SOLUBILISATION METHODS

The present invention relates to methods of solubilising an agent, eg biologically active materials, in an amphiphile. In particular the invention relates to methods of bringing biologically active substances used for topical administration into association with permeation aids.

It is a continuing objective of the pharmaceutical industry to achieve high degrees of solubilisation of biologically active materials in a variety of solvents. There are several reasons for this need to achieve solubilisation. For instance, achieving solubilisation in particular solvents may improve bioavailibility. An example of this would be the solubilisation of biologically active materials in oils. Examples of methods to achieve this can be found , for example, in WO 95/13795, WO 96/17593 and WO 96/17594.

The methods disclosed in the above-noted patent publications and applications include steps whereby the biologically active material is brought into association with an amphiphile. There are also circumstances where it would be desirable to achieve higher degrees of solubilisation of biologically active molecules, particularly water-soluble ones, in amphiphiles. Examples include:

i) to improve dissolution characteristics in aqueous media, eg. to aid in achieving rapid dissolution;

ii) to aid incorporation into low HLB systems such as oil mixtures; and

iii) to bring about association between biologically active substances and permeation aids, which are often amphiphiles, for topical use.

Thus, in a first aspect, the present invention provides a method of solubilising an agent in an amphiphile which method includes the steps of:

(i) bringing the agent and amphiphile into association with each other in a common solvent;

(ii) removing the common solvent; and

(iii) heating the residue from step (ii);

In step (i), the agent and the amphiphile can suitably be brought into association with each other by firstly dissolving each one separately in the common solvent, followed by mixing of the two resultant solutions.

The removal of the solvent should be carried out at a temperature such that the amphiphile/agent residue which remains is in the solid state. The heating step should then be sufficient to melt the solid amphiphile, and also to convert the amphiphile/agent array from an "open" form to one which is more condensed.

The common solvent can be water, for example, and it can be removed in step (ii) by, e.g. freeze drying, centrifugal vacuum drying or any other suitable method.

Suitably, in the above methods the amphiphile will be a phospholipid, for instance lecithin, a glycolipid, a

polyoxyethylene containing surfactant, a lipophilic sulphate, betaine, a sarcosine containing surfactant, Solulan 16, Solulan C24, polyoxyethylene 40 stearate, one of the Tween series of surfactants, one of the Span series of surfactants or a pegolated castor oil derivative, e.g. Cremaphor EL35.

The "agent" is suitably a hydophilic species which is generally soluble in aqueous solvents but insoluble in hydrophobic solvents. The range of hydrophilic species of use in the present invention is diverse but hydrophilic macromolecules represent an example of a species which may be used.

A wide variety of macromolecules is suitable for use in the present invention. In general, the macromolecular compound will be hydrophilic or will at least have hydrophilic regions since there is usually little difficulty in solubilising a hydrophobic macromolecule in oily solutions. Examples of suitable macromolecules include proteins and glycoproteins, oligo and polynucleic acids, for example DNA and RNA, polysaccharides and supramolecular assemblies of any of these including, in some cases, whole cells or organelles. It may also be convenient to co-solubilise a small molecule such as a vitamin in association with a macromolecule, particularly a polysaccharide such as a cyclodextrin. Small molecules such as vitamin B12 may also be chemically conjugated with macromolecules and may thus be included in the compositions.

Examples of particular proteins which may be successfully solubilised by the method of the present invention include insulin, calcitonin, haemoglobin, cytochrome C,

horseradish peroxidase, aprotinin, mushroom tyrosinase, erythropoietin, somatotropin, growth hormone, growth hormone releasing factor, galanin, urokinase, Factor IX, tissue plasminogen activator, superoxide dismutase, catalase, peroxidase, ferritin, interferon, Factor VIII and fragments thereof (all of the above proteins can be from any suitable source). Other macromolecules may be used are FITC-labelled dextran and RNA extract from Torulla yeast.

