Description



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Zinc-free and low-zinc insulin preparations having improved stability

The invention relates to stabilized pharmaceutical formulations comprising a polypeptide selected from a group comprising insulin (e.g. human insulin, bovine insulin or porcine insulin), an insulin analog, an insulin derivative, active insulin metabolites or combinations thereof; a surfactant or combinations of a number of surfactants and optionally a preservative or combinations of a number of preservatives and optionally an isotonicizing agent, buffer or further excipients or combinations thereof, the pharmaceutical formulation being low in zinc or free from zinc. These formulations can be employed for the treatment of diabetes and are particularly employable for use in insulin pumps, pens, injectors, inhalers or for preparations in which an increased physical stability 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 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



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endogenous insulin secretion by exogenous, mostly subcutaneous administration of insulin as a rule does not approximatey 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 acid residues and/or addition/removal of at least one amino acid residue from the corresponding, otherwise identical, naturally occurring insulin. The added and/or replaced amino acid residues can 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 have various amino acids, preferably proline, in position B29, but not glutamic acid.
EP 0 375 437 includes insulin analogs with lysine or arginine in B28, which can optionally additionally be 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 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. For some time, not only the naturally occurring insulins mentioned, but also preparations of insulin derivatives or insulin analogs have been on the market which show modified kinetics. Recombinant DNA technology today makes possible the preparation of such modified insulins. These include "monomeric insulin analogs" such as insulin Lispro, insulin Aspart and HMR

1964 (Lys(B3), Glu(B29) human insulin) having a rapid onset of action, and also insulin Glargin having a prolonged duration of action.

In addition to the duration of action, the stability of the preparation is very important for the patients. Stabilized insulin formulations having increased physical long-term stability are needed in particular for preparations which are exposed to particular mechanical stresses or relatively high temperatures. These include, for example, insulins in administration systems such as pens, inhalation systems, needleless injection systems or insulin pumps. Insulin pumps are either worn on or implanted in the body of the patient. In both cases, the preparation is exposed to the heat of the body and movement and to the delivery motion of the pump and thus to a very high thermomechanical stress. Since insulin pens too (disposable and reutilizable pens) are usually worn on the body, the same applies here. Previous preparations have only a limited stability under these conditions.

Insulin is present in neutral solution in pharmaceutical concentration in the form of stabilized zinc-containing hexamers, which are composed of 3 identical dimer units (Brange et al., Diabetes Care 13:923-954 (1990)). By modification of the amino acid sequence, the association of insulin can be decreased. Thus, the insulin analog Lispro, for example, mainly exists as a monomer and is thereby absorbed more rapidly and shows a shorter duration of action (HPT Ammon and C. Werning; Antidiabetika [Antidiabetics]; 2. Ed.; Wiss. Verl.-Ges. Stuttgart; 2000; p. 94.f). Especially the rapid-acting insulin analogs, which exist in the monomeric or dimeric form, show, however, a decreased stability and increased proneness to aggregation under thermal and mechanical stress. This makes itself noticeable in cloudiness and precipitates of insoluble aggregates. (Bakaysa et al, US patent no. 5474978). These higher molecular weight transformation products (dimers, trimers, polymers) and aggregates decrease not only the dose of insulin administered but can also induce irritation or immune reactions in the patient. Moreover, such insoluble aggregates can affect and block the cannulas and tubing of the pumps. Since zinc leads to an additional stabilization of the insulin, zinc-free or low-zinc preparations of insulin and insulin analogs are particularly susceptible to instability. In particular, monomeric insulin analogs having a rapid onset of action are prone very rapidly to aggregation and physical instability, because the formation of insoluble aggregates proceeds via monomers of insulin. In order to guarantee the quality of an insulin preparation, it is necessary to avoid the formation of aggregates.

There are various approaches for stabilizing insulin formulations. Thus, in international patent application WO98/56406 formulations stabilized by TRIS or arginine buffer have been described. US patent 5866538 describes an insulin preparation which contains glycerol and sodium chloride in concentrations of 5 – 100 mM and should have an increased stability. US patent 5948751 describes insulin preparations having increased physical stability, which is achieved by addition of mannitol or similar sugars. The addition of excess zinc to a zinc-containing insulin solution can likewise increase the stability (J. Brange et al., Diabetic Medicine, 3: 532-536, 1986). The influence of the pH and various excipients on the stability of insulin preparations has also been described in detail (J. Brange & L. Langkjaer, Acta Pharm. Nordica 4: 149-158).

