorbate 80;

at least one preservative; and

water,

wherein the pharmaceutical formulation has a pH in the acidic range from 1 to 6.8.

2. The pharmaceutical formulation as claimed in claim 1, wherein the at least one chemical entity comprises polysorbate 20.

3. The pharmaceutical formulation as claimed in claim 2, wherein the at least one preservative is chosen from phenols.

4. The pharmaceutical formulation as claimed in claim 3, wherein at least one preservative is cresol.

5. The pharmaceutical formulation as claimed in claim 4, further including zinc.

6. The pharmaceutical formulation as claimed in claim 1 further including at least one isotonicizing agent.

7. A pharmaceutical formulation comprising Gly(A21), Arg(B31), Arg(B32)-human insulin,

at least one chemical entity chosen from polysorbate and poloxamers;

at least one preservative; and

water,

wherein the pharmaceutical formulation has a pH in the acidic range from 1 to 6.8.

8. The pharmaceutical formulation as claimed in claim 2, wherein the polysorbate 20 is present in an effective amount to avoid turbidity.

9. The pharmaceutical formulation as claimed in claim 5, wherein the pharmaceutical formulation has a pH in the acidic range from 3.5 to 6.8.

10. The pharmaceutical formulation as claimed in claim 9, wherein the pharmaceutical formulation has a pH in the acidic range from 3.5 to 4.5.

11. The pharmaceutical formulation as claimed in claim 1, wherein the at least one preservative is chosen from phenol, cresol, chlorocresol, benzyl alcohol, and parabens.

12. The pharmaceutical formulation as claimed in claim 6, wherein the at least one isotonicizing agent is chosen from mannitol, sorbitol, lactose, dextrose, trehalose, sodium chloride, and glycerol.

13. The pharmaceutical formulation as claimed in claim 1, further comprising a buffer.

14. The pharmaceutical formulation as claimed in claim 13, wherein the buffer is chosen from TRIS, phosphate, citrate, acetate, and glycylglycine.

15. The pharmaceutical formulation as claimed in claim 1, wherein the Gly(A21), Arg(B31), Arg(B32)-human insulin is present in a concentration of 60-6000 nmol/ml.

16. The pharmaceutical formulation as claimed in claim 15, wherein the Gly(A21), Arg(B31), Arg(B32)-human insulin is present in a concentration of 240-3000 nmol/ml.

17. The pharmaceutical formulation as claimed in claim 1, wherein the at least one chemical entity is present in a concentration of 5-200 μg/ml.

18. The pharmaceutical formulation as claimed in claim 17, wherein the at least one chemical entity is present in a concentration of 5-120 μg/ml.

19. The pharmaceutical formulation as claimed in claim 18, wherein the at least one chemical entity is present in a concentration of 20-75 μg/ml.

20. The pharmaceutical formulation as claimed in claim 12, wherein at least one isotonicizing agent is chosen from glycerol and mannitol and wherein said at least one isotonicizing agent is present in a concentration of 100-250 mM.

21. The pharmaceutical formulation as claimed in claim 1, wherein NaCl is present in a concentration of up to 150 mM.

22. The pharmaceutical formulation as claimed in claim 13, wherein said buffer is present in a concentration of 5-250 mM.

23. The pharmaceutical formulation as claimed in claim 6, wherein the at least one chemical entity comprises polysorbate 20, at least one preservative is cresol, and the pharmaceutical formulation has a pH in the acidic range from 3.5 to 4.5.

24. A pharmaceutical formulation comprising Gly(A21), Arg(B31), Arg(B32)-human insulin:

at least one chemical entity chosen from polysorbate and poloxamers;

at least one preservative chosen from cresol; and

water,

wherein the pharmaceutical formulation has a pH in the acidic range from 3.5 to 4.5.

25. The pharmaceutical formulation as claimed in claim 1, further comprising one or more excipients chosen from acids, alkalis and salts.

* * * * *

EXHIBIT B

US007713930B2

(12) **United States Patent**
Brunner-Schwarz et al.

(10) **Patent No.: US 7,713,930 B2**
(45) **Date of Patent: *May 11, 2010**

(54) **ACIDIC INSULIN PREPARATIONS HAVING IMPROVED STABILITY**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **12/328,208**

(22) Filed: **Dec. 4, 2008**

(65) **Prior Publication Data**

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Related U.S. Application Data

(63) Continuation of application No. 11/089,777, filed on Mar. 25, 2005, now Pat. No. 7,476,652, which is a continuation of application No. 10/461,740, filed on Jun. 13, 2003, now abandoned.

