1 548 984

PATENT SPECIFICATION (11) 1 548 984

(21) Application No. 21405/76 (22) Filed 24 May 1976
(31) Convention Application No. 6719/75
(32) Filed 26 May 1975 in
(33) Switzerland (CH)
(44) Complete Specification published 18 July 1979
(51) INT CL² A61K 37/02 9/10
(52) Index at acceptance
A5B 180 315 316 317 31Y 38Y 394 39X 822 835 H M



(54) CALCITONIN PEPTIDE PREPARATIONS WITH PROLONGED ACTION

(71) We, CIBA-GEIGY AG, a body corporate organised according to the laws of Switzerland, of Basle, Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

Hitherto, calcitonins have been administered pharmacologically in the form of solutions, optionally in the presence of gelatin to prolong their activity. It has now been found that it is more advantageous to administer calcitonins in the form of suspensions of the sparingly soluble free base in a liquid. The base can be suspended in aqueous medium or in oil. The suspensions can be administered intramuscularly or subcutaneously.

The present invention therefore provides calcitonin preparations with prolonged action which contain a peptide of the calcitonin type, or calcitonin peptide with hypocalcaemic activity and an isoelectric point in the range of pH 5 to 9, in the form of a suspension of the sparingly soluble free base in a pharmaceutically suitable aqueous or oily medium, and a process for their manufacture by methods known per se.

The suspensions of the present invention have a substantially prolonged action. It has been established that, in the test on rats, I mg/kg of human calsitonin (Calcitonin M) in the form of the free base has a hypocalcaemic action of app. 40 hours duration, whereas Calcitonin M in salt form, for example as acetate, dissolved in water, has an action of 8 hours. In the presence of gelatin (16% weight/volume), the activity of 1 mg/kg of Calcitonin M (as hydrochloride) is app. 12 hours.

The preparations are obtained in a manner known per se, for example by suspending the calcitonin base, if appropriate already in concentrated aqueous suspension, in the isoelectric range in aqueous isotonic solutions. The oil suspensions are obtained, for example, by suspending the anhydrous base in the desired oily medium.

The term "isoelectric range" is used, because in the compounds of this invention the isoelectric point is not sharply defined, but extends over a wide pH range, cf. Kortüm, Lehrbuch der Elektrochemie, Verlag Chemie, Weinheim, 1966, p. 336. By isoelectric range is meant the pH range of pI ± app. 1 pH unit, where pI is the isoelectric point calculated theoretically.

The terms "peptides of the calcitonin type" or "calcitonin peptides" as used in this specification and in the claims means polypeptides with 32 amino amids as present in human calcitonin or in derivatives thereof wherein the asparagine and/or glutamine residues are replaced by the aspartic acid and glutamic acid residues respectively, or in hybrids of human calcitonin in which at least one and at most 16 of the amino-acids in the positions 2, 8, 10, 11, 12, 13, 14, 16, 18, 21, 23, 24, 25, 26, 27, 29, 30 and 31 are replaced by the amino-acids present in the corresponding positions of porcine thyrocalcitonin, or in which at least one of the amino acids in positions 11, 12, 16, 17, 19, 20, 22 and 24 are replaced by the amino acids as present in the corresponding positions of salmon calcitonin, and in which hybrids the amino acid in position 8, methionine, can be replaced by an α-lower alkyl-α-aminoacetic acid, i.e. one wherein lower alkyl has 2—4 carbon atoms, or human calcitonin-salmon calcitonin hybrids in which one or more of the amino acids in positions 8, 12, 16, 19, 22, 24, 26 and 27 of human calcitonin are substituted by the amino acids as present in the corresponding positions of salmon calcitonin, the amino acid in position 8 being optionally also, beyond methionine or valine, another α-lower alkyl-α-aminoacetic acid than valine, and in which at least in one of the positions 26 and 27 there is one of the amino acids as present in salmon calcitonin, and des-amino[1] derivatives and N^a-acyl derivatives of all these compounds.

Calcitonin peptides of the type just defined are disclosed for instance in UK patents 1 287 601, 1 287 125, 1 333 723, 1 459 303.

