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(54) Title: USES OF SPOROPOLLENIN

(57) Abstract: The invention provides the use of an exine shell of a naturally occurring spore, or a fragment thereof, as an antioxidant, for instance in a formulation containing an active substance. Also provided is a method for reducing rancidity, or other oxidative degradation, of a substance or composition, by encapsulating the substance or composition in, or chemically or physically binding it to, or mixing it with, an exine shell of a naturally occurring spore or a fragment thereof.

WO 2007/012856 A1

USES OF SPOROPOLLENIN

Field of the invention

This invention relates to new uses for exine shells of naturally occurring spores, as antioxidants and as delivery vehicles with antioxidant properties.

Background to the invention

Many active substances, for example pharmaceutically and nutraceutically active substances and food ingredients, are susceptible to oxidation on exposure to the air or to dissolved oxygen for instance in a humid or aqueous environment. For many, the oxidation process is induced by (ie, either initiated or accelerated by) exposure to UV radiation such as from natural daylight. Lipids such as oils tend to be particularly readily oxidised.

This susceptibility reduces the stability of the active substance and of any composition containing it. This can lead to reduced efficacy and/or to the generation of undesirable by-products which can for example spoil the flavour of a food product (eg, when fats and oils turn rancid), increase the toxicity of a drug formulation, compromise the appearance of a cosmetic product, cause operating problems for a fuel within an engine, or more generally impair performance during use.

Active substances can be protected from environmental influences such as oxygen and UV light by encapsulating them in suitable delivery vehicles. The preparation of such active-loaded delivery systems can often be complex, time consuming and expensive however. Problems can arise in ensuring that the encapsulating entities are sufficiently uniform in size and shape to ensure the resultant formulation meets quality control and regulatory standards and to provide homogeneity in active substance concentration. It can also be difficult to achieve adequately high active substance loadings in the encapsulating entities, without making those entities relatively large in size and in turn compromising the physical properties of the overall formulation.

It is moreover necessary to ensure that any encapsulated substances can be released to an adequate extent at the point of intended use. This is not always straightforward if the substance is also to be sufficiently well encapsulated as to protect it prior to use.

It is an object of the present invention to provide ways of formulating active substances which can improve their stability to oxidation and hence overcome or at least mitigate the above described problems.

Statements of the invention

According to a first aspect of the present invention there is provided the use of an exine shell of a naturally occurring spore, or a fragment thereof, as an antioxidant.

"Naturally occurring" means that the spore is produced by a living organism, whether prokaryote or eukaryote and whether plant or animal. The spore (which term includes pollen grains and also endospores of organisms such as bacteria) may for instance be derived from a plant, or in cases from a fungus, alga or bacterium or other micro-organism.

An exine shell of a spore is the outer coating from around the naturally occurring ("raw") spore. It can be isolated from the spore by successive treatments with organic solvents, alkali and acid so as to remove the other components of the spore such as the cellulosic intine layer and lipid, protein and nucleic acid components that may be attached to or contained within the exine shell. Enzymic methods have also been used to isolate exine coatings from spores.

The resulting exine shell, which takes the form of an essentially hollow capsule, typically contains sporopollenin, a substance which is known to be chemically and physically extremely stable (G. Shaw, "The Chemistry of Sporopollenin" in *Sporopollenin*, J. Brooks, M. Muir, P. Van Gijzel and G. Shaw (Eds), Academic Press, London and New York, 1971, 305-348) but which is also inert and non-toxic.

An exine shell may be obtained from a spore in known manner, for example by harsh treatment (eg, reflux) of the spore with a combination of organic solvent and strong acid and alkali. Suitable such methods are described for instance in WO-2005/000280 (see

page 10) and in the examples below. Other less severe methods may also be employed, for instance enzyme treatment (S. Gubatz, M. Rittscher, A. Meuter, A. Nagler, R. Wiermann, Grana, Suppl. 1 (1993) 12-17; K. Schultze Osthoff, R. Wiermann, J. Plant Physiol., 131 (1987) 5-15; F. Ahlers, J. Lambert, R. Wiermann, Z. Naturforsch., 54c (1999) 492-495; C. Jungfermann, F. Ahlers, M. Grote, S. Gubatz, S. Steuernagel, I. Thom, G. Wetzels and R. Wiermann, J. Plant Physiol., 151 (1997) 513-519). Alternatively, high pressure may be used to press out the internal contents of a spore through the naturally occurring pores in its outer exine layer. These methods may be used to remove proteins or carbohydrates to obtain the exine shell that retains the largely intact morphology of the original spore.

For *Lycopodium clavatum*, for example, the resultant exine shell may consist entirely or essentially of sporopollenin, optionally with a minor proportion of other materials such as chitin, glucans and/or mannans. The majority of the protein from the original spore will have been removed.

It is known from WO-2005/000280 to use spore-derived exine shells as delivery vehicles for drugs and dietetic substances. The active substance is either chemically or physically bound to, or encapsulated within, the exine shell. Reference is made in that document to the ability of the exine shells to provide a physical barrier between an encapsulated active substance and for example atmospheric oxygen, or against photolytic degradation.

However it has now surprisingly been found that an exine shell of a naturally occurring spore can itself act as an antioxidant to protect substances, in particular lipids and lipid-like substances, against oxidation. When oxygen is bubbled through an oil, for example, it rapidly becomes rancid as a result of oxidation processes. Yet when the oil is encapsulated within a spore-derived exine shell, it is much slower to oxidise, as shown in the examples below.