It seems that there is no upper limit of molecular weight for the macromolecular compound since dextran having a molecular weight of about 1,000,000 can easily be solubilised by the process of the present invention.

In addition to macromolecules, the process of the present invention is of use in solubilising smaller organic molecules. Examples of small organic molecules include glucose, carboxyfluorescin and many pharmaceutical agents, for example anti-cancer agents, but, of course, the process could equally be applied to other small organic molecules, for example vitamins or pharmaceutically or biologically active agents. In addition, compounds such as calcium chloride and sodium phosphate can also be solubilised using this process. Indeed, the present invention would be particularly advantageous for pharmaceutically and biologically active agents since the use of non aqueous solutions may enable the route by which the molecule enters the body to be varied, for example to increase bioavailability.

Another type of species which may be included in the hydrophobic compositions of the invention is an inorganic material such as a small inorganic molecule or a

colloidal substance, for example a colloidal metal. The process of the present invention enables some of the properties of a colloidal metal such as colloidal gold, palladium, platinum or rhodium, to be retained even in hydrophobic solvents in which the particles would, under normal circumstances, aggregate. This could be particularly useful for catalysis of reactions carried out in organic solvents.

The above-described method is particularly suitable for achieving association between an agent which is for topical administration and a permeation aid. An example of the former is Zinc Acetate ($ZnAc_2$).

Particularly suitable amphiphiles are those which are solid at room temperature, eg Solulan 16 and Solulan C24.

In other aspects the present invention provides:

i) a composition comprising an agent solubilised in an amphiphile obtainable by any of the methods described herein, particularly an agent for topical administration solubilised in an aphiphile which is a permeation aid; and

ii) the use of a composition of the invention in the preparation of a medicament for topical administration, particularly a composition for use in the treatment of inflammation and/or arthritis wherein the active agent is $ZnAc_2$.

Preferred features of each aspect of the invention are as for each other aspect *mutatis mutandis*.

The invention will now be described with reference to the following examples, which should not be construed as in any way limiting the invention.

Example 1

(1) A solution of zinc acetate at a concentration of 100mg/ml was prepared by addition of 100mg of $ZnAc_2$ to 1ml of distilled water, and mixing at RT until dissolution was achieved.

(2) A solution of Solulan 16 at a concentration of 100mg/ml was prepared by addition of 500mg of Solulan to 4.5ml of distilled water and mixing at 60°C until dissolution was achieved.

(3) Solutions from steps 1 & 2 were dispensed into 4ml glass screw-capped vials as follows, and mixed well:

	A	B	C
$ZnAc_2$ (Vol)	0.2ml	0.3ml	0.4ml
Solulan 16 (Vol)	1.8ml	1.7ml	1.6ml
$ZnAc_2$ (wt)	20mg	30mg	40mg
Solulan 16 (wt)	180mg	170mg	160mg
%Zn (wt:wt)	10	15	20
Ratio (wt:wt)	9:1	5.7:1	4:1

(4) The vials and contents were frozen in liquid nitrogen and lyophilised overnight with a condenser temperature of -40°C, and a vacuum of 0.1mBar.

(5) The following day, the lyophilates were incubated at +60°C on a heating block, to melt the solid cake of Solulan S16.

(6) Because of the fact that the solutions solidified at room temperature, dissolution or otherwise of $ZnAc_2$ in Solulan 16 was assessed visually by examining the clarity or turbidity of the resulting liquid formulations, rather than by recording optical densities. Results of visual observations are recorded in the table below:

Sample	Ratio S16:Zn (wt:wt)	Optical Clarity	Appearance
A	9:1	+++	Clear Solution
B	5.7:1	-	Cloudy Paste
C	4:1	-	Cloudy Paste

Example 2

(1) Solutions of Solulan 16 and $ZnAc_2$ were prepared as above, and dispensed into 2ml glass screw-capped vials as follows:

