

ANTI-OXIDANT COMPOSITIONS

5 The present invention relates to compositions comprising an anti-oxidant solubilised in a hydrophobic solvent in which it would not normally be soluble. In particular the present invention relates to compositions comprising ascorbic acid solubilised in a hydrophobic solvent in which it would not normally be soluble.

10 For many applications, e.g. in the pharmaceutical sciences, in food technology or the cosmetics industry, it is desired (and in certain cases essential) to employ anti-oxidants to limit oxidation of, for instance, an active ingredient or food ingredient.

15 Anti-oxidants can be divided into two main functional groups, the chelating agents which act by sequestering pro-oxidant ions, such as those of transition metals, while the second group are the free radical scavengers (chain-breakers), which have the effect of interrupting
20 oxidative chain reactions. The latter may operate in a hydrophilic (normally aqueous) or a hydrophobic (e.g. lipid) environment, depending on their solubility characteristics. Examples of lipid-soluble, free-radical scavengers include natural anti-oxidants such as α -tocopherol and β -carotene, as well as synthetic ones, e.g. BHA and BHT. Ascorbic acid (Vitamin C) is a water-soluble free-radical scavenger (and thus will normally
25 operate in this mode only in the aqueous phase), but it also has an important role as a chelating anti-oxidant. Yet another, very important anti-oxidant action of ascorbic acid is that it can interact synergistically with α -tocopherol, thus resulting in a greatly increased anti-oxidant activity which exceeds the sum of the
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component anti-oxidant activities. In this relationship, α -tocopherol functions as the primary anti-oxidant which is able to repair a lipid free radical, thus interrupting the oxidation chain reaction while itself being converted to a free radical in the process. Ascorbic acid acts by regenerating the tocopheroxyl radical, thus restoring its anti-oxidant function. Synergistic relationships are also known to occur between other anti-oxidant species, but often different mechanisms apply.

A requirement for ascorbic acid/ α -tocopherol synergistic action is that in order to interact, the two species must be able to come into close contact. This can be difficult in view of the fact that ascorbic acid is water soluble while α -tocopherol is lipid soluble. Such interactions may occur in a living cell since tocopherols are usually present in membranes which are in intimate contact with the aqueous cytoplasmic phase. It is therefore possible to duplicate this type of interaction in vitro using liposomes in place of biological membranes. A further available strategy is that of using a lipid-soluble derivative of ascorbic acid such as ascorbyl palmitate, which does interact synergistically with α -tocopherol. However, ascorbyl palmitate is not very soluble and can require heat to dissolve, which paradoxically increases oxidative susceptibility of vulnerable compounds, for example polyunsaturates, and this therefore mitigates against its use in situations where these compounds are required to be protected, for example in the preparation of certain foodstuffs.

Other approaches are possible, for example using microemulsions and reverse micelles. However, these will usually still involve the ascorbic acid being used in a

water solubilised form. The presence of water can, in fact, encourage oxidation by providing a medium for dispersing factors which can have pro-oxidant activity, e.g. molecular oxygen, metal ions, etc. It is thus
5 desirable to provide the ascorbic acid, if possible, in a water free environment. To date, no suitable method or approach has been disclosed or suggested.

UK patent application No. 9323588.5 discloses a process
10 by which a hydrophilic species can be solubilised in a hydrophobic solvent in which it would not normally be soluble. The process relies on the surprising discovery that if a hydrophilic species is mixed with an amphiphile under certain conditions, the resultant composition will
15 be readily soluble in lipophilic solvents such as oils.

It has now been surprisingly found that such compositions, comprising an anti-oxidant solubilised in a hydrophobic solvent in which it would not normally be
20 soluble, are effective as anti-oxidant compositions. Surprisingly it has been found that anti-oxidant species retain their anti-oxidant properties in such a non-aqueous environment.

25 Thus, in a first aspect the present invention provides an anti-oxidant composition comprising at least one anti-oxidant species solubilised in a hydrophobic solvent in which it would not normally be soluble.

30 In general, the compositions of the invention will be anhydrous. Thus, an anti-oxidant preparation is provided which does not contain water.

Suitably, the anti-oxidant species is selected from

ascorbic acid, citric acid, phytic acid, pyrophosphate, EDTA, transferrin, ceruplasmin, metallothionein, albumin, haptoglobin, cysteine, glutathione, conjugated bile pigments (e.g. bilirubin and biliverdin), uric acid,
5 vanillic acid, vanillin, and Trolox.

In a preferred embodiment, the anti-oxidant is selected from ascorbic acid, cysteine, glutathione, conjugated bile pigments and uric acid. A particularly preferred
10 anti-oxidant is ascorbic acid.

In the context of the present application, "solubilised" refers to the anti-oxidant species being held in the hydrophobic solvent, in the absence of water, i.e.
15 without the need for any water to be present.

The compositions of the present invention thus provide an anti-oxidant in a non-aqueous form to protect materials from oxidation, since it has been surprisingly found that
20 such compositions are effective as a means of protecting against oxidation notwithstanding that the anti-oxidant is solubilised in a hydrophobic solvent.

