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(54) **PROCEDE DE PREPARATION DE SOLUTIONS AQUEUSES CONTENANT UN MELANGE DE MICELLES**

(54) **PROCESS FOR THE PRODUCTION OF AQUEOUS MIXED MICELLE SOLUTIONS**

(57) A process for the production of aqueous mixed micelle solutions, containing mixed micelles formed from lipids and salts of bile acids, in which optionally slightly water-soluble or water-insoluble active ingredients are solubilized, is described, which is characterized in that mixtures are prepared from a) solutions which contain lipids, free bile acids and optionally slightly water-soluble or water-insoluble active ingredients in a water-soluble organic solvent and b) solutions which contain 0.05 to 3 equivalents of bases and optionally isotonized additives and/or water-soluble active ingredients in relation to the bile acids, the organic solvent is removed by ultrafiltration, freeze-drying, vacuum distillation or reverse osmosis, and the mixture obtained is optionally diluted with aqueous phase.

Abstract

A process for the production of aqueous mixed micelle solutions, containing mixed micelles formed from lipids and salts of bile acids, in which optionally slightly water-soluble or water-insoluble active ingredients are solubilized, is described, which is characterized in that mixtures are prepared from

a) solutions which contain lipids, free bile acids and optionally slightly water-soluble or water-insoluble active ingredients in a water-soluble organic solvent and

b) solutions which contain 0.05 to 3 equivalents of bases and optionally isotonized additives and/or water-soluble active ingredients in relation to the bile acids, the organic solvent is removed by ultrafiltration, freeze-drying, vacuum distillation or reverse osmosis, and the mixture obtained is optionally diluted with aqueous phase.

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Process For The Production Of Aqueous Mixed Micelle Solutions

The invention relates to a process for the production of aqueous mixed micelle solutions, containing mixed micelles formed from lipids and salts of bile acids, in which slightly water-soluble or water-insoluble active ingredients are optionally solubilized.

Processes for the production of such mixed micelle solutions are previously known, for example, from German patent specification 27 30 570.

In the previously known processes, the mixed micelle solutions are produced by the lipids, salts of bile acids and optionally slightly water-soluble or water-insoluble active ingredients being dissolved in an organic solvent (e.g. ethanol), and the solutions being concentrated by evaporation so that a lipid film forms on the vessel walls which is dissolved by aqueous solutions (Biochemistry 19, 1980, 602 ff. and 615 ff.; Naturforsch. 32c, 1977, 748 ff.).

But this process is quite complex and can be transferred on an industrially usable scale only with considerable equipment.

In addition to this preferred process, a process is known, for example, from example 3 of patent specification 27 30 570 which was already mentioned, in which such mixed micelle solutions are produced by mixing components and stirring the mixture.

But this process not only has the drawback that it takes several days but it is seen in the reworking of this example -- without active ingredient -- that only greatly clouded solutions, which contain mixed micelles with an average diameter of about 340 nm, are obtained in this way.

Clear solutions with mixed micelles of an average diameter of about 10 nm cannot be obtained in this way.

It has now been found that such clear aqueous solutions of mixed micelles can be produced in a simple way and in a