Often, these stabilization methods are not adequate for increased demands (improvement in ability to be kept at room or body temperature and under mechanical stress) or for "monomeric" insulin analogs or rapid-acting insulins, which are particularly susceptible to physical stress. Moreover, all commercial insulin preparations contain zinc, which is added to stabilize the preparation. Thus, Bakaysa et al. in US patent 5474978 describes stabilized formulations of insulin complexes which consist of 6 insulin analog monomers, 2 zinc atoms and at least 3 molecules of a phenolic preservative. These formulations can additionally contain a physiologically acceptable buffer and a preservative. If it is wished, however, to prepare zinc-free or low-zinc insulin preparations, the stabilization methods mentioned are not adequate for a marketable preparation. For example, it was not possible to develop a zinc-free preparation of insulin Lispro on account of inadequate physical stability (Bakaysa et al., Protein Science (1996), 5:2521-2531). Low-zinc or zinc-free insulin formulations having adequate stability, in particular physical stability, are not described in the prior art.

The present invention was thus based on the object of finding zinc-free preparations for insulins and their derivatives and analogs, which are distinguished by a high stability.

It has now surprisingly been found that the addition of surfactants (emulsifiers) such as, for example, poloxamers or polysorbates (Tween®) can drastically increase the stability of insulin preparations and thus even zinc-free preparations can be prepared which have a superior stability, in order to be capable of being used in infusion

pumps or other administration systems as well. These preparations show increased stability, particularly under stress conditions. This applies both to insulin, insulin analogs, insulin derivatives or mixtures thereof.

In neutral preparations, insulin forms complexes with zinc ions. Here, at an adequate zinc concentration, stable hexamers are formed from 6 insulin molecules and 2 zinc ions. For the formation of this structure, a zinc concentration of at least 0.4 % (w/w) relative to the insulin is necessary. This corresponds in the case of a preparation of 100 IU/ml of insulin to a concentration of about 13 μg/ml of zinc. An excess of zinc (e.g. 4 zinc ions per hexamer) again markedly stabilizes the preparation against physical stress (J. Brange et al., Neutral insulin solutions physically stabilized by the addition of Zn^{2+}. Diabetic Med. 3, 532-536 (1986)). In contrast to this, in preparations having lower zinc concentrations (< 0.4 percent by weight based on insulin), the formation of the hexamers is reduced. This leads to a dramatically reduced stability of the preparation (J. Brange and L. Langkjaer; Acta Pharm Nord, 4: 149-158 (1992)). "Zinc-free " or "low-zinc " within the meaning of this application therefore means the presence of less than 0.4 percent by weight of zinc based on the insulin content of the preparation, preferably less than 0.2 percent by weight based on the insulin content. For a customary insulin preparation containing 100 units per milliliter (0.6 μmol/ml), this means, for example, a concentration of less than 13 μg/ml of Zn^{++} ions (0.2 μmol/ml), preferably less than 6.5 μg/ml of Zn^{++} ions, in the pharmaceutical preparation, based on an insulin concentration of 100 units/ml. The freedom from zinc can also be achieved by addition of zinc-complexing substances, such as, for example, citrate or EDTA, so that sufficient zinc ions are not available for the formation of the insulin/zinc hexamer complex.

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. Surfactants which can be used are, inter alia, nonionic or ionic (anionic, cationic or amphoteric) surfactants. In particular, pharmaceutically customary surfactants are preferred, such as, for example:
alkali metal soaps, amine soaps and alkaline earth metal soaps (stearates, palmitates, oleates, ricinoleates), alkylsulfates and alkylsulfonates (sodium laurylsulfate, sodium cetylsulfate, sodium stearylsulfate), natural surfactants (bile

acid salts, saponins, gum arabic), cationic surfactants (alkonium bromides, cetylpyridinium chloride, cetrimide), fatty alcohols (cetyl alcohol, stearyl alcohol, cholesterol), partial and fatty acid esters of polyhydric alcohols such as of glycerol, sorbitol and the like (Span®, Tween®, Myrj®, Brij®), Cremophor® or poloxamers. The surfactants are present in the pharmaceutical composition in a concentration of 0.1 µg/ml – 10000 µg/ml, preferably 1 µg/ml – 1000 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 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, inter alia, be salts, arginine, protamine, or Surfen®.