(60) Provisional application No. 60/409,338, filed on Sep. 9, 2002.

(30) **Foreign Application Priority Data**

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A61K 38/28 (2006.01)

(52) **U.S. Cl.** **514/3**

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

The invention relates to a pharmaceutical formulation comprising a polypeptide selected from the group consisting of insulin, an insulin metabolite, an insulin analog, an insulin derivative and combinations thereof; a surfactant or combinations of two or more surfactants; optionally a preservative or combinations of two or more preservatives; and optionally an isotonicizing agent, buffers or further excipients or combinations thereof, the pharmaceutical formulation having a pH in the acidic range.

20 Claims, No Drawings

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ACIDIC INSULIN PREPARATIONS HAVING IMPROVED STABILITY

This application is a continuation of U.S. application Ser. No. 11/089,777, filed Mar. 25, 2005, now allowed, which is a continuation of Ser. No. 10/461,740, filed Jun. 13, 2003, now abandoned; both of which are incorporated herein by reference in their entirety which claims the benefit of U.S. Provisional Application No. 60/409,338, filed Sep. 9, 2002, and Federal Republic of Germany Application 10227232.8, filed Jun. 18, 2002.

SUMMARY OF THE INVENTION

The invention relates to a pharmaceutical formulation comprising a polypeptide selected from the group consisting of insulin, an insulin metabolite, an insulin analog, an insulin derivative or combinations thereof; a surfactant or combinations of two or more surfactants; optionally a preservative or combinations of two or more preservatives; and optionally an isotonicizing agent, buffers or further excipients or combinations thereof, the pharmaceutical formulation having a pH in the acidic range. These formulations can be employed for the treatment of diabetes, and are particularly suitable for preparations in which a high stability to thermal and/or physico-mechanical stress is necessary. The invention likewise relates to parenteral preparations which contain such formulations and can be used in diabetes and to methods for producing the preparations and for improving the stability of insulin preparations.

BACKGROUND OF THE INVENTION

Worldwide, approximately 120 million people suffer from diabetes mellitus. Among these, approximately 12 million are type I diabetics, for whom the substitution of the lacking endocrine insulin secretion is the only currently possible therapy. The affected persons are dependent lifelong on insulin injections, as a rule a number of times daily. In contrast to type I diabetes, there is not basically a deficiency of insulin in type II diabetes, but in a large number of cases, especially in the advanced stage, treatment with insulin, optionally in combination with an oral antidiabetic, is regarded as the most favorable form of therapy.

In the healthy person, the release of insulin by the pancreas is strictly coupled to the concentration of blood glucose. Elevated blood glucose levels, such as occur after meals, are rapidly compensated by a corresponding increase in insulin secretion. In the fasting state, the plasma insulin level falls to a basal value which is adequate to guarantee a continuous supply of insulin-sensitive organs and tissue with glucose and to keep hepatic glucose production low at night. The replacement of endogenous insulin secretion by exogenous, mostly subcutaneous administration of insulin, as a rule does not approximate the quality of the physiological regulation of the blood glucose described above. Often, deviations of blood glucose upward or downward occur, which in their severest forms can be life-threatening. In addition, however, blood glucose levels which are increased for years without initial symptoms are a considerable health risk. The large-scale DCCT study in the USA (The Diabetes Control and Complications Trial Research Group (1993) N. Engl. J. Med. 329, 977-986) demonstrated clearly that chronically elevated blood glucose levels are essentially responsible for the development of diabetic late damage. Diabetic late damage is microvascular and macrovascular damage which is manifested, under certain circumstances, as retinopathy, nephropathy or neuropathy and leads to loss of sight, kidney failure and the loss of extremities and is moreover accompanied by an increased risk of cardiovascular diseases. In view of this, an improved therapy of diabetes should be aimed at keeping the blood glucose as closely as possible in the physiological range. According to the concept of intensified insulin therapy, this should be achieved by repeated daily injections of rapid- and slow-acting insulin preparations. Rapid-acting formulations are given at meals in order to level out the postprandial increase in the blood glucose. Slow-acting basal insulins should ensure the basic supply with insulin, in particular during the night, without leading to hypoglycemia.