A variety of anti-oxidants can suitably be solubilised to produce compositions according to the invention. In particular, the compositions of the present invention are useful in that it is possible to provide lipid soluble anti-oxidants, e.g. vitamin E, in combination with one or more water soluble anti-oxidants, such as ascorbic acid,
25 which act synergistically with vitamin E, thus providing an enhanced anti-oxidant composition.
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Thus, in another aspect, the present invention provides an anti-oxidant composition comprising a lipid soluble

anti-oxidant species, together with one or more anti-oxidants solubilised in a hydrophobic solvent in which the one or more other anti-oxidants would not normally be soluble.

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The lipid soluble anti-oxidant species can be selected from tocopherols (e.g. α -tocopherol), β -carotene, $d\beta$ tocotrienol, quercetin, acacetin, BHA, BHT, TBHQ, propyl gallate and probucol. Preferably the lipid soluble anti-oxidant species is a tocopherol, particularly α -tocopherol, and the other anti-oxidant is one which can act synergistically with α -tocopherol, resulting in enhanced anti-oxidant activity, e.g. ascorbic acid, cysteine, glutathione, conjugated bile pigments or uric acid.

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Suitably the compositions of the present invention can be prepared using the processes described in UK patent application No. 9323588.5.

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Thus, in a further aspect the present invention provides a process for the preparation of a single phase hydrophobic anti-oxidant preparation comprising at least one antioxidant species solubilised in a hydrophobic solvent in which it would not normally be soluble, the process comprising:

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(i) associating the anti-oxidant species with an amphiphile in a liquid medium such that, in the liquid medium, there is no chemical interaction between the amphiphile and the anti-oxidant species;

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(ii) removing the liquid medium to leave an array of amphiphile molecules with their hydrophilic head

groups orientated towards the anti-oxidant species;
and optionally

(iii) providing a hydrophobic solvent around the
anti-oxidant species/amphiphile array.

Suitably, the process can be halted at the end of stage
(ii) and the resulting material can be stored under
appropriate conditions until it is needed to generate the
single-phase preparation by providing a hydrophobic
solvent.

In the context of the present invention, the term
"chemical interaction" relates to an interaction such as
a covalent or ionic bond or a hydrogen bond. It is not
intended to include van der Waals forces or other
interactions of that order of magnitude.

This process can also be used to produce a composition
comprising vitamin E together with one or more other
anti-oxidants which act synergistically with vitamin E,
and which are not normally soluble in a hydrophobic
solvent, to enhance anti-oxidant activity. In this case,
vitamin E can be added at either or both of stages (i)
and (iii) described above.

There are numerous amphiphiles which may be used to
prepare the compositions of 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 and halogenated amphiphiles, e.g. fluorinated phospholipids. 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 fact that the present inventors have found zwitterionic amphiphiles such as phospholipids to be particularly suitable for use in the process is a further indication of the significant differences between the present invention and the method of Okahata et al. Significantly, the authors of that prior art document concluded that anionic and zwitterionic lipids were completely unsuitable for use in their method and stated that they obtained zero yield of their complex using these lipids.

The hydrophobic solvent of choice will depend on the purpose for which the composition is intended, on the anti-oxidant species to be solubilised and on the amphiphile. Suitable solvents include non-polar oils such as mineral oils, squalane and squalene, 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, isoprenoids, e.g. nerol and geraniol, other alcohols such as t-butanol, terpineol, monoglycerides such as glycerol

monooleate (GMO), other esters, e.g. ethyl acetate, amyl acetate and bornyl acetate, diglycerides and triglycerides, particularly medium chain triglycerides and mixtures thereof, halogenated analogues of any of the above including halogenated oils, e.g. long chain fluorocarbons, and iodinated triglycerides, e.g. lipidiol. In particular, polyunsaturated oils or saturated oils are preferred.

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 effective choice of amphiphile than PC, whereas the converse is true when the hydrophobic solvent is a triglyceride.

In addition, in some cases it has been found to be advantageous to add a quantity of the amphiphile to the hydrophobic solvent before it is brought into contact with the anti-oxidant species/amphiphile array. This ensures that the amphiphile molecules are not stripped away from their positions around the anti-oxidant species because of the high affinity of the amphiphile for the hydrophobic solvent.

It is very much preferred that the preparations of the invention are optically clear and this can be monitored by measuring turbidity at visual wave lengths and, in some cases, by checking for sedimentation over a period of time.

The orientation of amphiphile molecules into an array with their hydrophilic head groups facing the moieties of an anti-oxidant species can be achieved in several ways

and examples of particularly suitable methods are discussed in more detail below.

5 In a first method, an anti-oxidant species is mixed with a dispersion of an amphiphile in a hydrophilic solvent, such that the amphiphile molecules form an assembly in which the hydrophilic head groups face outwards towards the hydrophilic phase which contains the anti-oxidant species. The hydrophilic solvent is then removed to
10 leave a dry composition in which the hydrophilic head groups of the amphiphile molecules are orientated towards the anti-oxidant species.

15 In this first method, it is preferred that the hydrophilic solvent is water although other polar solvents may be used.

The form taken by the amphiphile assembly may be micelles, unilamellar vesicles, preferably small
20 unilamellar vesicles which are generally understood to have a diameter of about 25 nm, multilamellar vesicles or tubular structures, for example cochleate cylinders, hexagonal phase, cubic phase or myelin type structures. The form adopted will depend upon the amphiphile which is
25 used and, for example, amphiphiles such as phosphatidyl choline (PC) tend to form small unilamellar vesicles whereas lyso-phosphatidyl choline forms micelles. However, in all of these structures, the hydrophobic tails of the amphiphile molecules face inwards towards
30 the centre of the structure while the hydrophilic head groups face outwards towards the solvent in which the anti-oxidant species is dispersed.

