PATENT SPECIFICATION (11) 1 587 076

1 587 076

(21) Application No. 29006/77 (22) Filed 11 July 1977
(31) Convention Application No. 5111/76
(32) Filed 12 July 1976
(31) Convention Application No. 6994/77
(32) Filed 7 June 1977 in
(33) Austria (AT) and Switzerland (CH)
(44) Complete Specification published 25 March 1981
(51) INT CL³ A61K 9/08
(52) Index at acceptance A5B 823 L

(19)



(54) INJECTION SOLUTIONS CONTAINING MICELLE-FORMING AGENTS

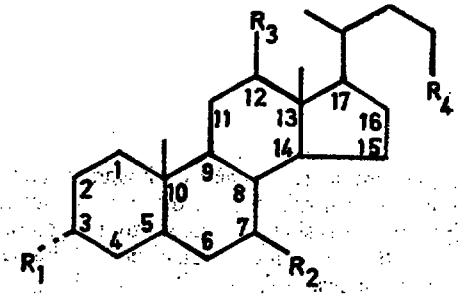
(71) We, F. HOFFMANN-LA ROCHE & CO., AKTIENGESELLSCHAFT, a Swiss Company of 124—184 Grenzacherstrasse, Basle, Switzerland, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with an injection solution, having an aqueous basis, comprising benzodiazepine. Previously, pharmaca have been brought into solution where necessary with the aid of synthetic solubilising agents such as, for example, propyleneglycol, polyethyleneglycols, "Cremophor" (Trade Mark) EL, the "Tweens" (Trade Mark) and the "Pluronics" (Trade Mark). The solubilisation by certain of these substances such as Cremophor EL, the Tweens and Pluronics is based on the formation of a colloidal system (micelles). However, these synthetic solubilising agents, including the micelle formers such as Cremophor EL, the Tweens and the Pluronics, in many cases show on parenteral administration side effects such as, for example, allergic reactions, anaphylactic shock, hemolysis and pyrogenic action.

Natural micelle formers such as, for example, cholanic acid derivatives are also known. These latter natural micelle formers are, however, ionic and accordingly strongly lytic. They produce hemolysis and have accordingly hitherto not been used in vehicles for parenteral administration.

It has now surprisingly been found in accordance with the present invention that the aforementioned disadvantages of the micelle formers, especially the hemolytic activity of the natural micelle formers, can be substantially reduced, or even eliminated, by the addition of lipids.

The present invention is based on the foregoing finding and is accordingly concerned with an injection solution comprising an aqueous basis, a cholanic acid derivative of the general formula



(I)

wherein

R_1, R_2 and R_3 represent hydrogen, hydroxy or an exocylic keto group and

R_4 represents a carboxy group or a carboxy group linked by an amide bond with the amino group of an amino acid,

a derivative thereof containing one or two double bonds in the steroid skeleton or a salt of such a compound, a lipid, a benzodiazepine and an additive for making the solution isotonic with blood and tissue fluid. If desired, the solution may also contain a pharmacon and/or pharmaceutical adjuvant soluble in water.

The present invention is also concerned with a process for the manufacture of

an injection solution aforesaid, which process comprises mixing together the individual components.

Especially preferred are those compounds of formula I in which R_1, R_2 and R_3 each represent a hydroxy group. These are the trihydroxy cholanic acids. Among these, the preferred compounds are those in which:

$R_1=R_2=R_3=$—OH, $R_4=$—COOH (cholic acid);

$R_1=R_2=R_3=$—OH, $R_4=$—CO—NH—CH_2—COOH (glycocholic acid); and

$R_1=R_2=R_3=$—OH, $R_4=$—CO—NH—CH_2—CH_2—SO_3H (taurocholic acid).

Furthermore, those compounds which have two hydroxy groups (i.e. the dihydroxycholanic acids) are preferred. Among these, the preferred compounds are those in which:

$R_1=R_3=$—OH, $R_2=$—H, $R_4=$—COOH (deoxycholic acid);

$R_1=R_3=$—OH, $R_2=$—H, $R_4=$—CO—NH—CH_2—COOH (glycodeoxycholic acid);

$R_1=R_3=$—OH, $R_2=$—H, $R_4=$—CO—NH—CH_2—CH_2—SO_3H (taurodeoxycholic acid);

$R_1=R_2=$—OH, $R_3=$—H, $R_4=$—COOH (chenodeoxycholic acid);

$R_1=R_2=$—OH, $R_3=$—H, $R_4=$—CO—NH—CH_2—COOH (glycochenodeoxycholic acid); and

$R_1=R_2=$—OH, $R_3=$—H, $R_4=$—CO—NH—CH_2—CH_2—SO_3H (taurochenodeoxycholic acid).