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 2 disulfide bridges. Insulin preparations have been employed for diabetes therapy for many years. Not only are naturally occurring insulins used, but recently also insulin derivatives and analogs.

Insulin analogs are analogs of naturally occurring insulins, namely human insulin or animal insulins, which differ by substitution of at least one naturally occurring amino acid residue with other amino acids and/or addition/removal of at least one amino acid residue from the corresponding, otherwise identical, naturally occurring insulin. The amino acids can in this case also be those which do not occur naturally.

Insulin derivatives are derivatives of naturally occurring insulin or an insulin analog which are obtained by chemical modification. This chemical modification can consist, for example, of the addition of one or more specific chemical groups to one or more amino acids. As a rule, insulin derivatives and insulin analogs have a somewhat modified action compared with human insulin.

Insulin analogs having an accelerated onset of action are described in EP 0 214 826, EP 0 375 437 and EP 0 678 522. EP 0 124 826 relates, inter alia, to substitutions of B27 and B28. EP 0 678 522 describes insulin analogs which in position B29 have various amino acids, preferably proline, but not glutamic acid.

EP 0 375 437 includes insulin analogs with lysine or arginine in B28, which can optionally be additionally modified in B3 and/or A21.

In EP 0 419 504, 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 of the positions B1-B6 is replaced by lysine or arginine. According to WO 92/00321, insulins of this type have a prolonged action. The insulin analogs described in EP-A 0 368 187 also have a delayed action.

The insulin preparations of naturally occurring insulins on the market for insulin substitution differ in the origin of the insulin (e.g. bovine, porcine, human insulin), and also the composition, whereby the profile of action (onset of action and duration of action) can be influenced. By combination of various insulin preparations, very different profiles of action can be obtained and blood sugar values which are as physiological as possible can be established. Recombinant DNA technology today makes possible the preparation of such modified insulins. These include insulin glargine (Gly(A21)-Arg(B31)-Arg(B32)-human insulin) with a prolonged duration of action. Insulin glargine is injected as an acidic, clear solution and precipitates on account of its solution properties in the physiological pH range of the subcutaneous tissue as a stable hexamer associate. Insulin glargine 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-Zsilavecz et al., 2:125-130(2001)).

The specific preparation of insulin glargine, which leads to the prolonged duration of action, is characterized, in contrast to previously described preparations, by a clear solution having an acidic pH. Especially at acidic pH, insulins, however, show a decreased stability and an increased proneness to aggregation on thermal and physicomechanical stress, which can make itself felt in the form of turbidity and precipitation (particle formation) (Brange et al., J. Ph. Sci 86:517-525 (1997)).

The proneness to aggregation can additionally be promoted by hydrophobic surfaces which are in contact with the solution (Sluzky et al., Proc. Natl. Acad. Sci. 88:9377-9381 (1991). Surfaces which can be considered as hydrophobic are the glass vessels of the preparations, the stopper material of the sealing caps or the boundary surface of the solution with the air supernatant. In addition, very fine silicone oil droplets can function as additional hydrophobic aggregation nuclei in the taking of the daily insulin dose by means of customary, siliconized insulin syringes and accelerate the process.

WO 01/43762 describes aqueous, parenteral pharmaceutical preparations comprising a polypeptide and glycerol, in which the stabilization of the preparation is to be achieved by purifying off destabilizing constituents of the glycerol.

WO 00/23098 describes insulin preparations stabilized using polysorbate 20 or poloxamer 188 for pulmonary administration, but does not describe the stabilization in an acidic solution against aggregation nuclei.

WO 02/076495 describes zinc-free and low-zinc insulin preparations having improved stability at room and body temperature and to mechanical stress by the addition of surfactants, but does not describe the stabilization of acidic insulin preparations against hydrophobic aggregation nuclei.

The present invention was thus based on the object of finding preparations for acid-soluble insulins containing surfactants, which are distinguished by a high long-term stability to stress due to temperature or physicomechanical stressing and tolerate a high stress with hydrophobic aggregation nuclei.

DETAILED DESCRIPTION OF THE INVENTION

It has now surprisingly been found that the addition of surfactants can greatly increase the stability of acidic insulin preparations and thus preparations can be produced which guarantee superior stability to hydrophobic aggregation nuclei for several months under temperature stress.