The weight ratio of amphiphile:anti-oxidant species will

generally be in the region of from 1:1 to 100:1, preferably from 2:1 to 20:1 and most preferably about 8:1 for PC and 4:1 for lyso-PC.

5 These ratios are preferred ratios only and, in particular, it should be pointed out that the upper limit is set by economic considerations which mean that it is preferable to use the minimum possible amount of amphiphile. The lower limit is somewhat more critical
10 and it is likely that ratios of 2:1 or below would only be used in cases where the anti-oxidant species has a significant hydrophobic portion or is exceptionally large.

15 Good performance is obtained when the solvent is removed quickly and a convenient method for the removal of the solvent is lyophilisation, although other methods can be used.

20 A second method for the preparation of a composition containing an array of amphiphiles with their head groups pointing towards the anti-oxidant species is to co-solubilise the anti-oxidant species and the amphiphile in a common solvent followed by removal of the solvent.

25 The solutions of the present invention 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.

30 In this aspect of the invention there is provided a two phase composition comprising a hydrophilic phase and a hydrophobic phase, the hydrophobic phase comprising a preparation of an anti-oxidant species as described

herein.

Generally, in this type of composition, the hydrophobic phase will be dispersed in the hydrophilic phase.

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The two phase compositions may be emulsions which may either be transient or stable, depending on the purpose for which they are required.

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The average size of the emulsion particles will depend on the exact nature of both the hydrophobic and the aqueous phases. However, it may be in the region of 2 μm

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Dispersion of the hydrophobic preparation in the aqueous phase can be achieved by mixing, for example either by vigorous vortexing for a short time for example about 10 to 60 seconds, usually about 15 seconds, or by gentle mixing for several hours, for example using an orbital shaker.

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Emulsions containing the hydrophobic preparations of the invention can also be used in the preparation of microcapsules. If the emulsion is formed from a gelatin-containing aqueous phase, the gelatin can be precipitated from the solution by coacervation by known methods and will form a film around the droplets of the anti-oxidant-containing hydrophobic phase. On removal of the hydrophilic phase, microcapsules will remain. This technology is known in the art, but has proved particularly useful in combination with the preparations of the present invention.

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In other aspects the invention provides:

(i) the use of an anti-oxidant composition of the invention in the preparation of a pharmaceutical or cosmetic formulation or a foodstuff;

5 (ii) a method for reducing oxidation of a pharmaceutical or cosmetic formulation or foodstuff which comprises adding an anti-oxidant composition of the invention to the pharmaceutical or cosmetic formulation or foodstuff;

10 (iii) a composition comprising at least one anti-oxidant species solubilised in a hydrophobic solvent in which it would not normally be soluble, for use as an anti-oxidation agent; and

15 (iv) the use of a composition of the invention in the preparation of an anti-oxidation agent.

The invention will now be described with reference to the following examples. Example 3 refers to the figures in
20 which:

FIGURE 1: shows a comparison of oxidation index (ratio of non-saturated fatty acid remaining in oil compared to amount of non-oxidisable interval
25 standard) for preparations with and without ascorbic acid alone or in combination with α -tocopherol.

FIGURE 2: shows a comparison of oxidation index for preparations with and without ascorbic acid in the
30 presence of linoleic acid and linoleic acid and Soy PC.

FIGURE 3: shows a comparison of oxidation index for preparations with and without ascorbic acid alone

and with linoleic acid.

EXAMPLE 1

5 2 rows of 4 small test-tubes were set up, and 0.2ml
 aliquots of 3.75, 7.5, 15 and 30mM ascorbic acid
 solutions were added to tubes 1, 2, 3 and 4 respectively,
 across each row. 0.2 ml of soy PC SUVs, prepared as in
 Example 2, was added to each tube in the front row, and
10 0.2 ml of distilled water to each tube in the second row.
 The tube contents were shell-frozen in liquid nitrogen
 and freeze-dried overnight. To each lyophilate in the
 first row was added 200mg of Miglyol 818, while 200mg of
 a solution comprising 10% soy PC in Miglyol 818 was added
15 to each tube in the second row. All of the mixtures were
 vortexed and left for several hours to disperse. At the
 end of this time, all of the first row tubes contained
 clear dispersions, while those in the second row were
 turbid, despite containing exactly the same components as
20 the corresponding tubes in the front row.

EXAMPLE 2

25 250mg of Soy phosphatidyl choline was dissolved in 5ml of
 diethyl ether in a glass boiling tube with a ground glass
 stopper. 80mg of ascorbic acid was dissolved in 1ml of
 distilled water. 300 μ L of ascorbic acid solution was
 added to the ethereal solution of phosphatidyl choline,
 shaken well, stoppered, and the mixture sonicated in a
30 bath for two minutes to give an almost clear water-in-oil
 emulsion. The ether was then removed in a rotary
 evaporator at 37°C with a slight vacuum, and the residue
 dried under a stream of nitrogen, followed by drying
 under high vacuum at room temperature in a lyophiliser

overnight. The following day, 3 ml of oleic acid was added to the residue with gentle mixing, to give a solution which was completely clear. The concentration of ascorbic acid in the oil was 8mg/ml.