Suitable derivatives of the compounds of formula I which may be used contain in the steroid structure one or two double bonds (e.g. in positions 7—8, 11—12 or 9—11).

Suitable salts of these cholanic acid derivatives are, in particular, alkali metal salts and especially sodium salts.

Lipids which can be used include the following substances:

Phosphatidylcholines,
glycerine ether phosphatides,
phosphatidylethanolamine,
phosphatidylinositol,
phosphatidylserines,
sphingomyelin,
plasmalogens,
cardiolipin,
sulphatides and
monoglycerides.

Preferred lipids are phosphatides, especially phosphatidylcholines.

In an injection solution in accordance with the present invention it is necessary—as is the case in conventional injection solutions—to use an isotonising additive to make the solution isotonic with blood and tissue fluids. Suitable isotonising additives include sodium chloride and glucose solution, Tris buffer, phosphate buffer, citrate buffer, glycine buffer and citrate-phosphate mixed buffer. The osmotic pressure of an injection solution in accordance with the invention should correspond approximately to that of the blood that is to say, it should amount to about 300 mOsm.

Preferred benzodiazepines are diazepam, clonazepam, flunitrazepam, nitrazepam, medazepam and bromazepam.

The molar ratio of the lipid component to the micelle former (the acid derivative of formula I) preferably lies in the range of from 0.1:1 to 2:1. Ratios of 0.1:1 to 0.8:1 and 1.5:1 and 2:1 are preferred. A particularly preferred ratio is 0.8:1 to 1.5:1.

The amount of lipid component plus micelle former contained in an injection

solution provided by the invention can vary within wide limits (e.g. up to 50—300 mg/ml of injection solution).

The amount of the benzodiazepine in an injection solution of this invention can likewise vary within wide limits (e.g. up 0.1—20 mg/ml of injection solution).

An injection solution in accordance with the present invention can be manufactured, as mentioned earlier, by simple admixture of the individual components. It is, however, preferred to dissolve the lipid component, the micelle former and the benzodiazepine in an organic solvent for these components, to evaporate off the organic solvent and then to add the water, the isotonising additive and, if desired, any further ingredients. Normally, the isotonising additive and, in most cases, also the possible further ingredients are mixed with the water prior to its addition to the product of the aforementioned evaporation. Suitable organic solvents which may be used are e.g. the alkanols with 1 to 4 carbon atoms, especially ethanol.

An alternative preferred process for the manufacture of an injection solution provided by the present invention comprises intensively stirring about one molar part of micelle former, about one molar part of lipid component, from about 50 to 250 molar parts of water and the benzodiazepine, until the mixture is homogenous. It is preferred to carry out this step in the presence of up to 2% of the water of an organic solvent for the components such as ethanol. When the mixture is homogenous, further water, the isotonising additives and, if desired, the further ingredients are added until the desired dilution or concentration is achieved. In principle, it is also possible to carry out this process initially without including the benzodiazepine and then dissolve the benzodiazepine in the resulting micellar solution.

The time which is necessary to form the aforementioned homogenous mixture depends upon the nature of the micelle former, the lipid component and the benzodiazepine. Normally, this time can be shortened by warming for a short period.

Since, on the one hand, certain lipids react very readily with oxygen, it is of advantage or convenient to work under oxygen-free conditions and especially under a nitrogen atmosphere. An antioxidant such as, for example, sodium ascorbate, sodium hydrogen sulphite or sodium pyrosulphite is preferred included in the finished injection solution.

Besides the advantages mentioned earlier, the novel injection solutions provided by this invention have the further advantage that their miscibility with plasma as well as with conventional infusion solutions (e.g. blood- and tissue fluid-isotonic glucose or sodium chloride solutions) is extraordinarily good.

As a further advantage it has been shown that the benzodiazepine is very readily liberated for physiological absorption from an injection solution of this invention.

It should also be noted that an injection solution in accordance with the present invention has a very low toxicity.

The following Examples illustrate the present invention:

Example 1.

a) 3 g of egg lecithin [isolated according to Singleton *et al.*, J. Am. Oil. Chem. Soc. *42* (1965) 53], 2 g of sodium glycocholate and 150 mg of diazepam are dissolved up in a round flask in 150 ml of ethanol saturated with nitrogen.

b) The ethanol is evaporated off on a rotary evaporator under a vacuum at 35° C. A lipid film results in the round flask.

c) 25 ml of 1/15-*M* phosphate buffer solution (pH 7) saturated with nitrogen are added under a nitrogen atmosphere. The micelles form spontaneously at room temperature.

d) 300 mg of sodium ascorbate powder are added and dissolved.

e) The solution is filtered sterile under laminar flow conditions and filled into ampoules containing nitrogen. These are sealed. During the entire procedure contact with air is avoided in order to obtain an oxygen-free solution in the ampoule.