The pharmaceutical preparations of the present invention contain 60-6000 nmol/ml, preferably 240-3000 nmol/ml, of an insulin, an insulin metabolite, an insulin analog or an insulin derivative.

The surfactants which can be used are, inter alia, nonionic surfactants. In particular, pharmaceutically customary surfactants are preferred, such as, for example: partial and fatty acid esters and ethers of polyhydric alcohols such as of glycerol, sorbitol and the like (SPAN®, TWEEN®, in particular TWEEN® 20 and TWEEN® 80, MYRJ®, BRIJ®), CREMOPHOR® or poloxamers. The surfactants are present in the pharmaceutical composition in a concentration of 5-200 μg/ml, preferably of 5-120 μg/ml and particularly preferably of 20-75 μg/ml.

The preparation can additionally optionally contain preservatives (e.g. phenol, cresol, parabens), isotonicizing agents (e.g. mannitol, sorbitol, lactose, dextrose, trehalose, sodium chloride, glycerol), buffer substances, salts, acids and alkalis and also further excipients. These substances can in each case be present individually or alternatively as mixtures.

Glycerol, dextrose, lactose, sorbitol and mannitol are customarily present in the pharmaceutical preparation in a concentration of 100-250 mM, NaCl in a concentration of up to 150 mM. Buffer substances, such as, for example, phosphate, acetate, citrate, arginine, glycylglycine or TRIS (i.e. 2-amino-2-hydroxymethyl-1,3-propanediol) buffer and corresponding salts, are present in a concentration of 5-250 mM, preferably 10-100 mM. Further excipients can be, inter alia, salts or arginine.

The invention therefore relates to a pharmaceutical formulation comprising a polypeptide selected from the group consisting of insulin, an insulin analog, an insulin derivative, an active insulin metabolite and combinations thereof; a surfactant or combinations of two or more surfactants; optionally a preservative or combinations of two or more preservatives; and optionally an isotonicizing agent, buffer substances and/or further excipients or combinations thereof, the pharmaceutical formulation being a clear solution which has a pH in the acidic range (pH 1-6.8), preferably pH 3.5-6.8, very particularly preferably 3.5-4.5.

Preferred pharmaceutical formulations of the present invention are those wherein the surfactant is selected from the group consisting of partial and fatty acid esters and ethers of polyhydric alcohols such as of glycerol and sorbitol, and polyols; the partial and fatty acid esters and ethers of glycerol and sorbitol being selected from the group consisting of SPAN®, TWEEN®, MYRJ®, BRIJ®, CREMOPHOR®; the polyols being selected from the group consisting of polypropylene glycols, polyethylene glycols, poloxamers, PLURONICS®, and TETRONICS®; the preservative being selected from the group consisting of phenol, cresol, and parabens; the isotonicizing agent being selected from the group consisting of mannitol, sorbitol, sodium chloride, and glycerol; the excipients being selected from the group consisting of buffer substances, acids, and alkalis; the insulin analog being selected from the group consisting of Gly(A21)-Arg(B31)-Arg(B32)-human insulin; Lys(B3)-Glu(B29)-human insulin; $Lys^{B28}Pro^{B29}$ human insulin, B28 Asp-human insulin, human insulin in which proline in position B28 has been substituted by Asp, Lys, Leu, Val or Ala and where in position B29 Lys can be substituted by Pro; AlaB26-human insulin; des(B28-B30)-human insulin; des(B27)-human insulin and des(B30)-human insulin; the insulin derivative being selected from the group consisting of B29-N-myristoyl-des(B30) human insulin, B29-N-palmitoyl-des(B30) human insulin, B29-N-myristoyl human insulin, B29-N-palmitoyl human insulin, B28-N-myristoyl $Lys^{B28}Pro^{B29}$ human insulin, B28-N-palmitoyl-$Lys^{B28}Pro^{B29}$ human insulin, B30-N-myristoyl-$Thr^{B29}Lys^{B30}$ human insulin, B30-N-palmitoyl-$Thr^{B29}Lys^{B30}$ human insulin, B29-N-(N-palmitoyl-γ-glutamyl)-des(B30) human insulin, B29-N-(N-lithocholyl-γ-glutamyl)-des(B30) human insulin, B29-N-(ω-carboxyheptadecanoyl)-des(B30) human insulin and B29-N-(ω-carboxyheptadecanoyl) human insulin.