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EXAMPLE 3

An aqueous dispersion of soy phosphatidyl choline (soy PC) was prepared, containing 50mg/g of suspension, flushed thoroughly with nitrogen, and sonicated in 3ml aliquots at an amplitude of 8 microns peak to peak. Each aliquot was subjected to a total sonication time of 4 minutes, in pulses of 30 seconds interspersed by cooling for 30 seconds in an ice slurry bath. The resulting opalescent dispersions of small unilamellar vesicles (SUV) were pooled and then centrifuged for 15 minutes to remove particles of titanium.

0.56g of SUV were mixed with 0.372g of 0.5% ascorbic acid solution, shell-frozen and freeze-dried overnight. To the resulting lyophilate was added 1.4g of trilinolein containing 5% by weight of linoleic acid and the mixture flushed with nitrogen, vortexed briefly and left to form a clear dispersion. This is termed the high ascorbate dispersion. Trilinolein is a model polyunsaturated triglyceride while linoleic acid is a solubilization facilitator.

A trilinolein/linoleic acid/PC oil phase of similar composition but lacking ascorbic acid was prepared by freeze-drying 0.7g of SUV and then dissolving the lyophilate in 1.75g of the same trilinolein/linoleic acid solution. A low ascorbate dispersion was prepared by diluting 0.25g of the high ascorbate one with 750mg of

the above oil phase. Into 3 small glass vials were added aliquots of 0.6, 0.6 and 0.5mg respectively of α -tocopherol. For reasons of accuracy, this was added as a 0.6% ethanolic solution, the ethanol being subsequently removed under a stream of nitrogen. To the above vials were then added 600mg of high ascorbate dispersion, 600mg of low ascorbate dispersion and 500mg of the above ascorbate-free oil phase respectively, mixing thoroughly to dissolve the α -tocopherol. A tray of 7 rows of crimpable glass gas chromatography vials were set up and to the vials in each row were added identical 50mg aliquots of the oil phases prepared above, according to the following scheme.

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Row no. Contents of each vial

- | | |
|----|---|
| 1 | Trilinolein/linoleic acid/PC oil phase |
| 20 | 2 High ascorbate oil phase |
| | 3 Low ascorbate oil phase |
| | 4 High ascorbate oil phase + α -tocopherol |
| 25 | 5 Low ascorbate oil phase + α -tocopherol |
| | 6 Trilinolein/linoleic acid/PC oil phase +
α -tocopherol |
| 30 | 7 Trilinolein/linoleic acid solution |

One vial from each row was retained as a zero time control and the remainder were transferred uncapped to a

37 C incubator and sampled after the incubation periods shown overleaf. At each sampling interval, 0.5ml of iso-octane containing 0.01% BHT was added to reduce further oxidative degradation, the contents mixed, sealed and the vials transferred to a minus 20 C freezer and stored for up to 2 weeks prior to GC analysis. At the time of sampling, 0.5 ml of a solution of a 0.125% heptadecanoic acid (a saturated, non-oxidisable, internal standard) in iso-octane was added to each vial and mixed. The fatty acid components in each vial were then converted to methyl ester derivatives by standard procedures and measured by GC. Results were expressed in terms of an Oxidation Index, which is defined here as the percentage of remaining 18 : 2 fatty acids (derived from trilinolein and linoleic acid) relative to 17 : 0 fatty acids (heptadecanoic acid).

	Incubation time (days)	Vial Row Number						
		1	2	3	4	5	6	7
20	Day zero	44.5	43.8	42.2	42.8	41.5	43.1	44.5
	Day 2	41.5	n/s	n/s	n/s	n/s	n/s	28.7
25	Day 4	31.7	40.4	39.0	37.8	40.0	29.8	21.7
	Day 6	21.0	38.8	36.8	n/s	n/s	33.7	7.2
	Day 8	9.0	53.9	45.6	44.4	50.2	39.0	0.6
30	Day 10	5.0	51.1	31.7	n/s	n/s	46.4	0.6
	Day 15	0	6.6	0	45.4	45.2	32.9	n/s
35	Day 20	0	0	0	n/s	n/s	15.9	n/s
	Day 30	n/s	0	0	41.5	35.9	0	n/s
	Day 48	n/s	n/s	n/s	52.5	11.3	n/s	n/s
40	Day 52	n/s	n/s	n/s	31.7	2.3	0.3	n/s

Day 56

n/s n/s n/s 4.1 0.2 n/s n/s

These results are plotted in Figure 1.

EXAMPLE 4

An aqueous phospholipid dispersion was prepared containing 100mg soy PC/g and converted to SUV as described in Example 3. Appropriate amounts of SUV were mixed with aqueous 1% ascorbic acid solutions to provide mixtures having PC : ascorbic acid ratios of 13.33 : 1, and the mixtures were shell-frozen and freeze-dried. The resulting lyophilates were mixed with refined fish oil containing 2% w/w of linoleic acid (as a solubilisation enhancer) to give clear dispersions containing 1.5 or 3.0 mg ascorbic acid/g of oil phase. Control oil phases were also prepared comprising pure fish oil, and also fish oil containing 2% linoleic acid both in the absence and presence of 2% w/w of dissolved soy PC (ie in the same concentration as in the dispersion containing 1.5mg ascorbic acid/g).