Example 2.

a) (2.9 g of egg lecithin, 2 g of sodium taurocholate and 150 mg of diazepam are dissolved up in a round flask in 150 ml of ethanol saturated with nitrogen.

Steps b to e) are carried out as in Example 1.

Example 3.

a) 40 mg of sodium glycocholate are dissolved in 1 mu of 1/15-*M* phosphate buffer.

b) 61.6 mg of egg lecithin are added thereto and stirring is carried out until the solution is clear (approximately 2 days).

c) 10 mg of diazepam are stirred in.
d) After 12 hours, the solution is centrifuged. In the supernatant the mixed micelles contain 2 mg/ml of diazepam.

Example 4.

The procedure described in Example 3 is followed, but in place of egg lecithin there are used 62.2 mg of phosphatidylinositol.

Example 5.

The procedure described in Example 3 is followed, but in place of egg lecithin there are used 65.4 mg of phosphatidylserine.

Example 6.

The procedure described in Example 3 is followed, but in place of egg lecithin there are used 60.6 mg of sphingomyelin.

Example 7.

The procedure described in Example 3 is followed, but in place of egg lecithin there are used 61.6 mg of sulphatide (bovine).

Example 8.

The procedure described in Example 3 is followed, but in place of egg lecithin there are used 57.2 mg of dimyristoyl lecithin.

Example 9.

The procedure described in Example 3 is followed, but in place of 61.6 mg of egg lecithin and 40 mg of sodium glycocholate there are used 11.8 mg of cardiolipin and 20 mg of sodium glycocholate.

Example 10.

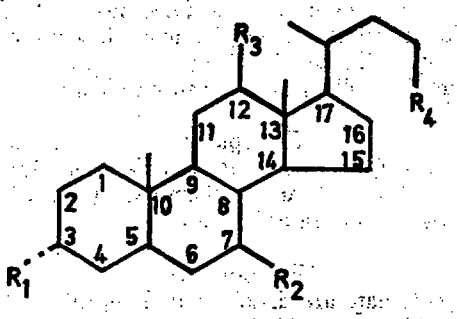
a) 75 mg of egg lecithin and 48.7 mg of sodium glycocholate are dissolved in 0.5 ml of methanol.
b) The methanol is evaporated off in a rotary evaporator at 35° C under a vacuum.
c) 1 ml of 1/15-*M* phosphate buffer is added.
d) 5 mg of diazepam are stirred in.
e) After stirring for 12 hours at room temperature, the solution is centrifuged. In the supernatant the mixed micelles contain 2.2 mg/ml of diazepam.

Example 11.

a) 5.58 g of glycocholic acid, 1.2 ml of 10-*M* sodium hydroxide solution and 24.4 ml of 1/15-*M* phosphate buffer (saturated with nitrogen; oxygen-free and potassium-free) are shaken until a solution is formed.
b) 9.6 g of soya lecithin, 200 mg of diazepam and 600 mg of sodium ascorbate are added and the mixture is shaken and stirred until a homogenous, honey-like phase is formed.
c) An additional 20 ml of oxygen-free phosphate buffer are added. There is formed a yellowish clear solution which is filtered sterile.

WHAT WE CLAIM IS:—

1. An injection solution comprising an aqueous basis, a cholanic acid derivative of the general formula



(I)

wherein
R_1, R_2 and R_3 represent hydrogen, hydroxy or an exocyclic keto group and

c) 10 mg of diazepam are stirred in.
d) After 12 hours, the solution is centrifuged. In the supernatant the mixed micelles contain 2 mg/ml of diazepam.

Example 4.

The procedure described in Example 3 is followed, but in place of egg lecithin there are used 62.2 mg of phosphatidylinositol.

Example 5.

The procedure described in Example 3 is followed, but in place of egg lecithin there are used 65.4 mg of phosphatidylserine.

Example 6.

The procedure described in Example 3 is followed, but in place of egg lecithin there are used 60.6 mg of sphingomyelin.

Example 7.

The procedure described in Example 3 is followed, but in place of egg lecithin there are used 61.6 mg of sulphatide (bovine).

Example 8.

The procedure described in Example 3 is followed, but in place of egg lecithin there are used 57.2 mg of dimyristoyl lecithin.

Example 9.