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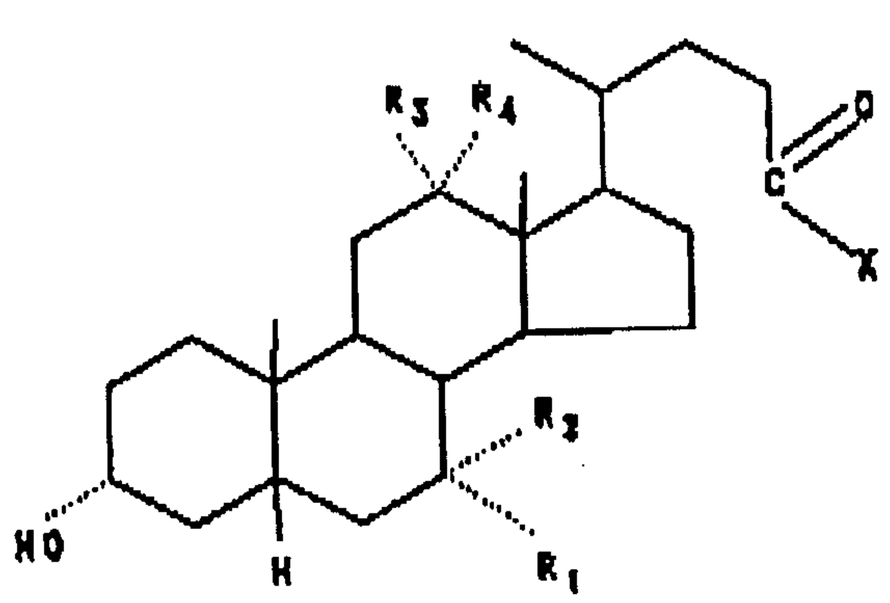
short time by a process which is characterized in that mixtures are prepared from

a) solutions, which contain lipids, free bile acids and optionally slightly water-soluble or water-insoluble active ingredients in a water-soluble organic solvent and

b) solutions, which contain 0.05 to 3 equivalent bases and optionally isotonized additives and/or water-soluble active ingredients relative to the bile acids,

the organic solvent is then removed preferably by ultrafiltration, freeze-drying, vacuum distillation or reverse osmosis, and the mixture obtained optionally is diluted with aqueous phase.

The process according to the invention can be performed by using the same bile acids as the previously known processes. Suitable bile acids are 5β-cholanic-24-acid derivatives of the general formula



in which

R_1 and R_2 as well as R_3 and R_4 jointly mean an oxo group, two hydrogen atoms, or a hydrogen atom and a hydroxy group, and

X represents a hydroxy group or a grouping of the formula $-NH-CH_2-CO_2H$ or $-NH-(CH_2)_2-SO_3H$.

As suitable bile acids, there can be mentioned, for example: cholic acid, glycocholic acid, taurocholic acid, deoxycholic acid, glycodeoxycholic acid, taurodeoxycholic acid, chenodeoxycholic acid, glycochenodeoxycholic acid and taurochenodeoxycholic acid.

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For the production of aqueous mixed micelle solutions, preferably 1 to 30 g and especially 2 g to 15 g of bile acid per 100 g of the aqueous solution containing optionally isotonized additives and water-soluble active ingredients is used.

For the production of aqueous mixed micelle solutions, the same lipids can be used in the process according to the invention as in the previously known processes.

Suitable lipids include, for example, monoglycerides, sulfatides, and especially phospholipids, such as sphingomyelins, plasmalogens, phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, phosphatidylinosites and cardiolipins, and also mixtures of these lipids (Dr. Otto-Albert Neumueller: Roempps Chemie-Lexikon; Franck'sche Verlagshandlung, Stuttgart (DE) 2665, 3159, 3920 and 4045).

For the production of aqueous mixed micelle solutions, preferably 3 to 40% and especially 5 to 20% of lipid per 100 g of the aqueous solution containing optionally isotonized additives and/or water-soluble active ingredients is used, the weight ratio between lipid and bile acid is preferably 0.1:1 to 2:1 and especially 0.8:1 to 2:1.

Suitable bases for the production of aqueous mixed micelle solutions according to the process of the invention include alkali hydroxides, such as lithium hydroxide, potassium hydroxide and especially also sodium hydroxide, and organic nitrogen bases which form physiologically acceptable salts. Such nitrogen bases include, for example, ammonia, primary, secondary or tertiary amines, ethanolamine, diethanolamine, piperazine, morpholine, lysine, ornithine, arginine, N,N-dimethylglucamine, choline and especially N-methylglucamine and tris(hydroxymethyl)aminomethane. These bases are used according to the invention in an amount so that the solutions contain 0.05 to 3 equivalent of base and especially 0.5 to 2 equivalent of base relative to the bile acids.