Acyl groups for the acylation of the N^{α}-amino group are the radicals of carboxylic acids, such as aliphatic, aromatic, araliphatic, heterocyclic and heterocyclyl - aliphatic carboxylic acids, in particular of lower univalent or bivalent alkane or alkene acids, primarily lower alkane acids containing 1 to 4 carbon atoms, such as formic acid, acetic acid, propionic acid, butyric acid, also acrylic acid, succinic acid; of alicyclic carboxylic acids, such as cycloalkylcarboxylic acids, for example unsubstituted and substituted benzoic acid or phthalic acid; of unsubstituted and aryl-substituted aryl-lower alkyl- or alkenylcarboxylic acids, for example phenylacetic acid; of unsubstituted or substituted univalent or bivalent 5- to 6-membered heterocyclic acids containing nitrogen, sulphur and/or oxygen as heteroatoms, such as pyridinecarboxylic acids, thiophenecarboxylic acids; or of heterocyclyl-lower alkane acids, such as pyridylacetic acid, imidazolylacetic acid, wherein the substituents of the rings are, for example, halogen atoms, nitro groups, lower alkyl or lower alkoxy groups or lower carbalkoxy groups. Further examples of acyl radicals are above all acyl radicals of amino acids, in particular of α-amino acids, for example glycyl-L-leucyl, L-pyroglutamyl, and acyl radicals which are derived from carbonic acid or thiocarbonic acid or the esters or amides thereof, for example lower alkyloxycarbonyl groups, such as ethoxycarbonyl, tert. butyloxy-carbonyl, and benzyloxycarbonyl, carbamoyl and thiocarbamoyl, which are unsubstituted or substituted as stated above, and N-substituted carbamoyl and thiocarbamoyl, for example N-lower alkylcarbamoyl, - N-phenyl-carbamoyl, N-phenyl-thiocarbamoyl.

Human calcitonin has an isoelectric range of pH 7 to 8. The change in the isoelectric range when replacing some amino acids by others can be determined experimentally, for example by electrophoresis, or can be calculated. The isoelectric point of the peptides can be calculated, for example, according to J. Greenstein and M. Winitz, "Chemistry of the Amino Acids", vol. 1, ed. John Wiley & Sons, Inc., New York, London, 1961, p. 482, ff.

The calcitonin type peptide base can be prepared advantageously by the process of Patent No. 1,490,079, i.e. the free base can be precipitated, for example, from a salt of the peptide with an acid or a base by adding a base or an acid (the free base is also defined in that Patent as the "inner salt" of the peptide). The base can also be precipitated with ion exchangers or with buffers or salt mixtures, such as phosphates, citrates or glycine, instead of with bases or acids. For example, the calcitonin base can be precipitated from a solution of a salt of the calcitonin with an acid, for example with acetic acid, by adding sodium hydroxide solution or ammonia until the pH which corresponds to the isoelectric range is attained. The pH increases during the precipitation of the peptide by app. one pH unit and then remains constant.

To obtain the preparations of the present invention it is possible to use the suspensions of the peptide base prepared by these methods direct, by diluting them, for example, with a further suspending agent, for example an aqueous suspending agent, such as one of the cited buffers or salt mixtures, and bringing them into the desired form, for example ampoules. It is, however, also possible to separate the precipitated base from the suspending agent and resuspend it separately in the desired suspending agent. A preferred method of obtaining the preparations in the form of ampoules or vials consists in freezing the concentrated suspension, which contains the peptide base which has been precipitated, for example, from a corresponding salt solution, with or without further dilution by adding a further suspending agent, in an appropriate lyophilisation apparatus and lyophilising it, and resuspending the dry lyophilisate in the desired suspending agent and then filling ampoules and vials with the resultant suspension. The lyophilisation is most preferably carried out direct in the ampoules or vials, as is also the reconstitution of the desired suspension. The suspension required for obtaining the preparations can also be prepared by mixing a salt of the calcitonin base whose isoelectric point is in the range of pH 5 to 9 with a buffer of the cited pH, or adding an acid or a base until this pH is attained, and using the resultant suspension direct. Finally, the preparations can be obtained by separating the pure base, which has been precipitated by the methods referred to, from the aqueous phase by known methods, for example those described in Patent No. 1,490,079 referred to hereinabove, and subsequently suspending them in the desired liquid.

