PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 9/107	A1	(11) International Publication Number: WO 97/34581
		(43) International Publication Date: 25 September 1997 (25.09.97)

(21) International Application Number: PCT/GB97/00749

(22) International Filing Date: 18 March 1997 (18.03.97)

(30) Priority Data:
9605709.6 19 March 1996 (19.03.96) GB

(71) Applicant *(for all designated States except US)*: CORTECS LIMITED [GB/GB]; The Old Blue School, Lower Square, Isleworth, Middlesex TW7 6RL (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: NEW, Roger, Randal, Charles [GB/GB]; Flat 10, Leinster Mansions, 1 Langland Gardens, Hampstead, London NW3 6QB (GB).

(74) Agents: CHAPMAN, Paul, William et al.; Kilburn & Strode, 30 John Street, London WC1N 2DD (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR SOLUBILISING HYDROPHYLIC MATERIALS (E.G. PROTEINS) IN A HYDROPHOBIC SOLVENT

(57) Abstract

Methods for the preparation of a single phase preparation comprising a hydrophilic species, e.g. a protein, solubilised in a hydrophobic phase are provided. The methods of the invention can be used in the preparation of a pharmaceutical, cosmetic, nutrient, foodstuff or food supplement composition.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

METHOD FOR SOLUBILISING HYDROPHYLIC MATERIALS (E.G. PROTEINS) IN A HYDROPHOBIC SOLVENT

The present invention relates to a novel method of solubilising a hydrophilic species, for example in a protein, in a hydrophobic solvent in which it would not normally be soluble.

There are many areas where it would be advantageous to bring hydrophilic molecules, for instance proteins, into association with a hydrophobic species, for example a lipid phase. Advantages such as increased stability can be conferred on hydrophilic molecules in a lipid environment. In addition, transport of such hydrophilic species across lipidic barriers, e.g. the skin, would be facilitated.

Early proposals for using hydrophobic carriers for protein delivery included the use of liposomes. Later systems were non-liposomal, but still relied on the provision of a hydrophilic phase which carried the protein or other hydrophilic molecule, this hydrophilic phase forming an emulsion with a hydrophobic phase. Examples of such systems are disclosed in EP-A-0366277 and EP-A-0521994.

WO-A-95/13795 disclosed a system whereby it was possible to obtain a true single-phase preparation where the hydrophilic species was truly "solubilised" in a hydrophobic phase. In the methods disclosed therein, the hydrophilic species is first associated with an amphiphile in a liquid medium such that, in the liquid medium, there is no chemical interaction between the amphiphile and the hydrophilic species. The liquid medium is then removed to leave an array of amphiphile molecules and the hydrophilic species. Finally, adding a hydrophobic solvent to the array results in a single

phase preparation where the hydrophilic species is solubilised in the hydrophobic solvent.

It has now surprisingly been found that it is possible to produce a single-phase preparation of a hydrophilic species in a hydrophobic solvent by a modified route which is often faster.

Thus, in a first aspect, the present invention provides a process for the preparation of a single phase hydrophobic preparation comprising a hydrophilic species, in a hydrophobic solvent, the process comprising:

(i) associating the hydrophilic species with an amphiphile in the presence of a hydrophobic phase; and

(ii) removing any hydrophilic solvent which is present;

wherein the hydrophilic solvent removal step is carried out under conditions which maintain the hydrophobic phase in a solid state.

In a preferred embodiment the hydrophilic species and the amphiphile are first dissolved in a hydrophilic solvent, eg an aqueous solvent, often water alone, and this solution is then brought into association with the hydrophobic solvent. The hydrophilic solvent removal step is conveniently achieved by lyophilisation, such that it is carried out at temperatures which will ensure that the hydrophobic solvent is maintained in the solid state until all the water has been removed. Under certain circumstances, the oil may become liquid during lyophilisation, as a result of local rises in temperature in parts of the solid block (usually at the surface and

edges) where all the hydrophilic solvent has already been removed. Here the cooling effect deriving from sublimation of hydrophilic solvent no longer exists, and in those areas the oil will melt. This situation will lead to the production of a satisfactory end-product providing that the oil is allowed to drain away from the remainder of the solid block as soon as it appears (if not, thenaccumulating oil will form a layer which prevents further removal of hydrophilic solvent).