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Suitable water-soluble organic solvents include, for example, lower alcohols, such as methanol, ethanol, propanol or isopropanol, or acetone. These solvents are preferably used in amounts so that the resulting mixtures are also solutions. The known solvents can be removed both by vacuum distillation and by reverse osmosis (T. H. Meltzer, Advances in Parenteral Science 13, Filtration in the Pharmaceutical Industry, Marcel, Dekker Verlag New York, etc., 1st edition, 72-74, 483-488 and 838-854; Chem.-Ing. Tech. [Chem. Eng. Tech.] 61, 1989, 535-544).

If the solvent is removed by freeze-drying, it is advisable to add 20 to 300 mg of a monosaccharide or disaccharide, such as glucose or galactose or a sugar alcohol, such as sorbitol or mannitol, to the solution ahead of time.

If the solvent is removed by ultrafiltration, a filter with an exclusion size of a maximum of 300,000 daltons is suitably used.

When the solvent is removed by reverse osmosis, which is already used industrially for water processing, the liquid to be removed, as is known, is removed through an asymmetrical membrane, which has no pores. Suitable membranes are, for example, those made from poly(dimethylsiloxane) or poly(vinyl alcohol) of about a 0.1 to 2 micron thickness which are applied to a sponge-like or tissue-like supporting layer. Suitable membrane modules are also capillary and tube modules or also plate modules or spirally wound modules. Relative to the development of solvent-selective membranes and their mode of operation, reference is made to the publication already mentioned in the journal Chem. Ing. Techn. 60, 1988, 590 ff.

The reverse osmosis can be used not only for removing solvents from aqueous dispersions, which have a higher vapor pressure than water, but it is also suitable for removing solvents with a lower vapor pressure than water, such as, for example, dimethyl formamide, dimethyl sulfoxide or acetonitrile.

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After the removal of the solvent has been performed, the mixture obtained can optionally be diluted with an aqueous phase. In this case, it can be advisable to use an aqueous phase which contains a maximum of 10 ml of a neutralized bile acid and optionally additionally also isotonizing additives.

Suitable slightly water-soluble or water-insoluble active ingredients are preferably those whose water-solubility does not exceed 2% at room temperature. Such active ingredients are, for example, plant protecting agents, such as, poorly soluble insecticides or herbicides and especially poorly soluble pharmaceutically active ingredients.

Poorly water-soluble or water-insoluble pharmaceutical active ingredients of the following active ingredient groups are suitable, for example, for the production of the pharmaceutical agents according to the invention:

Gestagenally effective steroid hormones such as, for example, 13-ethyl-17β-hydroxy-18,19-dinor-17α-pregn-4-en-2-yl-3-one (=levonorgestrel), 13-ethyl-17β-hydroxy-18,19-dinor-17α-pregna-4,15-dion-20-yn-3-one (=gestodene) or 13-ethyl-17β-hydroxy-11-methylene-18,19-dinor-17α-pregn-4-en-20-yne (desorgestrel); estrogenically effective steroid hormones such as 3-hydroxy-1,3,5(10)-estratrien-17-one (=estrone) or 1,9-nor-17α-pregna-1,3,5(10)-trien-20-yn-3,17β-diol (ethynylestradiol).

Androgenically effective steroid hormones such as 17β-hydroxy-4-androsten-3-one (=testosterone) and its esters or 17β-hydroxy-1α-methyl-5α-androsten-3-one (=mesterolone).

Antiandrogenically effective steroid hormones such as 17α-acetoxy-6-chloro-1β,2α-dihydro-3'H-cyclopropa[1,2]-pregna-1,4,6-triene-3,20-dione (cyproterone acetate).

Corticoids such as 11β,17α,21-trihydroxy-4-pregnene-3,20-dione (=hydrocortisone), 11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione (=prednisolone), 11β,17α-21-

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trihydroxy-6α-methyl-1,4-pregnatriene-3,20-dione (=methylprednisolone) and 6α-fluoro-11β,21-dihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione (=difluorocortolone).