The procedure described in Example 3 is followed, but in place of 61.6 mg of egg lecithin and 40 mg of sodium glycocholate there are used 11.8 mg of cardiolipin and 20 mg of sodium glycocholate.

Example 10.

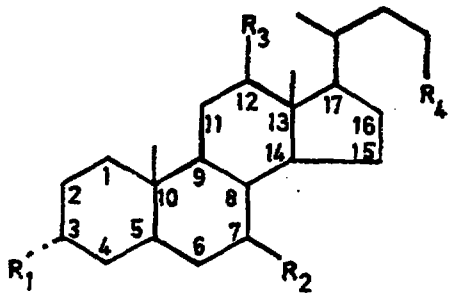
a) 75 mg of egg lecithin and 48.7 mg of sodium glycocholate are dissolved in 0.5 ml of methanol.
b) The methanol is evaporated off in a rotary evaporator at 35° C under a vacuum.
c) 1 ml of 1/15-*M* phosphate buffer is added.
d) 5 mg of diazepam are stirred in.
e) After stirring for 12 hours at room temperature, the solution is centrifuged. In the supernatant the mixed micelles contain 2.2 mg/ml of diazepam.

Example 11.

a) 5.58 g of glycocholic acid, 1.2 ml of 10-*M* sodium hydroxide solution and 24.4 ml of 1/15-*M* phosphate buffer (saturated with nitrogen; oxygen-free and potassium-free) are shaken until a solution is formed.
b) 9.6 g of soya lecithin, 200 mg of diazepam and 600 mg of sodium ascorbate are added and the mixture is shaken and stirred until a homogenous, honey-like phase is formed.
c) An additional 20 ml of oxygen-free phosphate buffer are added. There is formed a yellowish clear solution which is filtered sterile.

WHAT WE CLAIM IS:—

1. An injection solution comprising an aqueous basis, a cholanic acid derivative of the general formula



(I)

wherein

R_1, R_2 and R_3 represent hydrogen, hydroxy or an exocyclic keto group and

R_4 represents a carboxy group or a carboxy group linked by an amide bond with the amino group of an amino acid,

a derivative thereof containing one or two double bonds in the steroid skeleton or a salt of such a compound, a lipid, a benzodiazepine, and an additive for making the solution isotonic with blood and tissue fluid.

2. An injection solution according to claim 1 comprising additionally a pharmacon and/or pharmaceutical adjuvant soluble in water.

3. An injection solution according to claim 1 or claim 2 wherein the cholanic acid derivative is cholic acid, glycocholic acid, taurocholic acid, deoxycholic acid, flycodeoxycholic acid, taurodeoxycholic acid, chenodeoxycholic acid, glycochenodeoxycholic or taurochenodeoxycholic acid or a salt thereof.

4. An injection solution according to any one of claims 1 to 3 inclusive wherein a sodium salt of such cholanic acid derivative is present.

5. An injection solution according to any one of claims 1 to 4 inclusive, wherein the lipid is a phosphatidylcholine, a glycerine ether phosphatide, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyelin, plasmalogen, cardiolipin or a sulphatide or monoglyceride.

6. An injection solution according to any one of claims 1 to 5 inclusive, wherein the lipid is a phosphatide.

7. An injection solution according to claim 6, wherein the phosphatide is a phosphatidylcholine.

8. An injection solution according to any one of the claims 1 to 7 inclusive, wherein the additive is a glucose or sodium chloride solution, phosphate buffer, citrate buffer, glycine buffer, citrate-phosphate mixed buffer or Iris buffer.

9. An injection solution according to any one of claims 1 to 8 inclusive, wherein the benzodiazepine is diazepam, clonayepam, flunitrazepam, nitrazepam, medazepam or bromazepam.

10. An injection solution according to claim 9, wherein the benzodiazepine is diazepam.

11. An injection solution according to any one of claims 1 to 10 inclusive, wherein the molar ratio between the lipid component and the cholanic acid derivative is from 0.1:1 to 2:1.

12. An injection solution according to any one of claims 1 to 11 inclusive, wherein the amount of lipid component plus cholanic acid derivative is 50—300 mg/ml.

13. An injection solution according to any one of claims 1 to 11 inclusive, wherein the amount of the benzdiazepine is 0.1—20 mg/ml.

14. An injection solution according to any one of claims 1 to 13 inclusive, wherein an antioxidant is present.

15. An injection solution according to claim 14, wherein the antioxidant is sodium ascorbate, sodium hydrogen sulphite or sodium pyrosulphite.

16. A process for the manufacture of an injection solution having an aqueous basis, containing a cholanic acid derivative of formula I or a derivative thereof containing one or two double bonds in the steroid skeleton or a salt of such a compound, a lipid, a benzodiazepine, an additive for making the solution isotonic with blood and tissue fluid, which process comprises mixing togther the individual components.