A further subject of the invention is a pharmaceutical formulation such as described above, in which the insulin, the insulin analog, the active insulin metabolite and/or the insulin derivative is present in a concentration of 60-6000 nmol/ml, preferably in a concentration of 240-3000 nmol/ml (this corresponds approximately to a concentration of 1.4-35 mg/ml or 40-500 units/ml);

in which the surfactant is present in a concentration of 5-200 μg/ml, preferably of 5-120 μg/ml and particularly preferably of 20-75 μg/ml.

A further subject of the invention is a pharmaceutical formulation such as mentioned above, in which glycerol and/or mannitol is present in a concentration of 100-250 mM, and/or NaCl is preferably present in a concentration of up to 150 mM.

A further subject of the invention is a pharmaceutical formulation such as mentioned above, in which a buffer substance is present in a concentration of 5-250 mM.

A further subject of the invention is a pharmaceutical insulin formulation which contains further additives such as, for example, salts which delay the release of insulin. Mixtures of such delayed-release insulins with formulations described above are included therein.

A further subject of the invention is a method for the production of such pharmaceutical formulations. Likewise, a further subject of the invention is the use of such formulations for the treatment of diabetes mellitus.

A further subject of the invention is the use or the addition of surfactants as stabilizer during the process for the production of insulin, insulin analogs or insulin derivatives or their preparations.

EXAMPLES

The following examples illustrate, but by no means limit, the present invention.

Comparison investigations: Different preparations containing the insulin analog insulin glargine (Gly(A21), Arg(B31), Arg(B32)-human insulin) are prepared. To this end, insulin glargine is suspended in one part of water for injection, dissolved at pH 3-4, the other constituents are added, the pH is adjusted to 4.0+/−0.2 using hydrochloric acid/NaOH and the mixture is made up to the final volume. The concentration of insulin glargine in each of the experiments described below is 3.6378 mg/ml (corresponds to 100 units/ml). A second preparation is produced identically, but a specific amount of a surfactant is additionally added. The solutions are filled into 10 ml glass vessels (vials) and fitted with crimp caps. These vessels are now exposed to simulated in use or physicomechanical stress conditions:

1. In use test: The vessels are sorted into boxes with turned-up lids and stored during the investigation period of 28 days at +25° C. and controlled room humidity with exclusion of light. To simulate taking by the patient, once daily about 5 IU of the solutions are withdrawn using a customary insulin syringe and discarded. At the beginning and end of the working week this procedure is carried out twice in order to simulate taking at the weekend. Before each withdrawal, visual assessment of the solution in the vessels for turbidity and/or particle formation is carried out.
2. Shaking test: The vessels are placed in a box with a turned-up lid lying on a laboratory shaker having an incubator and thermostat and shaken at 25° C. with 90 movements/min parallel to the horizontal movement for a period of time of 10 days. After defined times, the turbidity value of the samples is determined by means of a laboratory turbidity photometer (nephelometer) in formaldazine nephelometric units (formaldazine nephelometric unit=FNU). The turbidity value corresponds to the intensity of the scattered radiation of the light incident on suspended particles in the sample.

Example 1

Stabilization of the in Use Period of Insulin Glargine Using Polysorbate 20 (Tween® 20)

a) The solution is sterile-filtered through a combination of 0.2 μm und 0.1 μm filters. It is then poured into 10 ml injection vials and sealed using crimp caps having an inserted sealing disk.

b) A comparison solution is prepared identically, but first a suitable amount of surfactant (10-30 ppm of polysorbate 20) is suspended in water for injection. The samples are stored at +5° C., 25° C. and 37° C. for a fixed period of time.

10 samples in each case are then subjected to an in use test. The results are shown in the table below.

Storage for 3 months at 5° C.

	Number of vials with particle formation after			
Test sample	7 days	14 days	21 days	28 days
Insulin glargine	7	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	1
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0

Storage for 6 months at 5° C.

	Number of vials with particle formation after			
Test sample	7 days	14 days	21 days	28 days
Insulin glargine	1	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	1
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	1
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	1	0

Storage for 3 months at 25° C.