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 a number of surfactants; optionally a preservative or combinations of a number of preservatives; and optionally an isotonicizing agent, buffer substances and/or further excipients or combinations thereof, the pharmaceutical formulation being free from or low in zinc; such a pharmaceutical preparation is preferred where the surfactant is selected from a group comprising alkali metal soaps, amine soaps, alkaline earth metal soaps, alkylsulfates, alkylsulfonates, natural surfactants, cationic surfactants, fatty alcohols, partial and fatty acid esters of polyhydric alcohols such as of glycerol and sorbitol, polyols; where the soaps mentioned are selected from a group comprising stearates, palmitates, oleates, ricinoleates; where the alkylsulfates are selected from a group comprising sodium laurylsulfate, sodium cetylsulfate, sodium stearylsulfate; where the natural surfactants are selected from a group

comprising bile acid salts, saponins, gum arabic, lecithins; where the cationic surfactants are selected from a group comprising alkonium bromides, cetylpyridinium chloride, Cetrimid®; where the fatty alcohols are selected from a group comprising cetyl alcohol, stearyl alcohol, cholesterol; where the partial and fatty acid esters and ethers of glycerol and sorbitol are selected from a group comprising Span®, Tween®, Myrj®, Brij®, Cremophor®; where the polyols are selected from the group comprising polypropylene glycols, polyethylene glycols, poloxamers, Pluronics, Tetronics; where the preservative is selected from a group comprising phenol, cresol, parabens; where the isotonicizing agent is selected from a group comprising mannitol, sorbitol, sodium chloride, glycerol; where the excipients are selected from a group comprising buffer substances, acids, alkalis; where the insulin is an insulin occurring in nature, for example human, bovine or porcine insulin; where the insulin analog is 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; where the insulin derivative is 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 and B29-N-(ω-carboxyheptadecanoyl) human insulin.

The invention further relates to a pharmaceutical formulation 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 0.1 – 10000 μg/ml, preferably in a concentration of 1 – 1000 μg/ml.

The invention further relates to a pharmaceutical formulation as mentioned above, in which glycerol and/or mannitol is present in a concentration of 100 – 250 mM, and/or chloride is preferably present in a concentration of up to 150 mM.

The invention further relates to a pharmaceutical formulation as mentioned above, in which a buffer substance is present in a concentration of 5 – 250 mM.
The invention further relates to a pharmaceutical insulin formulation which contains further additives such as, for example, salts, protamine or Surfen® which delay the release of insulin. Mixtures of such delayed-release insulins with formulations as described above are also included therein.
The invention further relates to a method for the preparation of such pharmaceutical formulations. The invention further likewise relates to the administration of such formulations for the treatment of diabetes mellitus.
The invention further relates to the use or the addition of surfactants as stabilizer during the process for the preparation of insulin, insulin analogs or insulin derivatives or their preparations.

In the pharmaceutical formulations described comprising a polypeptide selected from a group comprising insulin, an insulin analog, an insulin derivative, an active insulin metabolite or combinations thereof, the pH is between 2 and 12, preferably between 6 and 8.5 and particularly preferably between 7 and 7.8.

The application is described below with the aid of some examples, which should in no case act in a restrictive manner.

Examples:

Comparison investigations: Various zinc-free preparations containing the insulin analog HMR1964 (Lys(B3), Glu(B29), human insulin) are prepared. To this end, zinc-free HMR1964 and the other constituents are dissolved in one part of water for injection purposes and the pH is adjusted to 7.3 +/- 0.2 with hydrochloric acid/NaOH and made up to the final volume. The concentration of HMR 1964 in each of the experiments described below is 3.5 mg/ml (corresponds to 100 units/ml). A second

preparation is prepared identically, but a specific amount of a surfactant is additionally added. The solutions are dispensed into 5 ml or 10 ml glass vessels (vials) and fitted with crimp caps. These vessels are then exposed to stress conditions:

1. Rotation test: In each case 5 vessels of a batch and 5 vessels of the comparison batch are subjected to a rotation test. To this end, the vessels are mounted in a rotator and rotated top over bottom (360°) at 37°C at 60 rpm. After defined times, the turbidity of the preparations situated in the vessels is compared with turbidity standards or determined in formazine nephelometric units (FNU) using a laboratory turbidity photometer (nephelometer). The experiment is carried out until a turbidity value of 18 FNU is exceeded in all vessels.

2. Shaking test: The vessels are placed on a laboratory shaker in an incubator and shaken at 30°C at 100 movements/min. After defined times, the turbidity value of the samples is determined by means of a laboratory turbidity photometer (nephelometer) in formazine nephelometric units (FNU).

