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(56) Related Art
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Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

(54) Title: ACIDIC INSULIN PREPARATIONS WITH IMPROVED STABILITY

(54) Bezeichnung: SAURE INSULINZUBEREITUNGEN MIT VERBESSERTER STABILITÄT

(57) Abstract: The invention relates to a pharmaceutical formulation comprising a polypeptide selected from a group containing insulin, an insulin metabolite, an insulin analogue, an insulin derivative or combinations thereof and also comprising a surfactant or combinations of several surfactants, optionally a preservative or combinations of several preservatives and optionally an isotonic agent, buffers or additional auxiliary agents or combinations thereof. According to the invention, said pharmaceutical formulation has an acidic pH value.

(57) Zusammenfassung: Die Erfindung betrifft eine pharmazeutische Formulierung enthaltend ein Polypeptid ausgewählt aus einer Gruppe enthaltend Insulin, einen Insulinmetaboliten, ein Insulinanalogon, ein Insulinderivat oder Kombinationen davon; ein Tensid oder Kombinationen mehrerer Tenside; optional ein Konservierungsmittel oder Kombinationen mehrerer Konservierungsmittel; und optional ein Isotonisierungsmittel, Puffer oder weitere Hilfsstoffe oder Kombinationen davon, wobei die pharmazeutische Formulierung einen pH-Wert im sauren Bereich aufweist.

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Description

Acidic insulin preparations with improved stability

The invention relates to a pharmaceutical formulation comprising a polypeptide selected from a group comprising 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 physicomechanical 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.

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 the 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 in the night. The replacement of the endogenous insulin secretion by exogenous, mostly

subcutaneous administration of insulin as a rule does not approximately achieve the quality of the physiological regulation of the blood glucose described above. Often, deviations of the 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. It is to be derived from this that an improved therapy of diabetes is primarily to 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 naturally occurring insulins are used here, 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. The chemical modification can consist, for example, in 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.

As a rule, insulin derivatives and insulin analogs have a somewhat modified action compared with human insulin.

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.

International patent application PCT/EP02/02625 (unpublished) describes zinc-free and low-zinc insulin preparations having stability improved by the addition of surfactants at room and body temperature and mechanical stress, but does not describe the stabilization of acidic insulin preparations against hydrophobic aggregation nuclei.

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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.

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

Accordingly, in a first aspect of the invention there is provided a pharmaceutical formulation comprising:

(a) Gly(A21), Arg(B31), Arg(B32)-human insulin;

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

(c) at least one preservative; and

(d) water,

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

In a second aspect of the invention there is provided a pharmaceutical formulation comprising:

(a) Gly(A21), Arg(B31), Arg(B32)-human insulin;

(b) at least one chemical entity chosen from polysorbate and poloxamers;

(c) at least one preservative; and

(d) water.

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

The pharmaceutical preparations 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:

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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 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 a group comprising insulin, an insulin analog, an insulin derivative, an active insulin metabolite 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, 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.

Such a pharmaceutical formulation is preferred where the surfactant is selected from a group comprising partial and fatty acid esters and ethers of polyhydric alcohols such as of glycerol and sorbitol, polyols; the partial and fatty acid esters and ethers of glycerol and sorbitol being selected from a group comprising Span®, Tween®, Myrj®, Brij®, Cremophor®; the polyols being selected from the group polypropylene glycols, polyethylene glycols, poloxamers, Pluronics, Tetronics; the preservative being selected from a group comprising phenol, cresol, parabens; the isotonicizing agent being selected from a group comprising mannitol, sorbitol, sodium chloride, glycerol; the excipients being selected from a group comprising buffer substances, acids, alkalis; the insulin analog being selected from a group comprising 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 or des(B30)-human insulin;
the insulin derivative being selected from a group comprising 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 und B29-N-(ω-carboxyheptadecanoyl) human insulin.

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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.

The application is described below with the aid of some examples, which are in no way intended to act restrictively.

Comprises/comprising and grammatical variations thereof when used in this specification are to be taken to specify the presence of stated features, integers, steps or components or groups thereof, but do not preclude the presence or addition of one or more other features, integers, steps, components or groups thereof.

Examples:

The following examples are intended to illustrate the concept of the invention in greater detail, without acting restrictively.

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 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 und 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
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

	Number of vials with particle formation after			
Test sample	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

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	Number of vials with particle formation after			
Test sample	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

	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	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 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 0.01 mg/ml are equally able to stabilize the solution against particle formation during the in use period.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A pharmaceutical formulation comprising:

a) Gly(A21), Arg(B31), Arg(B32)-human insulin;

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

c) at least one preservative; and

d) 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 phenol, cresol, chlorocresol, benzyl, alcohol and parabens.

3. The pharmaceutical formulation of claim 2, wherein the at least one preservative is cresol.

4. The pharmaceutical formulation as claimed in any one of claims 1 to 3, further including at least one isotonicizing agent.

5. The pharmaceutical formulation as claimed in any one of claims 1 to 4, wherein the polysorbate 20 is present in an effective amount to avoid turbidity.

6. The pharmaceutical formulation as claimed in any one of claims 1 to 5, wherein the pharmaceutical formulation has a pH in the acidic range from 3.5 to 6.8.

7. The pharmaceutical formulation of claim 6, wherein the pharmaceutical formulation has a pH in the acidic range from 3.5 to 4.5.

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8. The pharmaceutical formulation of any one of claim 4 to 7, wherein the at least one isotonicizing agent is chosen from mannitol, sorbitol, lactose, dextrose, trehalose, sodium chloride, and glycerol.

9. The pharmaceutical formulation as claimed in any one of claims 1 to 8, further comprising a buffer.

10. The pharmaceutical formulation of claim 9, wherein the buffer is selected from the group consisting of TRIS, phosphate, citrate, acetate, and glycylglycine.

11. The pharmaceutical formulation as claimed in any one of claims 1 to 10, wherein the Gly(A21), Arg(B31), Arg(B32)-human insulin is present in a concentration of 60 - 6000 nmol/ml.

12. The pharmaceutical formulation of claim 11, wherein the Gly(A21), Arg(B31), Arg(B32)-human insulin is present in a concentration of 240 – 3000 nmol/ml.

13. The pharmaceutical formulation as claimed in any one of claims 1 to 12, wherein the at least one chemical entity is present in a concentration of 5 – 200 µg/ml.

14. The pharmaceutical formulation of claim 13, wherein the at least one chemical entity is present in a concentration of 5 – 120 µg/ml.

15. The pharmaceutical formulation of claim 14, wherein the at least one chemical entity is present in a concentration of 20 – 75 µg/ml.

16. The pharmaceutical formulation as claimed in any one of claims 4 to 15, wherein the 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.

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17. The pharmaceutical formulation as claimed in any one of claims 8 to 16, wherein sodium chloride is present in a concentration of up to 150 mM.

18. The pharmaceutical formulation as claimed in any one of claims 9 to 17, wherein said buffer is present in a concentration of 5 – 250 mM.

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

20. The pharmaceutical formulation as claimed in any one of claims 1 to 19, further comprising one or more excipients chosen from acids, alkalis and salts.

21. A pharmaceutical formulation comprising Gly(A21), Arg (B31), Arg(B32)-human insulin and the at least one chemical entity is chosen from polysorbates substantially as hereinbefore described with reference to the Examples.

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