Alternatively, the temperature during lyophilisation can be maintained such that the oil remains solid even after the hydrophilic solvent has been driven off. In this case following lyophilisation, the temperature of the preparation is elevated to produce the single phase preparation. This can often simply be achieved by bringing the lyophilised preparation up to room temperature which in turn will cause the hydrophobic phase to return to the liquid state. Other methods for removal of hydrophilic solvent may also be employed, eg spray drying.

In the context of the present invention, the term "hydrophilic species" relates to any species which is generally soluble in hydrophilic solvents, eg 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.

The method of the present invention provides a convenient and relatively fast way of producing single phase preparations as described. The speed of the process is a particular advantage when one is concerned with the stability of the oil, the hydrophilic species or both,

particularly when the concentration of amphiphile is high. Furthermore, lower ratios of amphiphile to hydrophilic species can be achieved, e.g. as low as 7:1, even when using , for instance, triglycerides as the hydrophobic phase, which permit a higher loading capacity for the preparations.

As mentioned above, in one preferred embodiment the amphiphile is "presented" in the hydrophilic phase. However, where appropriate, the amphiphile can first be dissolved or dispersed in the oil. This is particularly appropriate when using amphiphiles which do not disperse well in water. A further advantage of the method of the present invention is that it allows the use of amphiphiles which do not lyophilise well, for instance ones which are liquid at temperatures at which lyophilisation is normally carried out.

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 complex of a small molecule such as a vitamin in association with a macromolecule, for example 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, microbial toxins, peptide and protein antigens 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.

There are numerous amphiphiles which may be used in the present invention and zwitterionic amphiphiles such as phospholipids are among those which have been found to be especially suitable. Phospholipids having a phosphatidyl choline head group have been used with particular success and examples of such phospholipids include phosphatidyl choline (PC) itself, lyso-phosphatidyl choline (lyso-PC), sphingomyelin, derivatives of any of these, for example hexadecylphosphocholine or amphiphilic polymers containing phosphoryl choline. In the present application, the terms phosphatidyl choline (PC) and lecithin are used interchangeably. Suitable natural lecithins may be derived from any convenient source, for example egg and, in particular, soya. In most cases, it is preferable to select an amphiphile which is chemically similar to the chosen hydrophobic solvent and this is discussed in greater detail below.

The hydrophobic solvent of choice will depend on the purpose for which the composition is intended, on the type of species to be solubilised and on the amphiphile. Suitable solvents include long chain fatty acids with unsaturated fatty acids such as oleic and linoleic acids

being preferred, alcohols, particularly medium chain alcohols such as octanol and branched long chain alcohols such as phytol, monoglycerides such as glycerol monooleate (GMO), diglycerides and triglycerides, particularly medium chain triglycerides and mixtures thereof.

Optimum results are generally obtained when the hydrophobic solvent and the amphiphile are appropriately matched. For example, with a solvent such as oleic acid, lyso-PC is a more suitable choice of amphiphile than PC, whereas the converse is true when the hydrophobic solvent is a triglyceride.

Alternatively, in the case of hydrophobic solvents with amphiphilic properies (eg free fatty acids such as oleic acid, which can ionise in the presence of a base to form sodium oleate), inclusion of an additional amphiphile may be unnecessary if a proportion of the solvent molecules are converted to the amphiphile form prior to or during the mixing process. In particular, the base used is a volatile base such as triethylamine. This approach is particularly successful when solubilising calcitonin or insulin.

Thus, in a second aspect the present invention provides a process for the preparation of a single phase hydrophobic preparation comprising a hydrophilic species, in a hydrophobic solvent, the process comprising:

(i) associating the hydrophilic species with a base in the presence of a hydrophobic phase; and

(ii) removing any hydrophilic solvent which

is present;

wherein the hydrophobic phase is one with hydrophobic properties and wherein the hydrophilic solvent removal step is carried out under conditions which maintain the hydrophobic phase in a solid state. As discussed above, preferably the base ia volatile base such as triethylamine. Suitable hydrophobic solvents include medium and long chain fatty acids examples of which include oleic acid and linoleic acid. This method is particularly useful for solubilising calcitonin or insulin.