Ergolines such as 3-(9,10-dihydro-6-methyl-8α-ergolinyl)-1,1-diethyl urea (=ergoline), 3-(2-bromo-9,10-dihydro-6-methyl-8α-ergolinyl)-1,1-diethyl urea (=bromoergoline) or 3-(6-methyl-8α-ergolinyl)-1,1-diethyl urea (=terguride).

Antihypertensives such as 7α-acetylthio-17α-hydroxy-3-oxo-4-pregnene-21-carboxylic acid- -lactone (=spironolactone) or 7α-acetylthio-15β,16β-methylene-3-oxo-17α-pregna-1,4-diene-21,17-carbolactone (=mespirenone).

Anticoagulants such as 5-[hexahydro-5-hydroxy-4-(3-hydroxy-4-methyl-1-octen-6-ynyl)-2(1H)-pentalenylidene)]-pentanoic acid (=iloprost).

Psychopharmaceutical agents such as 4-(3-cyclopentyloxy-4-methoxy-phenyl-2-pyrrolidone (=rolipram) and 7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one (=diazepam).

Carotinoids such as α-carotene and β-carotene.

Liposoluble vitamins, such as vitamins of the vitamin A, vitamin D, vitamin E and vitamin K groups.

The β-carbolines are an especially preferred group, as they are described, for example, in European patent applications 234,173 and 239,667. As β-carbolines, there can be mentioned, for example, 6-benzoyloxy-4-methoxymethyl-β-carboline-3-carboxylic acid-isopropylester (=becarnil) and 5-(4-chlorophenoxy)-4-methoxymethyl-β-carboline-3-carboxylic acid-isopropyl ester (=C1-Phocip).

The aqueous mixed micelle solutions produced according to the process of the invention can optionally contain isotonic additives to increase their osmotic pressure. Suitable additives are, for example, inorganic or organic salts or buffer substances, such as sodium chloride,

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phosphate buffer, citrate buffer, glycine buffer, citrate-phosphate buffer, TRIS-HCl buffer, maleate buffer, etc.; monosaccharides or disaccharides, such as glucose, lactose, saccharose, sugar alcohols, such as mannitol, sorbitol, xylitol or glycerine; or water-soluble polymers, such as dextran or polyethylene glycol.

These isotonized substances are usually added in concentrations so that the resulting aqueous mixed micelle solution exhibits an osmotic pressure of 5-1000 mosm, with injection solutions of optimally 300 mosm.

Further, the aqueous mixed micelle solutions can contain additional water-soluble active ingredients to produce combination preparations. Examples of such combination preparations are mixtures of water-soluble and fat-soluble vitamins or preparations which contain water-soluble antibiotics, in addition to corticoids.

The micelles of this invention can be used conventionally, e.g., for pharmaceutical purposes, e.g., in accordance with German patent 27 30 570, Acta Anaesthesiol. Scand. 1986, 337-340 and J. Pharm. Pharmacol. 1988, 85-88.

The aqueous mixed micelle solutions of this invention contain mixed micelles with an average diameter of 2 to 100 nm, preferably 3 to 50 nm. Especially in the case of solutions suitable for injection, the mixed micelles will optimally have an average diameter of 3 to 20 nm.

Since the lipids and also some active ingredients are sensitive to oxidation, the process is suitably performed under an inert gas atmosphere, such as nitrogen or argon, and the aqueous mixed micelle solutions obtained are stabilized by adding antioxidants, such as sodium ascorbate, tocopherol or sodium hydrogen sulfite.

The process according to the invention has the advantage that on an industrial scale, it is substantially simpler to perform than the previously known processes. The dissolving, mixing and vacuum distillation or reverse osmosis process steps required in its performance are industrially

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continuously performable and not costly. Moreover, the process according to the invention has the advantage that the thermal stress of the components is less than in the previously known processes, especially so if the process of reverse osmosis is used to remove the solvent.

After the production has been performed, the aqueous mixed micelle solution obtained can be sterilized by filtration and/or heat-sterilized at 100° to 140°C.