	Number of vials with particle formation after			
Test sample	7 days	14 days	21 days	28 days
Insulin glargine	9	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	2	2	2	2

-continued

Test sample	Number of vials with particle formation after 7 days	14 days	21 days	28 days
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	1
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0

Storage for 6 months at 25° C.

Test sample	Number of vials with particle formation after 7 days	14 days	21 days	28 days
Insulin glargine	10	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	1
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	1	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0

Storage for 1 month at 37° C.

Test sample	Number of vials with particle formation after 7 days	14 days	21 days	28 days
Insulin glargine	0	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	3	3	5
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0

Storage for 3 months at 37° C.

Test sample	Number of vials with particle formation after 7 days	14 days	21 days	28 days
Insulin glargine	5	9	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	1	1	1	1

-continued

Test sample	Number of vials with particle formation after 7 days	14 days	21 days	28 days
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0

Storage for 6 months at 37° C.

Test sample	Number of vials with particle formation after 7 days	14 days	21 days	28 days
Insulin glargine	10	10	10	10
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	0
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	1	0
Insulin glargine + 0.020 mg/mlof polysorbate 20	0	0	0	0
Insulin glargine + 0.0030 mg/ml of polysorbate 20	1	1	1	1

Without addition of polysorbate 20, particle formation can occur in the solution even after 7 days in use. By addition of polysorbate 20, the particle formation can be markedly suppressed during the in use period.

The stabilizing action of polysorbate 20 is retained even on storage at elevated temperatures for a period of 3 months.

A decline in the stabilizing action due to possible hydrolysis of the polysorbate in the acidic medium of the solution cannot be determined in comparison with the data after storage for 1 month.

Example 2

Stabilization of Insulin Glargine Using Polysorbate 20 Under Physico-mechanical Stress Loading

a) The solution is sterile-filtered through a combination of 0.2 μm und 0.1 μm filters. It is then poured into 10 ml injection vials and sealed using crimp caps having an inserted sealing disk.

b) A comparison solution is prepared identically, but first a suitable amount of surfactant (0.010-0.030 mg/ml of polysorbate 20) is suspended in water for injection.

The samples are stored at +5° C., 25° C. und 37° C. for a fixed period of time. 5 samples in each case are then subjected to a shaking test. The results are shown in the table below, the limit 15 FNU corresponds to turbidities which are discernible in daylight.

Storage for 1 month at 5° C.

	Number of vials > 15 FNU								
Test sample	0 days	0.5 days	1 day	2 days	3 days	4 days	6 days	8 days	10 days
Insulin glargine	0	0	0	2	3	3	4	4	4
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	0	0	1	3	4	5
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0

Storage for 1 month at 25° C.

	Number of vials > 15 FNU								
Test sample	0 days	0.5 days	1 day	2 days	3 days	4 days	6 days	8 days	10 days
Insulin glargine	0	0	0	1	1	1	1	2	3
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	0	0	0	1	2	3
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0

Storage for 1 month at 37° C.

	Number of vials > 15 FNU								
Test sample	0 days	0.5 days	1 day	2 days	3 days	4 days	6 days	8 days	10 days
Insulin glargine	0	0	0	2	5	5	5	5	5
Insulin glargine + 0.010 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.015 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.020 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0
Insulin glargine + 0.030 mg/ml of polysorbate 20	0	0	0	0	0	0	0	0	0

Without addition of polysorbate 20, even after 2 days of severe physicomechanical stress, a visible turbidity can occur in the solution. By addition of polysorbate 20, the formation of turbidity during physicomechanical stressing can be markedly delayed. The stabilizing action of polysorbate 20 is retained even on storage at elevated temperatures.

A decline in the stabilizing action due to possible hydrolysis of the polysorbate in the acidic medium of the solution cannot be detected.

Example 3

Comparison of the Stabilization of the in Use Period of Insulin Glargine using Polysorbate 20 (Tween® 20) Und Using Polysorbate 80 (Tween® 20)

Open 10 vials in each case to give 5 ml of insulin glargine injection solution and

a) addition of 0.001 mg/ml of polysorbate 20
b) addition of 0.01 mg/ml of polysorbate 20
c) addition of 0.001 mg/ml of polysorbate 80
d) addition of 0.01 mg/ml of polysorbate 80

in the form of a concentrated stock solution.

The samples are then subjected to an in use test.

The results are shown in the table below.