The particle size and the degree of agglomeration of the suspensions obtainable by the described methods depend on the chosen concentrations of the starting solutions, the temperature, the precipitation conditions of the peptide bases, for example stirring speed, and on the nature of the precipitant. By observing certain conditions, for example those described in the illustrative Examples, it is possible to obtain suspensions which can easily be made homogeneous by shaking with the advantageous particle size of 1 to 100 μ, in particular of 10 to 50 μ.

The aqueous suspensions can contain substances which increase the viscosity, such as sodium carboxymethyl cellulose, carboxymethyl cellulose, dextran, polyvinyl pyrrolidone, and gelatin. If desired, the suspensions can be stabilised by the customary wetting

agents for injection preparations or suitable preservatives can be added to them.

Suspensions in oil contain as oily components the customary vegetable, synthetic or semi-synthetic oils for injection purposes. As such particular mention may be made of liquid fatty acid esters which contain as acid component a long-chain fatty acid with 8 to 22, in particular 12 to 22 carbon atoms, for example lauric acid, tridecyl acid, myristic acid, pentadecyl acid, palmitic acid, margaric acid, stearic acid, arachinic acid, behenic acid, or corresponding unsaturated acids, for example oleic acid, elaidic acid, erucic acid, brasidic acid or linolic acid. The alcohol component contains a maximum of 6 carbon atoms and is a monohydric or polyhydric alcohol, for example a monohydric, dihydric or trihydric alcohol, for example methanol, ethanol, propanol, butanol or pentanol or isomers thereof, principally, however, glycol or glycerol. As fatty acid esters there are therefore to be mentioned for example: ethyl oleate, isopropyl myristate, isopropyl palmitate, "Labrafil (Registered Trade Mark) M 2735" (polyoxyethylene glycerol trioleate product of Gattefossé, Paris), "Miglyol (Registered Trade Mark) 812" (triglyceride of saturated fatty acids having a chain length of 8 to 12 carbon atoms, product of Chemische Werke Witten/Ruhr, Germany), and especially vegetable oils, such as cotton seed oil, almond oil, olive oil, castor oil, sesame oil, soya bean oil, above all ground nut oil.

The suspensions contain 0.1 to 5 mg/ml of the calcitonin base. The amount normally administered by injection is 1 to 2 ml once to seven times per week and contains preferably 0.2 mg to 2 mg of calcitonin base, in particular Calcitonin M base.

The injection preparations are obtained in conventional manner under antimicrobial conditions, likewise the filling and sealing of the ampoules and vials.

The preparation of the present invention may be used for treating pathological conditions in humans for whom the administration of a calcitonin, in particular of Calcitonin M, is indicated, for example hypercalcaemic conditions, bone diseases which are characterised by an increased rate of metabolism, for example Paget's disease, or osteroporosis. The method consists in administering the calcitonin intramuscularly or subcutaneously in the form of one of the preparations with prolonged action of the present invention. Suitable doses, for example in the treatment with Calcitonin M, are those referred to hereinabove. Preferably the preparations described hereinabove or in the illustrative Examples are used.

The following Examples will serve to illustrate the invention. Percentages are weight by volume when referred to in connection with solutions or suspensions.

Example 1

Preparation of aqueous injection suspensions with calcitonin base

In an atmosphere of nitrogen, the calcitonin base is precipitated from a 5% solution of Calcitonin M acetate at room temperature to 65°C, preferably at 40°C, by adding 1 normal NaOH over a period of 15 to 30 minutes until a pH of 7.7 is attained. The batch is subsequently cooled to room temperature in the course of 1 to 3 minutes and the pH rises to app. 8.2.