The two ascorbic acid-containing dispersions, and the three oil phase controls, were each distributed as 50mg aliquots into crimpable glass chromatography vials as in Example 3, and again stored uncapped in a 37 C incubator, ie under conditions for accelerated oxidation of lipids. At appropriate intervals, samples were removed from the incubator and 1ml of a solution containing 10mg BHT and 625mg heptadecanoic acid in 200ml iso-octane was added (see Example 3 for rationale). The vials were sealed, shaken and stored in the freezer prior to GC analysis of the remaining fatty acids. Lipid oxidation

was monitored according to the rate of disappearance of the 22:6 polyunsaturated fatty acids. Results are shown in Figure 2 and are again plotted as the Oxidation Index which is the percentage of remaining 22:6 fatty acids relative to the remaining saturated (non-oxidising) C17:0 fatty acid (heptadecanoic acid). The time course of oxidation of pure fish oil was effectively identical to that for fish oil + linoleic acid and is not plotted. The delayed oxidation of the soy PC-containing control oil phase, may well have been due to the additional - tocopherol present in the soy PC (over and above that in the fish oil).

EXAMPLE 5

A lyophilate of soy PC and ascorbic acid was prepared as described in Example 4 and then mixed with sunflower oil containing 2% w/w of added linoleic acid to form a clear dispersion containing 1.5mg ascorbic acid/g of oil phase. Oil phase controls were prepared comprising pure sunflower oil and also sunflower oil containing 2% linoleic acid, in the absence and presence of 2% w/w of soy PC. 50mg aliquots of the ascorbic acid dispersion and of the 3 oil phase controls, were incubated under conditions for accelerated lipid oxidation as described in Example 4, and were sampled periodically and analysed in the same way. In this case however, the 18 : 2 fatty acid (linoleic acid) content was monitored. Results are plotted in Figure 3. The time course of oxidation of the soy PC-containing control oil phase was effectively identical to the equivalent control lacking PC, and was not plotted. The reason for the accelerated oxidation of the oil controls containing free linoleic acid, compared with pure sunflower oil where the endogenous linoleic

acid is in a conjugated form, is not clear.

CLAIMS

1. An anti-oxidant composition comprising at least one anti-oxidant species solubilised in a hydrophobic solvent in which it would not normally be soluble.
2. A composition as claimed in claim 1 which is anhydrous.
3. A composition as claimed in claim 1 or claim 2 wherein the anti-oxidant species is solubilised in the hydrophobic solvent to form a single phase.
4. A composition as claimed in any one of claims 1 to 3 wherein the anti-oxidant species is ascorbic acid, phytic acid, pyrophosphate, EDTA, transferrin, ceruplasmin, metallothionein, albumin, haptoglobin, cysteine, glutathione, a conjugated bile pigment, uric acid, vanillic acid, vanillin or trolox.
5. A composition as claimed in claim 4 wherein the anti-oxidant species is ascorbic acid, cysteine, glutathione, a conjugated bile pigment or uric acid.
6. An anti-oxidant composition comprising a lipid soluble anti-oxidant species together with one or more other anti-oxidant species, solubilised in an hydrophobic solvent in which the one or more other anti-oxidant species would not normally be soluble.
7. A composition as claimed in claim 6 wherein the lipid soluble anti-oxidant species is vitamin E and the other anti-oxidant species is one which acts synergistically with vitamin E, resulting in enhanced



anti-oxidant activity.

8. A composition as claimed in claim 6 wherein the other anti-oxidant species is ascorbic acid, cysteine, glutathione, a conjugated bile pigment or uric acid.

9. A composition as claimed in any one of claims 6 to 8 which is anhydrous.

10. A process for the preparation of a single phase hydrophobic anti-oxidant preparation comprising at least one anti-oxidant species solubilised in a hydrophobic solvent in which it would not normally be soluble, the process comprising:

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(i) associating the anti-oxidant species with an amphiphile in a liquid medium such that in the liquid medium there is no chemical interaction between the amphiphile and the anti-oxidant species;

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(ii) removing the liquid medium to leave an array of amphiphilic head groups orientated towards the anti-oxidant species; and optionally

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(iii) providing a hydrophobic solvent around the anti-oxidant species/amphiphile array.

11. A process as claimed in claim 10 wherein the anti-oxidant is ascorbic acid, phytic acid, pyrophosphate, EDTA, transferrin, ceruplasmin, metallothionein, albumin, haptoglobin, cysteine, glutathione, a conjugated bile pigment, uric acid, vanillic acid, vanillin or trolox.

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12. A process as claimed in claim 11 wherein the anti-



oxidant is ascorbic acid, cysteine, glutathione, a conjugated bile pigment or uric acid.

5 13. A process as claimed in any one of claims 10 to 12 wherein vitamin E is also present in the preparation.

14. A process as claimed in claim 13 wherein vitamin E is added at either or both of stages (i) and (iii).

10 15. A process as claimed in any one of claims 10 to 14 wherein the amphiphile is a phospholipid.

15 16. A process as claimed in claim 15, wherein the phospholipid has a phosphatidyl choline head group.

17. A process as claimed in claim 16, 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.

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18. A process as claimed in any one of claims 10 to 17, wherein the hydrophobic solvent comprises a long chain fatty acid, a medium chain alcohol, a branched long chain alcohol, a monoglyceride, diglyceride, medium chain triglyceride or long chain triglyceride.

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19. A process as claimed in any one of claims 10 to 18, wherein the amphiphile comprises PC and the hydrophobic solvent is a triglyceride or wherein the amphiphile comprises lyso-PC and the hydrophobic solvent is oleic acid.