The following embodiments are used to explain the process according to the invention in more detail.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

In the foregoing and in the following examples, all temperatures are set forth uncorrected in degrees Celsius and unless otherwise indicated, all parts and percentages are by weight.

Examples

Example 1

400 ml of an aqueous solution, which contains 2.138 of sodium hydroxide, is introduced in a round-bottom flask. Then, it is mixed with 100 ml of an ethanol solution, which contains 45 g of phospholipid (phospholipon 100 L, manufacturer A. Nattermann & Cie., DE-5000 Cologne) and 27.1 g of glycocholic acid. A clear mixture results slightly with yellowish coloring and a pH of 6.5.

The ethanol is removed by reverse osmosis, by the solution being concentrated from 500 ml to 250 ml in an apparatus for the reverse osmosis (Membra-Fil P-28. Buechi company, Goeppingen, Membrane DRC-1000). The pressure is 35 bars. In a second step, the concentrate is diluted with a

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3.5 mM solution of neutralized glycocholic acid to the initial volume. The mixed micelle formulation obtained then contains less than 0.01 mg/ml of ethanol.

The composition is as follows:

Phospholipon:	Manufacturer Nattermann AG,	
DE-5000 Cologne:		90.0 mg
Glycocholic acid:		52.1 mg
Sodium hydroxide:		4.3 mg
Water	to	1.0 ml
pH:		6.6

Example 2

800 ml of an aqueous solution with 2.8 g of potassium hydroxide is mixed with 120 ml of an ethanol solution, which contains 45 g of phospholipon and 27 g of glycocholic acid. A clear solution results whose pH is adjusted to 6.5 with 0.1 N potassium hydroxide solution. 18 g of sorbitol is added to this solution and stirred to complete dissolution.

The mixed micelle solution containing ethanol is converted in an ultrafiltration unit (Amicon GmbH, DE 8510 Witten; Type DC 2, Membrane: HIP 30-20). It is ultrafiltered at a membrane differential pressure of at most 1 bar. The volume of the removed ultrafiltrate is continuously replaced by 10 mM neutralized glycocholic acid solution. If the ultrafiltrate volume is 2.5 l, the continuous supply is interrupted and the mixed micelle solution is concentrated from 1 l to 500 ml.

The formulation has the following composition:

Phospholipon:		88.9 mg
Glycocholic acid:		58.0 mg
Potassium hydroxide:		6.4 mg
Water:	to	1.0 ml
pH:		6.8

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Example 3

Under the conditions of example 2, a mixed micelle solution is produced, with the difference, however, that the aqueous solution additionally contains 50 mg of sodium EDTA and the ethanol solution additionally contains 580 mg of active ingredients (C1-Phocip).

The mixed micelle formulation obtained has the following composition:

Phospholipon:		89.2 mg
Glycocholic acid:		52.3 mg
Potassium hydroxide:		6.1 mg
C1-Phocip:		1.01 mg
Water:	to	1.0 ml
pH:		6.7

The preceding examples can be repeated with similar success by substituting the generically or specifically described reactants and/or operating conditions of this invention for those used in the preceding examples.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions.

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The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A process for the preparation of an aqueous solution of mixed micelles containing a lipid and a salt of a bile acid, comprising

mixing

(a) a solution comprising a lipid and a free bile acid in a water-soluble organic solvent with

(b) a solution comprising 0.05 to 3 equivalents, relative to the bile acid, of a base; and

removing said organic solvent.

2. The process of claim 1, wherein solution (a) further contains an active agent which is sparingly soluble or insoluble in water.

3. The process of claim 2, wherein the active agent which is sparingly soluble or insoluble in water is a pharmaceutical active agent.

4. The process of claim 3, wherein the pharmaceutical active agent is a β-carboline.

5. The process of claim 2, 3 or 4, wherein the resultant micelles have an average diameter of 3 to 50 nm.

6. The process of any one of claims 1 to 5, wherein solution (b) further contains an isotonizing additive and/or water soluble active agent.