One advantage of the preparations of the present invention is that they are essentially anhydrous and therefore stable to hydrolysis. They are also stable to freeze-thawing and have greater stability at high temperatures, probably because water must be present in order for the protein to unfold and become denatured. This means that they may be expected to have a much longer shelf life than aqueous preparations of the hydrophilic species.

The solutions provided using the methods of the invention are extremely versatile and have many applications. They may either be used alone or they may be combined with an aqueous phase to form an emulsion or similar two phase composition which forms yet a further aspect of the invention.

One way in which the compositions produced using the methods of the invention may be used is for the oral delivery to mammals, including man, of substances which would not, under normal circumstances, be soluble in lipophilic solvents. This may be of use for the delivery

of dietary supplements such as vitamins or for the delivery of biologically active substances, particularly proteins or glycoproteins, including insulin and growth hormones.

In a further application, it is possible to encapsulate or microencapsulate, for example by the method described above, nutrients such as vitamins which can then be used, not only as human food supplements but also in agriculture and aquaculture, one example of the latter being in the production of a food stuff for the culture of larval shrimps.

In addition, the compositions find application in the preparation of pharmaceutical or other formulations for parenteral administration, as well as formulations for topical or ophthalmic use. For this application, it is often preferable to use an emulsion of the oil solution and an aqueous phase as described above.

Many therapeutic and prophylactic treatments are intended for sustained or delayed release or involve a two component system, for example including a component for immediate release together with a component for delayed or sustained release. Because of their high stability, the preparations of the invention are particularly useful for the formulation of a macromolecule intended for sustained or delayed release.

The longer shelf life of the compositions produced by the methods disclosed herein is a particular advantage in the pharmaceutical area.

The hydrophile-in-oil preparations may find application in the pharmaceutical or similar industries for flavour

masking. This is a particular problem in the pharmaceutical industry since many drugs have unpleasant flavours and are thus unpopular with patients, especially children.

A further use is in the cosmetics industry where, again, hydrophobic preparations of hydrophilic compounds can very easily be incorporated into a cosmetic formulation. Examples of macromolecules which may be used in this way include those with moisturising or enzymatic action of some sort. The invention can also be used for the incorporation of proteins such as collagen into dermatological creams and lotions.

Thus, in a third aspect, the present invention provides the use of a process of the invention in the preparation of a pharmaceutical, cosmetic, nutrient, foodstuff or food supplement composition. In particular, when the processes of the invention are used in the preparation of a pharmaceutical composition, it will be one for topical, oral or opthalmic use.

Finally, the invention has numerous uses in the field of chemical and biological synthesis, for example, non-aqueous enzymatic synthesis.

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

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

EXAMPLE 1

(i) 20 mg of aprotinin was dissolved in 1 ml of distilled water.

(ii) 1 g of Soya PC was dissolved in 9 ml of Miglyol 818 to give a concentration of PC in M818 close to 100 mg/ml.

(iii) Three B10 glass-screw capped vials were prepared containing the following quantities of the two solutions described above. The contents were mixed well.

	A	B	C
Distilled water	600 µl	600 µl	600 µl
Aprotinin (20 mg/ml)	125 µl	63 µl	0 µl
M818/PC (9:1)	1 ml	1 ml	1 ml
PC (wt in mg)	100	100	100
Aprotinin (wt in mg)	2.5	1.25	0

(iv) The vials were then frozen in liquid N2 and lyophilised at temperature of -45°C for two days.

(v) After lyophilisation, the contents of the vials were brought to room temperature upon which clear or slightly opalescent oils were obtained.

(vi) The optical densities of 200 µl samples were measured at 600 nm, using the sample C (containing no aprotinin) as a blank.