	A	B	C	D	E	F	G
S16 (μl)	0	20	40	80	120	160	200
$ZnAc_2$ (μl)	20	20	20	20	20	20	20
S16 (mg)	0	2	4	8	12	16	20
$ZnAc_2$ (mg)	2	2	2	2	2	2	2
Ratio S16:Zn	0:1	1:1	2:1	4:1	6:1	8:1	10:1

(2) After lyophilisation and heating to 60°C, the solubility of $ZnAc_2$ in S16 was assessed visually as described in Example 1. The results of observations are given in the table below:

Sample	A	B	C	D	E	F	G
Ratio (S16:Zn)	0	1	2	4	6	8	10
Observations after heating	*	*	*	+	+	-	-

*= Samples remained as white solids
+= Samples turned to a viscous glassy fluid
-= essentially clear free-flowing fluid

Example 3

(1) A solution of Solulan C24 at a concentration of 100mg/ml was prepared by addition of 500mg of Solulan C24 to 4.5ml of distilled water and mixing at 60°C until dissolution was achieved.

(2) 500mg $CuAc_2$ was dissolved in 10ml of distilled water

to give a concentration of 50mg/ml.

(3) 150µl of $CuAc_2$ solution (7.5mg solid) and 925µl of Solulan C24 solution (92.5mg solid) were dispensed into a 4ml glass screw-capped vial. The vial contents were mixed well, frozen in liquid nitrogen and lyophilised overnight.

(4) 10mg of $CuAc_2$ was dispensed into a 2ml glass vial and 100mg of Solulan C24 was added. The vials was capped and heated to 60°C to melt the oil. The contents of the tube were vortexed to disperse the $CuAc_2$ in the oil, then incubated at 60°C for eight hours. After incubation, the contents of the vial consisted of a colourless oil solution on top of solid undissolved crystals of $CuAc_2$.

(5) The following day, the contents of the lyophilised tube was converted to a clear strong blue-coloured solution by incubating in a heating block for 2 minutes at 60°C.

(6) The vials from steps (4) and (5) were allowed to stand at room temperature to allow any undissolved $CuAc_2$ to sediment, before the oil solidified.

(7) 20µg of solid oil was taken off the surface of each sample in step (6), and dissolved in 180µl of distilled water.

(8) The optical densities of the aqueous solutions obtained in step (7) were measured at 650nm, and compared against reference solutions prepared by dilution of $CuAc_2$ solution from step 2 in distilled water. The results are reported in the table below, where it is seen

that a higher concentration of $CuAc_2$ dissolved in the oil can be measured by following the lyophilisation procedure described above, than by simple mixing of the components.

	Concentration in aqueous solution (mg/ml)	
	Measured	Theoretical
After lyophilisation	6.7	7.5
After simple mixing	0.8	10.0

Example 4

A virus suspension (Sabin strains, Types 1, 2, 3) containing $5x10^8$ particles/ml (spun to remove contaminating protein) was diluted 50-fold by addition of 200µl of the suspension to 9.9ml of distilled water, yielding a concentration of 10^7 particles/ml. The suspension was divided into four equal aliquots of 2.5ml, and dispensed into 7ml screw-capped glass vials. 2.5ml of distilled water was added to one aliquot of virus particles and this group was labelled "W". 2.5ml of Solulan C24 (100mg/ml) was added to another aliquot and mixed gently. This group was labelled "S".

200µl of each preparation was dispensed into 10 freeze-drying vials, and the remainder in 100µl aliquots into other tubes as "pre-drying" controls. The controls were stored overnight at +4°C. The freeze-drying vials were placed in the centrifugal rotor of the freeze-dryer and lyophilised overnight.

On the following day 100µl of culture medium was added to

each vial in group "W" and mixed gently. The vials in group "S" were sealed and heated to 60°C in a hot water bath for 5 seconds to melt the Solulan C24, which resulted in a claer solution. Upon cooling to room temperature this material solidified. 90µl of medium was added to the vials of the "S" group to make the total volume up to 100µl. 10µl of sample was then transferred from each of groups "S" and "W" to fresh 1ml vials and 1ml of medium was added to each and mixed well.