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20. A process as claimed in any one of claims 10 to 18,
wherein the anti-oxidant/amphiphile array is formed by
mixing the anti-oxidant with a dispersion of an
amphiphile in a hydrophilic solvent and removing the
5 hydrophilic solvent.

21. A process as claimed in claim 20, wherein the
hydrophilic solvent is water.

10 22. A process as claimed in claim 20 or claim 21,
wherein the amphiphile assembly comprises micelles,
unilamellar vesicles, multilamellar vesicles or a tubular
structure such as cochleate cylinders, hexagonal phase,
cubic phase or myelin type structures.

15 23. A process as claimed in any one of claims 20 to 22,
wherein the hydrophilic solvent is removed by
lyophilisation.

20 24. A process as claimed in any one of claims 10 to 20,
wherein the antioxidant/amphiphile array is formed by co-
solubilising the anti-oxidant and the amphiphile in a
common solvent and subsequently removing the common
solvent.

25 25. A process as claimed in any one of claims 10 to 19,
wherein the hydrophilic anti-oxidant/amphiphile array is
formed by emulsifying a solution of the amphiphile in a
hydrophobic solvent with a solution of the anti-oxidant
30 in a hydrophilic solvent to give an emulsion and removing
the hydrophobic solvent.

26. A process as claimed in claim 24 or claim 25,
wherein the weight ratio of amphiphile to hydrophilic



species is from about 1:1 to 50:1.

27. A process as claimed in claim 26, wherein the emulsion is water-in-oil emulsion.

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28. A process as claimed in claim 26 or claim 27, wherein the hydrophobic solvent is a low boiling point organic solvent such as diethyl ether.

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29. A single phase hydrophobic preparation of an anti-oxidant in a hydrophobic solvent, obtainable by a process as claimed in any one of claims 10 to 28.

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30. A single phase hydrophobic preparation as claimed in claim 29 which is anhydrous.

31. An array of amphiphilic head groups orientated towards an anti-oxidant species in a lyophilised form.

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32. A lyophilised array as claimed in claim 30 which is obtainable by a process as defined in any one of claims 10 to 28.

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33. A lyophilised array as claimed in claim 30 or claim 31 wherein the anti-oxidant species is ascorbic acid, cysteine, glutathione, a conjugated bile pigment or uric acid.

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34. A lyophilised array as claimed in claim 33 wherein the anti-oxidant species is ascorbic acid.

35. A two phase composition comprising a hydrophilic phase and a hydrophobic phase, wherein the hydrophobic phase comprises a composition as claimed in any one of



claims 1 to 8 or a preparation as claimed in claim 29.

5 36. A composition as claimed in claim 35, wherein the hydrophobic phase is dispersed in a continuous hydrophilic phase.

37. A composition as claimed in claim 35 or claim 36 which is an emulsion.

10 38. The use of an anti-oxidant composition as defined in any one of claims 1 to 8, of a lyophilised array as defined in any one of claims 31 to 34 or of a two phase composition as defined in any one of claims 35 to 37 in the preparation of a pharmaceutical or cosmetic
15 formulation or foodstuff.

39. A method for reducing oxidation of a pharmaceutical or cosmetic formulation or of a foodstuff which comprises the step of adding an anti-oxidant composition as defined
20 in any one of claims 1 to 8, of a lyophilised array as defined in any one of claims 31 to 34 or a two phase composition as defined in any one of claims 35 to 37 to the pharmaceutical or cosmetic formulation or to the foodstuff.