Example 1: Stabilization of HMR1964 by addition of zinc in the rotation test

a) Zinc-free HMR1964 (calculated such that a concentration of 3.5 mg/ml results in the finished formulation) is dissolved in an aqueous solution, which in the final formulation contains 2.7 mg/ml of m-cresol, 20 mg/ml of glycerol and 6 mg/ml of trometamol (tris), and the pH is adjusted to 7.2 – 7.4 (measured at room temperature) using 1 N hydrochloric acid/1 N NaOH. The solution is made up to the final volume with water and sterile-filtered through a 0.2 μm filter. It is then filled into 5 ml injection vials and sealed using caps.
b) A comparison solution is prepared identically, but before making up with water a corresponding amount of a 0.1 % strength zinc chloride stock solution is added, so that a zinc content of 15 μg/ml results in the finished formulation.

In each case, 5 samples are then stressed in the rotation test and the turbidity is determined after various periods of time. The results are shown in the following table.

	Number of test samples with turbidity > 18 FNU after:					
Description	0 h	8 h	16 h	32 h	40 h	56 h
HMR1964 Without addition	0	5	-	-	-	-
HMR1964 +15 µg/ml of Zn	0	0	0	0	4	5

The addition of zinc can markedly delay the resulting turbidity of the solution in terms of time and thereby stabilizes the HMR1964 formulation. Without addition of zinc, the preparation has a marked turbidity in the rotation test even after 8 hours.

Example 2: Stabilization of HMR1964 by addition of polysorbate 20 (Tween® 20) in the rotation test

a) Zinc-free HMR1964 (calculated such that a concentration of 3.5 mg/ml results in the finished formulation) is dissolved to an aqueous solution which contains 3.15 mg/ml of m-cresol, 5 mg/ml of NaCl and 6 mg/ml of trometamol in the final formulation and the pH is adjusted to 7.2 – 7.4 (measured at room temperature) using 1 N hydrochloric acid/1 N NaOH. The solution is made up to the final volume with water and sterile-filtered through a 0.2 µm filter. It is then filled into 5 ml injection vials and sealed using caps.

b) A comparison solution is prepared identically, but before making up with water a corresponding amount of a 0.1 % strength polysorbate 20 (Tween® 20) stock solution is added, so that a concentration of 10 µg/ml results in the finished formulation.

In each case 5 samples are then stressed in the rotation test and the turbidity is determined after various periods of time. The results are shown in the following table.

	Number of test samples with turbidity > 18 FNU after:					
Description	0 h	8 h	16 h	24 h	32 h	40 h
HMR1964 Without addition	0	5	-	-	-	-
HMR1964 + 10 µg/ml of Tween® 20	0	0	0	0	5	-

The addition of polysorbate 20 delays the occurrence of turbidity very markedly.

Example 3: Stabilization of HMR1964 by addition of poloxamer in the rotation test

a) Zinc-free HMR1964 (calculated such that a concentration of 3.5 mg/ml results in the finished formulation) is dissolved to an aqueous solution which contains 4.5 mg/ml of phenol, 5 mg/ml of NaCl and 6 mg/ml of trometamol in the final formulation and the pH is adjusted to 7.2 – 7.4 (measured at room temperature) using 1 N hydrochloric acid/1 N NaOH. The solution is made up to the final volume with water and sterile-filtered through a 0.2 μm filter. It is then filled into 5 ml injection vials and sealed using caps.

b) A comparison solution is prepared identically, but before making up with water a corresponding amount of a 0.1 % strength poloxamer 171 (e.g. Genapol®) stock solution is added, such that a concentration of 10 μg/ml results in the finished formulation.

In each case 5 samples are then stressed in the rotation test and the turbidity is determined after various periods of time. The results are shown in the following table.

	Number of test samples with turbidity > 18 FNU after:					
Description	0 h	8 h	16 h	24 h	32 h	40 h
HMR1964 Without addition	0	5	-	-	-	-
HMR1964 + 0.01 mg/ml of poloxamer 171	0	0	0	2	5	-

The addition of poloxamer 171 also delays the occurrence of turbidity markedly and stabilizes the preparation.