	Vials with particle formation after			
Test sample	7 days	14 days	21 days	28 days
Insulin glargine + 0.001 mg/ml of polysorbate 20	no	yes	Yes, particles increasingly occur	Yes, particles increasingly occur
Insulin glargine + 0.010 mg/ml of polysorbate 20	no	no	no	no
Insulin glargine + 0.001 mg/ml of polysorbate 80	no	yes	Yes, particles increasingly occur	Yes, particles increasingly occur
Insulin glargine + 0.010 mg/ml of polysorbate 80	no	no	no	no

An addition of polysorbate 20 or of polysorbate 80 in a concentration of 0.001 mg/ml are equally able to stabilize the solution against particle formation during the in use period.

What is claimed is:

1. A pharmaceutical formulation comprising Gly(A21), Arg(B31), Arg(B32)-human insulin;

at least one chemical entity chosen from esters and ethers of polyhydric alcohols;

at least one preservative; and

water,

wherein the pharmaceutical formulation has a pH in the acidic range from 1 to 6.8.

2. The pharmaceutical formulation as claimed in claim **1**, wherein the at least one preservative is chosen from phenols.

3. The pharmaceutical formulation as claimed in claim **1**, wherein the at least one preservative is cresol.

4. The pharmaceutical formulation as claimed in claim **3**, further including zinc.

5. The pharmaceutical formulation as claimed in claim **1**, further including at least one isotonicizing agent.

6. The pharmaceutical formulation as claimed in claim **4**, wherein the pharmaceutical formulation has a pH in the acidic range from 3.5 to 6.8.

7. The pharmaceutical formulation as claimed in claimed **6**, wherein the pharmaceutical formulation has a pH in the acidic range from 3.5 to 4.5.

8. The pharmaceutical formulation as claimed in claim **1**, wherein the at least one preservative is chosen from phenol, cresol, chlorocresol, benzyl alcohol, and parabens.

9. The pharmaceutical formulation as claimed in claim **5**, wherein the at least one isotonicizing agent is chosen from mannitol, sorbitol, lactose, dextrose, trehalose, sodium chloride, and glycerol.

10. The pharmaceutical formulation as claimed in claim **1**, further comprising a buffer.

11. The pharmaceutical formulation as claimed in claim **10**, wherein the buffer is chosen from TRIS, phosphate, citrate, acetate, and glycylglycine.

12. The pharmaceutical formulation as claimed in claim **1**, wherein the Gly(A21), Arg(B31), Arg(B32)-human insulin is present in a concentration of 60-6000 nmol/ml.

13. The pharmaceutical formulation as claimed in claim **12**, wherein the Gly(A21), Arg(B31), Arg(B32)-human insulin is present in a concentration of 240-3000 nmol/ml.

14. The pharmaceutical formulation as claimed in claim **1**, wherein the at least one chemical entity is present in a concentration of 5-200 μg/ml.

15. The pharmaceutical formulation as claimed in claim **14**, wherein the at least one chemical entity is present in a concentration of 5-120 μg/ml.

16. The pharmaceutical formulation as claimed in claim **15**, wherein the at least one chemical entity is present in a concentration of 20-75 μg/ml.

17. The pharmaceutical formulation as claimed in claim **9**, wherein at least one isotonicizing agent is chosen from glycerol and mannitol and wherein said at least one isotonicizing agent is present in a concentration of 100-250 mM.

18. The pharmaceutical formulation as claimed in claim **1**, further comprising one or more excipients chosen from acids, alkalis and salts.

19. The pharmaceutical formulation as claimed in claim **18**, wherein the excipient is NaCl which is present in a concentration of up to 150 mM.

20. The pharmaceutical formulation as claimed in claim **10**, wherein said buffer is present in a concentration of 5-250 mM.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,713,930 B2
APPLICATION NO. : 12/328208
DATED : May 11, 2010
INVENTOR(S) : Anette Brunner-Schwarz et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On Title Page 2, Item (56) under "Other Publications", line 1, delete "Glargin" and insert -- Glargine --, therefor.

In column 8, line 31, delete "mg/mlof" and insert -- mg/ml of --, therefor.

In column 8, line 34, delete "0.0030" and insert -- 0.030 --, therefor.

In column 11, line 35, delete "Und" and insert -- And --, therefor.

Signed and Sealed this
Thirty-first Day of May, 2011

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office