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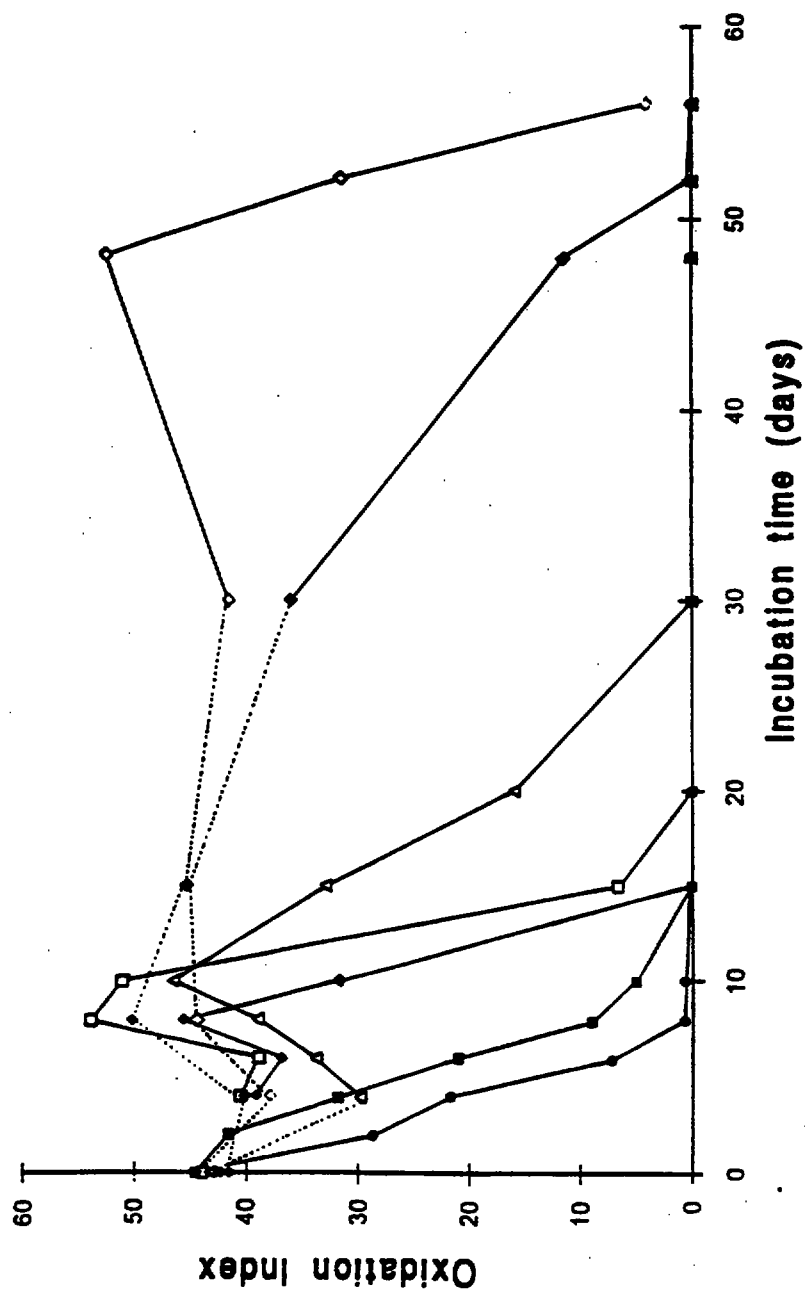
40. The use of an anti-oxidant composition as defined in any one of claims 1 to 8, of a lyophilised array as defined in any one of claims 31 to 34 or of a two phase composition as defined in any one of claims 35 to 37 in
30 the preparation of an anti-oxidation agent.

41. An anti-oxidant composition as defined in any one of claims 1 to 8, a lyophilised array as defined in any one of claims 31 to 34 or a two phase composition as defined

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FIG. 1.