Example 4: Stabilization of HMR1964 by addition of polysorbate 20 or polysorbate 80 in the shaking test

a) Zinc-free HMR1964 (calculated such that a concentration of 3.5 mg/ml results in the finished formulation) is dissolved to an aqueous solution which contains 3.15 mg/ml of m-cresol, 5 mg/ml of NaCl and 6 mg/ml of trometamol in the final formulation and the pH is adjusted to 7.2 – 7.4 (measured at room temperature) using 1 N hydrochloric acid/1 N NaOH. The solution is made up to the final volume with water

and sterile-filtered through a 0.2 µm filter. It is then filled into 5 ml injection vials and sealed using caps.

b) A comparison solution is prepared identically, but before making up with water a corresponding amount of a 0.1 % strength polysorbate 20 (Tween® 20) stock solution is added, such that a concentration of 10 µg/ml results in the finished formulation.

c) A further comparison solution is prepared identically as in b), but this time polysorbate 80 (Tween® 80) is used instead of polysorbate 20.

The samples are shaken at 30°C on a laboratory shaker (60 rpm) and the turbidity of the samples is measured after specific times. The results are shown in the following table.

	Shaking test, turbidity (FNU) after				
Addition	**Start**	**1 week**	**2 weeks**	**3 weeks**	**4 weeks**
Without addition	0.55	2.04	4.86	6.12	10.51
0.01 mg/ml of Tween 20	1.75	2.60	2.44	2.44	3.80
0.01 mg/ml of Tween 80	2.38	2.98	2.86	3.01	4.14

Both the addition of polysorbate 20 and of polysorbate 80 have a stabilizing effect on HMR1964 in the shaking test.

Example 5: Stabilization of HMR1964 by addition of zinc or poloxamer (Genapol®) in the shaking test

a) Zinc-free HMR1964 (calculated such that a concentration of 3.5 mg/ml results in the finished formulation) is dissolved to an aqueous solution which contains 3.3 mg/ml of phenol, 5 mg/ml of NaCl and 6 mg/ml of trometamol in the final formulation and the pH is adjusted to 7.2 – 7.4 (measured at room temperature) using 1 N hydrochloric acid/1 N NaOH. The solution is made up to the final volume with water

and sterile-filtered through a 0.2 µm filter. It is then filled into 5 ml injection vials and sealed using caps.

b) A comparison solution is prepared identically, but before making up with water a corresponding amount of a 0.1 % strength poloxamer 171 (Genapol®) stock solution is added, such that a concentration of 10 µg/ml results in the finished formulation.

c) A further comparison solution is prepared as described in a), but instead of poloxamer, a corresponding amount of a 0.1% strength zinc chloride stock solution is added to the solution before making up with water, so that a concentration of 15 µg/ml of zinc results in the finished formulation.

	Shaking test, turbidity (FNU) after				
Addition	**Start**	**1 week**	**2 weeks**	**3 weeks**	**4 weeks**
None	0.39	0.70	4.46	8.74	14.11
0.01 mg/ml of poloxamer	0.36	0.57	0.52	1.59	0.89
0.015 mg/ml of Zn	1.02	0.68	0.70	0.56	0.86

Both an addition of zinc and the addition of poloxamer prevent the occurrence of turbidity in the shaking test.

Example 6: Stabilization of HMR1964 by addition of poloxamer in the rotation test

a) Zinc-free HMR1964 (calculated such that a concentration of 3.5 mg/ml results in the finished formulation) is dissolved to an aqueous solution which contains 3.3 mg/ml of phenol, 5 mg/ml of NaCl and 6 mg/ml of trometamol in the final formulation and the pH is adjusted to 7.2 – 7.4 (measured at room temperature) using 1 N hydrochloric acid/1 N NaOH. The solution is made up to the final volume with water and sterile-filtered through a 0.2 µm filter. It is then filled into 5 ml injection vials and sealed using caps.

b) A comparison solution is prepared identically, but before making up with water a corresponding amount of a 0.1 % strength poloxamer 171 (Genapol®) stock solution is added, such that a concentration of 100 µg/ml results in the finished formulation.

In each case 5 samples are then stressed in the rotation test and the turbidity is determined after various periods of time. The results are shown in the following table.

	Number of test samples with turbidity > 18 FNU after:					
Description	0 h	8 h	16 h	24 h	32 h	40 h
HMR1964 Without addition	0	5	-	-	-	-
HMR1964 + 0.10 mg/ml of poloxamer 171	0	0	0	0	1	5

The addition of 100 μg/ml of poloxamer likewise stabilizes the HMR1964 preparation very markedly.

Patent claims:

1. 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 a number of surfactants;
optionally a preservative or combinations of a number of preservatives; and
optionally an isotonicizing agent, buffer or further excipients or combinations thereof, the pharmaceutical formulation being free from or low in zinc.