Ao the suspension of the calcitonin base (10 mg per 0.2 ml) obtained in this way are added, with stirring, NaH_2PO_4, Na_2HPO_4 and NaCl and distilled water in such amounts as to yield a suspension of the composition:

Calcitonin M (base) (100%)	0.2 %
$NaH_2PO_4 . 2 H_2O$	0.4 %
$Na_2HPO_4 . 12 H_2O$	0.14%
NaCl	0.49%

The above injection suspension has a pH of 7, can be readily rendered homogeneous by shaking and has a particle size of 10 μ and loose agglomerates of 25 to 50 μ. It is filled into ampoules of the composition

Calcitonin M (base) (100%)	2.0 mg
$NaH_2PO_4 . 2 H_2O$	4.0 mg
$Na_2HPO_4 . 12 H_2O$	14.5 mg
NaCl	4.9 mg
distilled water	to 1.0 ml

Example 2

Calcitonin M is precipitated with NaOH from the acetate solution as in Example 1 and the suspension is mixed, with stirring, with glycine, NaCl, NaOH and distilled water in such amounts as to yield a suspension of the composition:

Calcitonin M (base) (100%)	0.1%
glycine	0.5%
NaCl	0.7%
NaOH to pH 7.3	

This suspension is filled into ampoules containing 1 mg of Calcitonin M and having the composition

Calcitonin M (base) (100%)	1.0 mg
glycine	5.0 mg
NaCl	7.0 mg
NaOH to pH 7.3	
distilled water	to 1.0 ml

Particle size and structure are as indicated in Example 1, and a homogeneous suspension can be readily reconstituted.

Example 3

Preparation of aqueous injection suspensions with Calcitonin M

The Calcitonin M base, which has been precipitated as concentrated suspension (for example, 100 mg/2 ml), from a solution of the acetate in accordance with the particulars of Example 1, is frozen and lyophilised in a suitable lyophilisation apparatus.

The dry lyophilisate is resuspended in suitable suspending agents, for example those of Examples 1 and 2, with stirring, and filled for example into ampoules of the composition indicated in Examples 1 and 2. These injection suspensions can be readily reconstituted in a homogeneous form by shaking and have a particle size of 10 to 50 μ.

Example 4

A dry lyophilisate of Calcitonin M base as prepared and described in Example 3 is resuspended in a 5% mannitol solution with stirring and filled into ampoules of the composition

Calcitonin M (base) (100%)		1.0 mg
mannitol, free of pyrogens		50.0 mg
distilled water	to	1.0 ml

This suspension has a pH of 8.2, can be readily reconstituted in a homogeneous form by shaking and has a particle size of 10 to 50 μ.

Example 5

Ampoules containing Calcitonin M base are prepared in a manner similar to that described in Example 4, except that they also contain L-methionine and have the composition

Calcitonin M (base) (100%)		1.0 mg
L-methionine		1.0 mg
mannitol, free of pyrogens		50.0 mg
NaOH to pH 7.5 to 8		
distilled water	to	1.0 ml

This suspension has the same particle size as in Example 4 and can also be readily reconstituted in homogeneous form.

Example 6

75 mg of Calcitonin M acetate (calculated on the base) are dissolved in 3 ml of water. To this solution is added 0.3 ml of 1% ammonia to a pH of 7.4. The solution is stirred until the precipitate has formed and then 12 ml of water or phosphate buffer are added. The suspension contains 5 mg/ml of Calcitonin M as base.

Example 7

Calcitonin M (base) (100%)		0.2 mg
NaCl		0.9 mg
distilled water	to	1.0 ml

Example 8

Preparation of dry vials with Calcitonin M base.

With stirring, 2 ml of a concentrated suspension of Calcitonin M which is prepared according to the particulars of Example 1 and contains 100 mg of pure base, is treated with 50 ml of an aqueous solution containing 5% of mannitol and 0.2% of methionine and the batch is diluted with 50 ml of distilled water. Vials of 2 ml capacity are filled with 1 ml of this solution, frozen in a suitable lyophilisation apparatus and lyophilised. An injection suspension can be reconstituted from the dry lyophilisate in the vials by adding an aqueous solvent, for example 1 ml of distilled water. Injection suspensions with a particle size of <10 μ and loose agglomerates of 25—50 μ are obtained. They have the composition:

Calcitonin M (base) (100%)	1.0 mg
L-methionine	1.0 mg
mannitol, free of pyrogens	25.0 mg

WHAT WE CLAIM IS:—

1. Calcitonin preparations with prolonged action which contain a peptide of the calcitonin type wtih hypocalcemic action and an isoelectric point in the range of pH 5 to 9, in the form of a suspension of the sparingly soluble free base in a pharmaceutically suitable aqueous or oily medium.