17. A process according to claim 16 in which the components of the injection solution also comprise a pharmacon, and/or pharmaceutical adjuvant soluble in water.

18. A process according to claim 16 or 17, wherein the lipid component, the cholanic acid derivative and the benzodiazepine are dissolved in an organic solvent, the organic solvent is thereupon evaporated off and then water the additive for making the solution isotonic with blood and tissue fluid as well as, if desired, a pharmacon and/or pharmaceutical adjuvant soluble in water is added.

19. A process according to claim 18, wherein a C_1—C_4 alkanol is used as the solvent.

20. A process according to claim 19, wherein the C_1—C_4 alkanol is ethanol.

21. A process as claimed in claim 16 of claim 17 wherein about one molar part of a cholanic acid derivative, about one molar part of lipid, about 50 to 250 molar parts of water and the benzodiazepine are intensively stirred until the mixture is homogenous, whereupon further water, the additive for making the solution isotonic with blood and tissue fluid and, if desired, a pharmacon and/or pharmaceutical adjuvant soluble in water is added until the desired dilution or concentration is achieved.

22. A process according to claim 21, wherein up to 2% of an organic solvent

for the components present in the 50 to 250 molar parts of water is used.

23. A process according to claim 22, wherein the organic solvent is ethanol.

24. A modification of the process as claimed in any one of claims 21 to 23 inclusive, wherein the components other than the benzodiazepine are dissolved in the water and then said benzodiazepine is dissolved in the resulting micellar solution.

25. A process according to claim 16 or 17, wherein cholic acid, glycholic acid, taurocholic acid, deoxycholic acid, glycodeoxycholic acid, taurodeoxycholic acid, chenodeoxycholic acid, glycochenodeoxycholic acid or taurochenodeoxycholic acid, or a salt thereof, is used as the cholanic acid derivative.

26. A process according to any one of claims 16, 17 and 25 wherein the sodium salt of the cholanic acid derivative is used.

used is a phosphatidylcholine, glycerine ether phosphatide, phosphatidylethanolamine,

27. A process according to any one of claims 16 to 26 inclusive, wherein the lipid used is a phosphatidylcholine, glycerine ether phosphatide, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, sphingomyselin, plasmalogen, cardiolipin, a sulphatide or a monoglyceride.

28. A process according to any one of claims 16 to 27 inclusive, wherein the lipid used is a phosphatide.

29. A process according to claim 28, wherein the phosphatide used is phosphatidylcholine.

30. A process according to any one of claims 16 to 29 inclusive, wherein the additive for making the solution isotonic with blood and tissue fluid used is a glucose or sodium chloride solution, phosphate buffer, citrate buffer, glycine buffer, citrate-phospate mixed buffer or Iris-buffer.

31. A process according to any one of claims 16 to 30 inclusive, wherein the benzodiazepine used is diazepam, clonazepam, flunitrazepam, nitrazepam, medazepam or bromazepam.

32. A process according to claim 31, wherein the benzodiazepine used is diazepam.

33. A process according to any one of claims 16 to 32 inclusive, wherein the ratio between the lipid component and the cholanic acid derivative is from 0.1:1 to 2:1.

34. A process according to any one of claims 16 to 33 inclusive, wherein the amount of lipid component plus cholanic acid derivative used provides 50—300 mg/ml thereof in the final injection column.

35. A process according to any one of claims 16 to 34 inclusive, wherein the injection solution contains from 0.1 to 20 mg/ml of benzodiazepine.

36. A process according to any one of claims 16 to 35 inclusive, wherein an antioxidant is added as a pharmaceutical adjuvant.

37. A process according to claim 36, wherein the antioxidant is sodium ascorbate, sodium hydrogen sulphite or sodium pyrosulphite.

38. A process for the manufacture of an injection solution, substantially as hereinbefore described with reference to any one of the foregoing Examples.

39. An injection solution comprising an aqueous basis, a cholanic acid derivative of formula I or a derivative thereof containing one or two double bonds in the steroid skeleton or a salt of such a compound, a lipid, a benzodiazepine, an additive for making the solution isotonic with blood and tissue fluid as well as, if desired, a pharmacon and/or pharmaceutical adjuvant soluble in water, when manufactured by the process claimed in any one of claims 16 to 38 inclusive.

For the Applicants,
CARPMAELS & RANSFORD,
Chartered Patent Agents,
43 Bloomsbury Square,
London, WC1A 2RA.

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1981.
Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.

* C L M S *