2. The pharmaceutical formulation as claimed in claim 1, the surfactant being selected from a group comprising alkali metal soaps, amine soaps, alkaline earth metal soaps, alkylsulfates, alkylsulfonates, natural surfactants, cationic surfactants, fatty alcohols, fatty acids, partial and fatty acid esters and ethers of polyhydric alcohols, of glycerol, sorbitol and of sucrose, polyols.

3. The pharmaceutical formulation as claimed in claim 2, the soaps mentioned being selected from a group comprising stearates, palmitates, oleates, ricinoleates.

4. The pharmaceutical formulation as claimed in one of claims 2 and 3, the sulfates being selected from a group comprising sodium laurylsulfate, sodium cetylsulfate, sodium stearylsulfate.

5. The pharmaceutical formulation as claimed in one of claims 2 to 4, the natural surfactants being selected from a group comprising bile acid salts, saponins, gum arabic, lecithins.

6. The pharmaceutical formulation as claimed in one of claims 2 to 5, the cationic surfactants being selected from a group comprising alkonium halides, cetylpyridinium chloride, Cetrimid®.

7. The pharmaceutical formulation as claimed in one of claims 2 to 6, the fatty alcohols being selected from a group comprising cetyl alcohol, stearyl alcohol, cholesterol.

8. The pharmaceutical formulation as claimed in one of claims 2 to 7, the partial and fatty acid esters and ethers of the polyhydric alcohols, of glycerol and sorbitol being selected from a group comprising Span®, Tween® (polysorbate), Myrj®, Brij®, Triton®, Cremophor®.

9. The pharmaceutical formulation as claimed in one of claims 2 to 8, the polyols being selected from a group comprising polypropylene glycols, polyethylene glycols, poloxamers, Pluronics, Tetronics.

10. The pharmaceutical formulation as claimed in one of claims 1 to 9, the preservative being selected from a group comprising phenol, cresol, chlorocresol, benzyl alcohol, parabens.

11. The pharmaceutical formulation as claimed in one of claims 1 to 10, the isotonicizing agent being selected from a group comprising mannitol, sorbitol, lactose, dextrose, trehalose, sodium chloride, glycerol.

12. The pharmaceutical formulation as claimed in one of claims 1 to 11, the excipients being selected from a group comprising buffer substances such as, for example, TRIS, phosphate, citrate, acetate, glycylglycine or further substances such as acids, alkalis, salts, protamine, arginine, Surfen®.

13. The pharmaceutical formulation as claimed in one of claims 1 to 12, the insulin analog being selected from a group comprising Gly(A21), Arg(B31), Arg(B32), human insulin, Lys(B3), Glu(B29), human insulin, Asp(B28) human insulin, Lys(B28) Pro(B29) human insulin, des(B30) human insulin.

14. The pharmaceutical formulation as claimed in one of claims 1 to 13, 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(B39) 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.

15. The pharmaceutical formulation as claimed in one of the preceding claims, in which the insulin, the insulin analog, the insulin derivative and/or the insulin metabolite is present in a concentration of 60 – 6000 nmol/ml.

16. The pharmaceutical formulation as claimed in claim 15, the insulin, the insulin analog, the insulin derivative and/or the insulin metabolite being present in a concentration of 240 – 3000 nmol/ml.

17. The pharmaceutical formulation as claimed in one of the preceding claims, in which the surfactant is present in a concentration of 0.1 – 10000 μg/ml.

18. The pharmaceutical formulation as claimed in claim 17, in which the surfactant is present in a concentration of 1 – 1000 μg/ml.

19. The pharmaceutical formulation as claimed in one or more of claims 10 – 18, in which glycerol and/or mannitol is present in a concentration of 100 – 250 mM.

20. The pharmaceutical formulation as claimed in claim 19, in which chloride is present in a concentration of up to 150 mM.

21. The pharmaceutical formulation as claimed in one of the preceding claims, in which a buffer substance is present in a concentration of 5 – 250 mM.

22. A process for the production of a pharmaceutical formulation as claimed in one or more of claims 1 to 21, where the components are mixed together in

the form of aqueous solutions, then the desired pH is adjusted and the mixture is made up to the final volume with water.

23. The process for the production of the pharmaceutical formulation as claimed in claim 22, the final concentration of 3.15 mg/ml of cresol, 3.5 mg/ml of HMR 1964, 6.0 mg/ml of trometamol, 5.0 mg/ml of NaCl, and 0.1 mg/ml of Tween® 20 being achieved.

24. A pharmaceutical formulation obtainable as claimed in one of claims 22 or 23.