Oxidation Experiment 1



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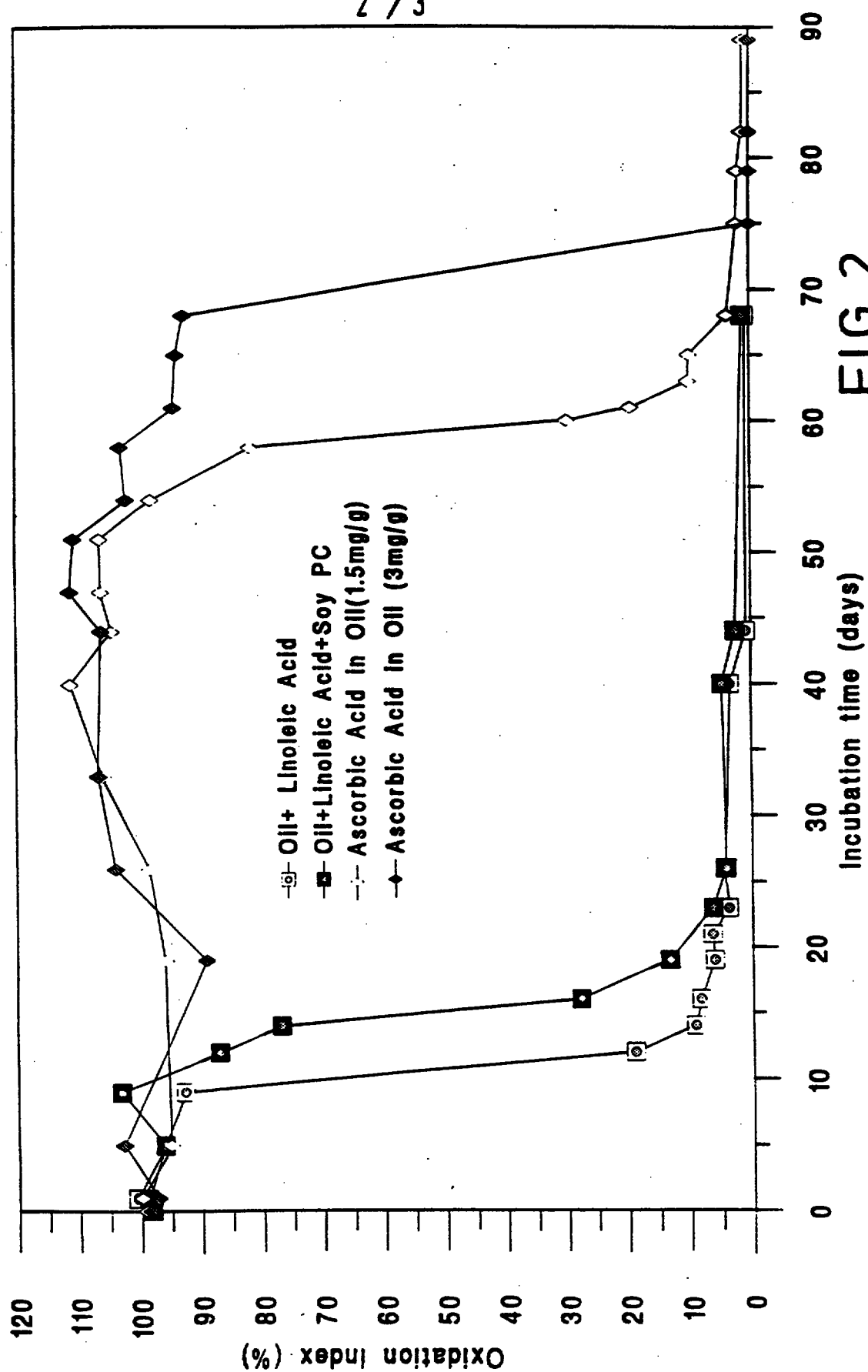
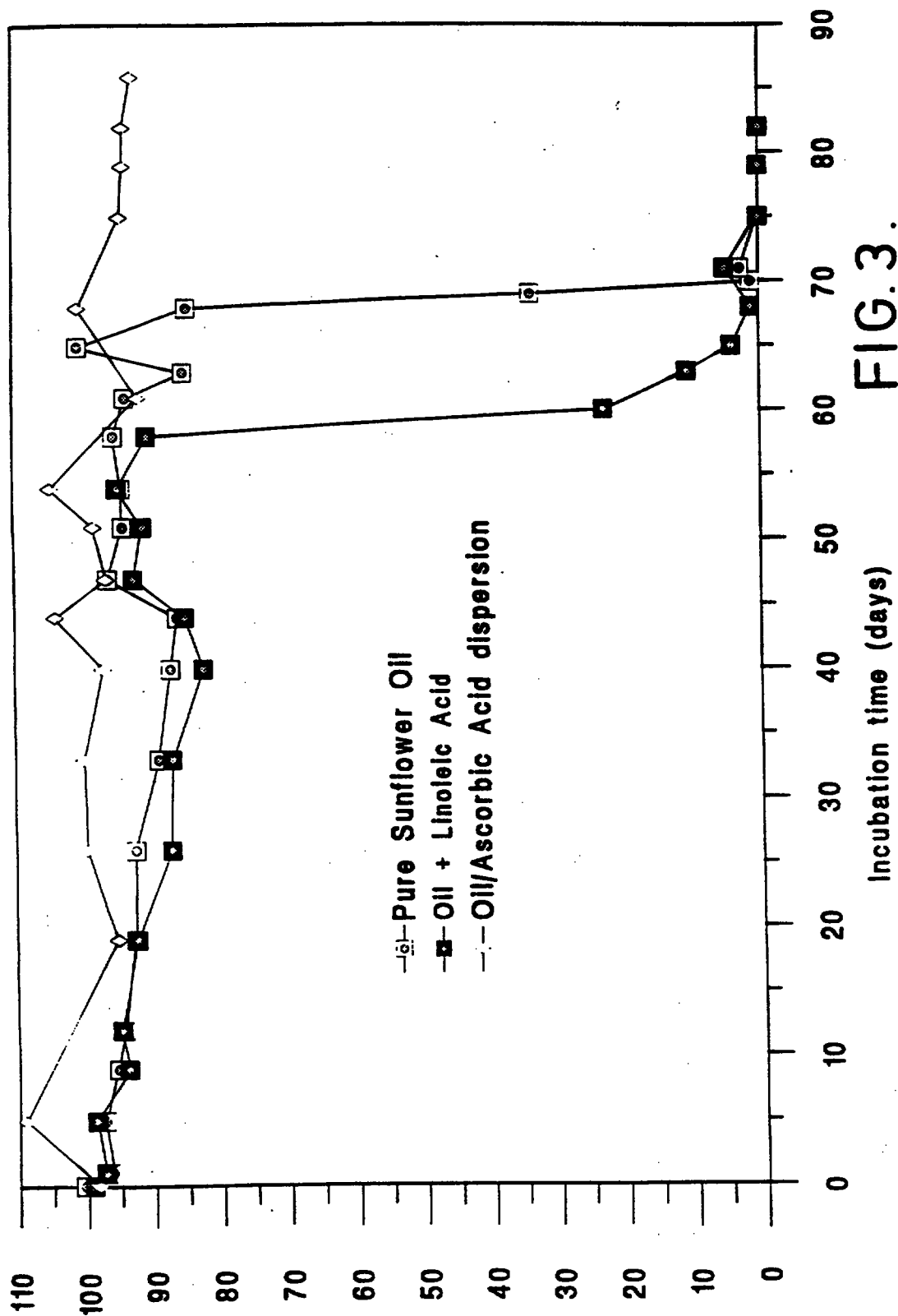


FIG.2.

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(21) International Application Number: PCT/GB95/02888 (22) International Filing Date: 8 December 1995 (08.12.95) (30) Priority Data: 9424908.3 9 December 1994 (09.12.94) 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): KIRBY, Christopher, John [GB/GB]; 95 Oxford Road, Wokingham, Berkshire RG11 2YL (GB). (74) Agents: CHAPMAN, Paul, William et al.; Kilburn & Strode, 30 John Street, London WC1N 2DD (GB).		(81) Designated States: AL, AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, 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, TT, UA, UG, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, 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), ARIPO patent (KE, LS, MW, SD, SZ, UG). Published <i>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.</i>
(54) Title: ANTI-OXIDANT COMPOSITIONS (57) Abstract An anti-oxidant composition is provided comprising at least one anti-oxidant species solubilized in a hydrophobic solvent in which it would not normally be soluble. Processes for preparing such a composition are also provided.		