7. The process of any one of claims 1 to 6, wherein the organic solvent is removed by ultrafiltration, freeze-drying, vacuum distillation or reverse osmosis.

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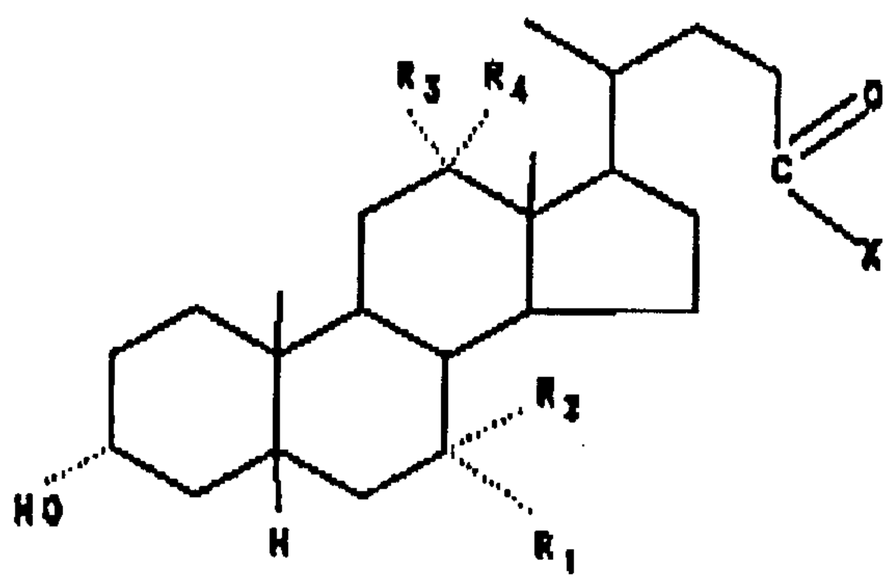
8. The process of any one of claims 1 to 7, further comprising diluting with an aqueous phase the mixture obtained after removing the organic solvent.

9. The process of any one of claims 1 to 8, wherein solution (a) is diluted such that the mixture obtained with solution (b) is also a solution.

10. The process for the preparation of an aqueous mixed micelle solution according to any one of claims 1 to 9, wherein the bile acid is a 5β-cholanic-24-acid derivative of the formula



wherein

R_1 and R_2 are two hydrogens, hydrogen and hydroxy, or jointly are oxo,

R_3 and R_4 are two hydrogens, hydrogen and hydroxy, or jointly are oxo, and

X is hydroxy, $-NH-CH_2-CO_2H$ or $-NH-(CH_2)_2-SO_3H$.

11. The process of any one of claims 1 to 10, wherein the lipid is a phospholipid.

12. The process of any one of claims 1 to 11, wherein the base is sodium hydroxide or potassium hydroxide.

13. A process for the preparation of an aqueous solution of mixed micelles containing a lipid and a salt of a bile acid, comprising

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removing organic solvent from a solution formed by mixing

(a) a solution comprising a lipid and a free bile acid in a water-soluble organic solvent

with

(b) a solution comprising 0.05 to 3 equivalents, relative to the bile acid, of a base.

14. A solution of aqueous mixed micelles prepared by the process of claim 1, 2 or 3.

15. A solution of aqueous mixed micelles prepared by a process of claim 4.

16. The solution of claim 15, wherein the β-carboline is 6-benzoyloxy-4-methoxymethyl-β-carboline-3-carboxylic acid-isopropyl ester or 5-(4-chlorophenoxy)-4-methoxymethyl-β-carboline-3-carboxylic acid-isopropyl ester.

17. A solution of aqueous mixed micelles prepared by a process of any one of claims 5 to 13.

18. A solution of aqueous mixed micelles containing a lipid and a salt of a bile acid, substantially free of thermally stressed components, wherein the mixed micelles have an average diameter of 3 to 20 nm.

19. The solution of any one of claims 14 to 18, which is in a form suitable for injection.