	A	B	C
Aprotinin concentration in oil (mg/ml)	2.5	1.25	0
OD_{600}	0.330	0.096	0.00

In parallel experiments, 2.5 mg of aprotinin added to oil alone gives an optical density of >1.0, indicating that in this example solubilisation had occurred in both samples A & B as a result of the procedure adopted.

EXAMPLE 2

(i) 80 mg of aprotinin was dissolved in 4 ml of distilled water.

(ii) 100 mg of salicylic acid was dissolved in 10 ml of Miglyol 818.

(iii) Soya phosphatidyl choline was dispersed in distilled water by probe ultrasonication at a concentration of 250 mg/ml.

(iv) The solutions prepared above were dispersed into B10 glass screw-capped vials as in the table below:

	A	B	C	D	E	A'	B'	C'	D'	E'
Aprotinin (20 mg/ml)	0	250	375	500	750	0	250	350	500	750
Distilled water	600	350	-	-	-	600	350	-	-	-
PC (250 mg/ml)	400	400	400	400	400	400	400	400	400	400
M818	1	1	1	1	1	-	-	-	-	-
M818 + salicylic acid	-	-	-	-	-	1	1	1	1	1
Aprotinin (mg)	0	5	7.5	10	15	0	5	7.5	10	15

(v) The mixtures were all vortexed for ten seconds, frozen in liquid nitrogen and lyophilised for two days at -45°C.

(vi) After lyophilisation, the tubes were warmed at 37°C for fifteen minutes.

(vii) Clarity or otherwise of the solution was assessed by measurement of the optical density of the oils at 600 nm.

Results are given in the table below.

	A	B	C	D	E
Aprotinin (mg/ml)	0	5	7.5	10	15
M818 alone	0.009	0.191	0.566	0.390	1.576
M818 + salicylic acid	0.000	0.006	0.000	0.012	0.021

The results show that effective solubilisation of protein in the oil can be achieved, particularly in the presence of facilitating agents such as salicylic acid (described in International patent application no. PCT/GB95/02891), using the procedure described here.

EXAMPLE 3

(1) Disperse into each of three glass 7ml vials 1ml of salmon calcitonin solution in distilled water at a concentration of 1mg/ml.

(2) To one vial from (1), labelled "A", add dropwise, with vortexing, 0.4ml of oleic acid. Freeze the dispersion immediately in liquid nitrogen.

(3) To one vial from (1), labelled "B", add 5µl of glacial acetic acid, then add dropwise, with vortexing, 0.4ml of oleic acid. Freeze the dispersion immediately in liquid nitrogen.

(4) To one vial from (1), labelled "C", add 5µl of triethylamine, then add dropwise, with vortexing, 0.4ml of oleic acid. Freeze the dipersion immediately in liquid nitrogen.

(5) Lyophilise all the vials overnight in a lyophiliser with a shelf temperature of +4°C.

(6) The following day, warm the vials to obtain all the oils in a liquid state, and measure the optical density of each oil at 600nm.

RESULTS

	A	B	C
OD_{600nm}	0.096	0.096	0.005

A clear solution is obtained

EXAMPLE 4

(1) Into a glass 7ml vial was dispensed 1mg bovine insulin and 1ml of distilled water was added.

(2) 5µl of triethylamine was added to the vial from (1), then , dropwise, with vortexing, was added 0.4ml of oleic acid. The dispersion was frozen immediately in liquid nitrogen.

(3) The vial was lyophilised overnight in a lyophiliser with a shelf temperature of +4°C.

(4) The following day, the vial was warmed to obtain the oil in a liquid state, in which insulin is dissolved as a clear solution in an identical fashionn to that described with calcitonin in example 3 above.

CLAIMS:

1. A process for the preparation of a single phase hydrophobic preparation comprising a hydrophilic species, in a hydrophobic solvent, the process comprising:

(i) associating the hydrophilic species with an amphiphile in the presence of a hydrophobic phase; and

(ii) removing any hydrophilic solvent which is present;

wherein the hydrophilic solvent removal step is carried out under conditions which maintain the hydrophobic phase in a solid state.

2. A process as claimed in claim 1 wherein the hydrophilic species and the amphiphile are first dissolved in a hydrophilic solvent and this solution is then brought into association with the hydrophobic phase.