2. Calcitonin type peptides preparations according to claim 1, which contain human calcitonin (Calcitonin M) as calcitonin base.

3. Calcitonin type peptides preparations according to claim 1, which contain a derivative or an analog of Calcitonin M as calcitonin base.

4. Calcitonin type peptides preparations according to one or more claims 1 to 3, which contain the free base suspended in an aqueous isotonic solution with a pH within the isoelectric range.

5. Calcitonin type peptides preparations according to one or more of claims 1 to 4, which contain a buffer.

6. Calcitonin type peptides preparations according to one or more of preceding claims, which contain 0.1 to 5 mg/ml of the calcitonin base.

7. Calcitonin type peptides preparations according to one or more of the preceding claims, which contain 0.2 to 2 mg of base suspended in 1 to 2 ml.

8. Calcitonin type peptides preparations according to one or more of the preceding claims, which have a particle size between 1 μ and 100 μ.

9. Calcitonin type peptides preparations according to one or more of the preceding claims in the form of injection solutions in ampoules or vials for intramuscular or subcutaneous administration.

Example 3

Preparation of aqueous injection suspensions with Calcitonin M

The Calcitonin M base, which has been precipitated as concentrated suspension (for example, 100 mg/2 ml), from a solution of the acetate in accordance with the particulars of Example 1, is frozen and lyophilised in a suitable lyophilisation apparatus.

The dry lyophilisate is resuspended in suitable suspending agents, for example those of Examples 1 and 2, with stirring, and filled for example into ampoules of the composition indicated in Examples 1 and 2. These injection suspensions can be readily reconstituted in a homogeneous form by shaking and have a particle size of 10 to 50 μ.

Example 4

A dry lyophilisate of Calcitonin M base as prepared and described in Example 3 is resuspended in a 5% mannitol solution with stirring and filled into ampoules of the composition

Calcitonin M (base) (100%)		1.0 mg
mannitol, free of pyrogens		50.0 mg
distilled water	to	1.0 ml

This suspension has a pH of 8.2, can be readily reconstituted in a homogeneous form by shaking and has a particle size of 10 to 50 μ.

Example 5

Ampoules containing Calcitonin M base are prepared in a manner similar to that described in Example 4, except that they also contain L-methionine and have the composition

Calcitonin M (base) (100%)		1.0 mg
L-methionine		1.0 mg
mannitol, free of pyrogens		50.0 mg
NaOH to pH 7.5 to 8		
distilled water	to	1.0 ml

This suspension has the same particle size as in Example 4 and can also be readily reconstituted in homogeneous form.

Example 6

75 mg of Calcitonin M acetate (calculated on the base) are dissolved in 3 ml of water. To this solution is added 0.3 ml of 1% ammonia to a pH of 7.4. The solution is stirred until the precipitate has formed and then 12 ml of water or phosphate buffer are added. The suspension contains 5 mg/ml of Calcitonin M as base.

Example 7

Calcitonin M (base) (100%)		0.2 mg
NaCl		0.9 mg
distilled water	to	1.0 ml

Example 8

Preparation of dry vials with Calcitonin M base.

With stirring, 2 ml of a concentrated suspension of Calcitonin M which is prepared according to the particulars of Example 1 and contains 100 mg of pure base, is treated with 50 ml of an aqueous solution containing 5% of mannitol and 0.2% of methionine and the batch is diluted with 50 ml of distilled water. Vials of 2 ml capacity are filled with 1 ml of this solution, frozen in a suitable lyophilisation apparatus and lyophilised. An injection suspension can be reconstituted from the dry lyophilisate in the vials by adding an aqueous solvent, for example 1 ml of distilled water. Injection suspensions with a particle size of <10 μ and loose agglomerates of 25—50 μ are obtained. They have the composition:

Calcitonin M (base) (100%)	1.0 mg
L-methionine	1.0 mg
mannitol, free of pyrogens	25.0 mg

WHAT WE CLAIM IS:—

1. Calcitonin preparations with prolonged action which contain a peptide of the calcitonin type wtih hypocalcemic action and an isoelectric point in the range of pH 5 to 9, in the form of a suspension of the sparingly soluble free base in a pharmaceutically suitable aqueous or oily medium.