To fresh 1ml vials was added 4 x 20µl of samples from each of the pre-drying groups and 1ml of medium was added to each. The contents of each vial were mixed well.

The suspensions prepared as described herein were used to perform 10-fold dilutions in Vero cell cultures,to measure the viability of the polio virus present. The results were expressed as the highest dilution at which 50% cytopathic effects were observed.

Nature of Sample	**Highest Dilution at which 50% CPE observed**
Non-dried control + water	$10^{-4}/10^{-6}$
Non-dried control + Solulan C24	$10^{-5}/10^{-5}$
Freeze-dried control + water	$10^{-2}/10^{-2}$
Freeze-dried control + Solulan C24	$10^{-6}/10^{-8}$

CLAIMS:

1. A method of solubilising an agent in an amphiphile which method includes the steps of:-

(i) bringing the agent and amphiphile into association with each other in a common solvent;

(ii) removing the common solvent; and

(iii) heating the residue from step (ii);

2. A method as claimed in claim 1 wherein the agent and the amphiphile are brought into association with each other by firstly dissolving each one separately in the common solvent, followed by mixing of the two resultant solutions.

3. A method as claimed in claim 1 or claim 2 wherein the common solvent is water.

4. A method as claimed in claim 3 wherein the water is removed in step (iii) by freeze drying, centrifugal vacuum drying or any other suitable method.

5. A method as claimed in any one of claims 1 to 4 wherein the amphiphile is lecithin, a glycolipid, a polyoxyethylene containing surfactant, a lipophilic sulphate, betaine, a sarcosine containing surfactant, Solulan 16, Solulan C24, polyoxyethylene 40 stearate, one of the Tween series of surfactants, one of the Span series of surfactants or a pegolated castor oil derivative, e.g. Cremaphor EL35.

6. A method as claimed in any one of claims 1 to 4 wherein the amphiphile is a permeation aid.

7. A method as claimed in any one of claims 1 to 6 wherein the agent is one administered topically.

8. A method as claimed in claim 7 wherein the agent is Zinc Acetate.

9. A method as claimed in claim 8 wherein the amphiphile is Solulan 16 or Solulan C24.

10. A method as claimed in any one of claims 1 to 5 wherein the agent is a macromolecule, a small organic or inorganic molecule or a colloidal substance.

11. A method as claimed in claim 10, wherein the macromolecule comprises a protein, glycoprotein, oligo- or polynucleic acid, polysaccharide or supramolecular assembly thereof.

12. A method as claimed in claim 11, wherein the protein is insulin, calcitonin, haemoglobin, cytochrome C, horseradish peroxidase, aprotinin, mushroom tyrosinase, erythropoietin, somatotropin, growth hormone, growth hormone releasing factor, galanin, urokinase, Factor IX, tissue plasminogen activator, superoxide dismutase, catalase, peroxidase, ferritin, interferon, Factor VIII or fragments thereof.

13. A method as claimed in any one of claims 10 to 12 wherein the agent is for oral administration.

14. A method as claimed in claim 13 wherein the agent is

a virus.

15. A composition comprising an agent solubilised in an amphiphile obtainable by a method as defined in any one of claims 1 to 14.

16. The use of a composition as defined in claim 15 in the preparation of a medicament for topical administration.

17. The use as claimed in claim 16 wherein the medicament is for the treatment or prevention of inflammation and/or arthritis.

18. The use of a composition as defined in claim 15 in the preparation of a medicament for manipulating the immune response.

19. The use as claimed in claim 18 wherein the medicament is a vaccine.

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(57) Abstract

Improved methods for solubilising biologically active materials, e.g. proteins, in a hydrophobic solvent are provided.