3. A process as claimed in claim 1 wherein the amphiphile is dissolved or otherwise brought into association with the hydrophobic phase before the hydrophobic phase is brought into association with the hydrophilic species.

4. A process as claimed in any one of claims 1 to 3 wherein the hydrophilic solvent removal step is achieved using lyophilistion.

5. A process as claimed in any one of claims 1 to 3 wherein the hydrophilic species comprises a macromolecule, a small organic or inorganic molecule or

a colloidal substance.

6. A process as claimed in claim 5 wherein the macromolecule comprises a protein, glycoprotein, oligo- or polynucleic acid, polysaccharide or supramolecular assembly thereof.

7. A process as claimed in claim 6 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, microbial toxins, peptide and protein antigens or fragments thereof.

8. A process as claimed in any one of claims 1 to 7 wherein the amphiphile is a phospholipid.

9. A process as claimed in claim 8 wherein the phospholipid has a phosphatidyl choline head group.

10. A process as claimed in claim 9 wherein the phospholipid is phosphatidyl choline (PC), lyso-phosphatidyl choline (lyso-PC), sphingomyelin, a derivative of one of the above such as hexadecyl phosphocholine or an amphiphile polymer containing phosphoryl choline.

11. A process as claimed in any one of claims 1 to 10 wherein the hydrophobic phase comprises a long chain fatty acid, a medium chain alcohol, a branched long chain alcohol, a monoglyceride or medium chain triglyceride.

12. A process as claimed in any one of claims 1 to 11 wherein the amphiphile comprises PC and the hydrophobic phase is a triglyceride or wherein the amphiphile comprises lyso-PC and the hydrophobic phase is oleic acid.

13. A process as claimed in any one of claims 2 to 12 wherein the hydophilic solvent is an aqueous solvent, preferably water.

14. A process for the preparation of a single phase hydrophobic preparation comprising a hydrophilic species, in a hydrophobic solvent, the process comprising:

(i) associating the hydrophilic species with a base in the presence of a hydrophobic phase; and

(ii) removing any hydrophilic solvent which is present;

wherein the hydrophobic phase is one with hydrophobic properties and wherein the hydrophilic solvent removal step is carried out under conditions which maintain the hydrophobic phase in a solid state.

15. A process as claimed in claim 14 wherein the base is a volatile base.

16. A process as claimed in claim 15 wherein the base is triethylamine.

17. A process as claimed in any one of claims 14 to 16 wherein the hydrophobic solvent is a medium or long chain fatty acids or mixtures of one or other of these.

18. A process as claimed in any one of claims 14 to 17 modified by any one or more of the features of claims 4 to 7.

19. A process as claimed in any one of claims 14 to 18 wherein the hydrophilic species is calcitonin.

20. A process as claimed in any one of claims 14 to 18 wherein the hydrophilic species is insulin.

21. The use of a process as defined in any one of claims 1 to 20 in the preparation of a pharmaceutical, cosmetic, nutrient, foodstuff or food supplement composition.

22. The use as claimed in claim 21 wherein the pharmaceutical composition is for topical, oral or opthalmic use.

INTERNATIONAL SEARCH REPORT

Int ›nal Application No
PCT/GB 97/00749

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/107

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 95 13795 A (CORTECS LTD.) 26 May 1995 cited in the application see the whole document ---	1-22
T	WO 96 14781 A (CORTECS LTD.) 23 May 1996 see the whole document ---	1-22
E	WO 97 15289 A (CORTECS LTD.) 1 May 1997 see the whole document -----	1-22

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
15 July 1997	24.07.1997
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Masturzo, P

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. ional Application No
PCT/GB 97/00749

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9513795 A	26-05-95	AU 8149694 A	06-06-95
		CA 2176577 A	26-05-95
		CN 1137751 A	11-12-96
		EP 0729350 A	04-09-96
		ZA 9409109 A	16-05-96
WO 9614781 A	23-05-96	US 5553924 A	10-09-96
		AU 3720595 A	06-06-96
WO 9715289 A	01-05-97	NONE	