2. Calcitonin type peptides, preparations according to claim 1, which contain human calcitonin (Calcitonin M) as calcitonin base.

3. Calcitonin type peptides preparations according to claim 1, which contain a derivative or an analog of Calcitonin M as calcitonin base.

4. Calcitonin type peptides preparations according to one or more claims 1 to 3, which contain the free base suspended in an aqueous isotonic solution with a pH within the isoelectric range.

5. Calcitonin type peptides preparations according to one or more of claims 1 to 4, which contain a buffer.

6. Calcitonin type peptides preparations according to one or more of preceding claims, which contain 0.1 to 5 mg/ml of the calcitonin base.

7. Calcitonin type peptides preparations according to one or more of the preceding claims, which contain 0.2 to 2 mg of base suspended in 1 to 2 ml.

8. Calcitonin type peptides preparations according to one or more of the preceding claims, which have a particle size between 1 μ and 100 μ.

9. Calcitonin type peptides preparations according to one or more of the preceding claims in the form of injection solutions in ampoules or vials for intramuscular or subcutaneous administration.

10. Calcitonin type peptides preparations according to one or more of claims 4 to 7, which contain Calcitonin M.

11. Calcitonin type peptides preparations according to claim 8, which contain Calcitonin M.

12. Calcitonin type peptides preparations as claimed in one or more of the preceding claims, in the form of aqueous suspensions containing calcitonin M in the form of a precipitate as obtainable by adjustment of the pH of an aqueous solution of a calcitonin M salt to the isoelectric range.

13. Calcitonin type peptides preparations as claimed in claim 12 formed by dilution of the suspension obtained by adjustment of the pH of an aqueous solution of a calcitonin M salt to the isoelectric range with an aqueous medium.

14. Calcitonin type peptides preparations as claimed in claim 13 in which the aqueous medium used for dilution contains a pharmaceutically acceptable buffer or salt or salt mixture.

15. Calcitonin type peptides preparations as claimed in claim 12 formed by dilution of the separated precipitate as obtainable by adjustment of the pH of an aqueous solution of a calcitonin M salt to the isoelectric range.

16. Calcitonin type peptides preparations as claimed in one or more of claims 12—15 the suspended particles of Calcitonins M having a size in between 10 and 50 μ.

17. Calcitonin type peptides preparations according to claim 1 as described in the Examples 1, 2 and 7.

18. Calcitonin type peptides preparations according to claim 1 as described in the Examples 3—6 and 8.

19. A process for the manufacture of calcitonin type peptides preparations for intramuscular or subcutaneous administration, wherein suspensions which contain, as sparingly soluble free base, a calcitonin with hypocalcemic action and an isoelectric point in the range of pH 5 to 9, are prepared in known manner.

20. A process according to claim 19, wherein a sparingly soluble peptides base which is precipitated from a salt of calcitonin with an acid or a base by adjusting the pH of the solution to the isoelectric range, by addition of a base or an acid respectively or by treatment with an appropriate ion exchanger or with buffers or salt mixtures, is used as starting material.

21. A process according to claim 20, wherein the base obtained in suspension is used for the manufacture of the preparations direct by itself or after it has been mixed with a further suspending agent.

22. A process according to claim 20, wherein the base obtained in suspension is separated and brought together separately with the desired suspending agent.

23. A process according to claim 20, wherein the suspension obtained from the precipitation of the peptide base is subjected to lyophilisation and the dry lyophilisate is used for obtaining the desired preparations.

24. A process for the manufacture of calcitonin type peptides preparations according to claim 14 described in the Examples.

ABEL & IMRAY,
Chartered Patent Agents,
Northumberland House,
303—306, High Holborn,
London, WC1V 7LH.

Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1979
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.