This antioxidant effect is not merely due to the shell providing a physical barrier against the ingress of oxygen, since it can be observed even when a substance is outside of, though ideally still in contact with or in a mixture with, the exine shell. Moreover it is already known that spore-derived exine shells are at least partially porous, having micropores through which lipids, proteins, nucleic acids and carbohydrates can be

removed during isolation of the exine layer and through which active substances may pass when impregnating the shells, as described in WO-2005/000280. These pores would be expected to allow ambient oxygen to contact any substance encapsulated within the shell, yet in fact it has been found that even if oxygen is able to pass through the pores, oxidation of the encapsulated substance is still inhibited.

It appears therefore that the exine shell itself, or at least its surface, is capable of acting as an antioxidant. Thus in the context of the present invention, use as an antioxidant means use to increase the inherent resistance of a substance or composition to oxidation. It is not intended to encompass mere physical protection against contact with oxygen, although such protection may accompany the antioxidant effect provided by the invention.

According to the present invention, the exine shell may be derived from any suitable naturally occurring spore, whether plant or animal in origin. In this context, the term "plant" is to be construed in its broadest sense, and embraces for example mosses, fungi, algae, gymnosperms, angiosperms and pteridosperms. Moreover the term "spore" is used to encompass not only true spores such as are produced by ferns, mosses and fungi, but also pollen grains, as are produced by seed-bearing plants (spermatophytes) and also endospores of organisms such as bacteria.

Suitable species from which such spores may be obtained include the following, the diameters of their spores being shown in the second column:

Bacillus subtilis	1.2 μm
Myosotis ("forget-me-not")	2.4 – 5 μm
Aspergillus niger	4 μm
Penicillium	3 – 5 μm
Cantharellus minor	4 – 6 μm
Ganomerma	5 – 6.5 μm
Agrocybe	10 – 14 μm
Urtica dioica	10 – 12 μm

Periconia	16 – 18 μm
Epicoccum	20 μm
Lycopodium clavatum	25 μm
Lycopodium clavatum	40 μm
Abies	125 μm
Cucurbitapapo	200 μm
Cuburbita	250 μm.

The exine shell used in the present invention may be of a type described in WO-2005/000280, in particular at pages 4, 8 and 9 and in Example 1. Other spores from which exine shells can be extracted are disclosed in the publications referred to at page 8 of WO-2005/000280.

According to the invention, the exine shell may be used to increase the oxidative stability of a substance to which it is added or a composition (which includes a formulation) in which it is used. It may therefore be used to reduce the oxidation rate of the substance or composition.

The exine shell may be used to protect a substance or composition against UV-induced oxidation. Again this is not intended to mean mere physical protection (ie, screening) from UV radiation. Rather, it has been found that exine shells of naturally occurring spores can be capable of reducing the tendency of a substance to oxidise in the presence of UV radiation, even though the inherent sun screening capability of such shells has been found to be relatively low. (For example, the sporopollenin of exine shells from *Lycopodium clavatum* and *Ambrosia trifida* has an approximately flat spectrum over the wavelength range 190-900 nm, the absolute value of the extinction coefficient being 1-2 x 105 m^{-1} and not significantly different for the two types of shells. A single exine shell transmits approximately 45 % of light at 450 nm. The transmission of wavelengths between 190 and 900 nm is similar (Stephen L. Atkin, Sylvain Barrier, Zhengang Cui, Paul D.I. Fletcher, Grahame Mackenzie and Vincent Panel, unpublished work).)

In accordance with the invention, the exine shell will suitably be used as an antioxidant in a formulation containing an active substance. The active substance may be

encapsulated within, or chemically or physically bound to, the exine shell. Thus the exine shell may be used as a delivery vehicle for a substance. Alternatively the active substance and exine shell may be present as a simple physical mixture in the formulation.

Because of its inherent non-toxicity, a spore-derived exine shell can be particularly suitable for use as a delivery vehicle in the context of formulations which are likely to come into contact with, or be ingested by, the human or animal body. The proteinaceous materials which can otherwise cause allergic reactions to pollens are preferably removed during the processes used to isolate the exine component.

Sporopollenin, a major component of many exine shells, is one of the most resistant naturally occurring organic materials known to man, and can survive very harsh conditions of pressure, temperature and pH as well as being insoluble in most organic solvents. This too makes it highly suitable for use as a delivery vehicle.

Further potential advantages to using spore-derived exine shells as delivery vehicles, in addition to their antioxidant activity, include:

- The shells can be very effective at protecting an encapsulated substance from atmospheric effects, in particular from light and/or oxygen, and therefore from premature degradation.

- The physical protection they provide can help reduce loss of the substance by for instance evaporation, diffusion or leaching.

- The exine shells prepared from any given organism are very uniform in size, shape and surface properties, unlike typical synthetic encapsulating entities.

- There is however significant variation in spore size and shape between different species, allowing a formulation to be tailored dependent on the nature and desired concentration of the active substance, the site and manner of its intended application, the desired active release rate, the likely storage conditions prior to use, etc...

- It can also be possible to encapsulate relatively high quantities of an active substance within even a small exine shell. The combination of high active loadings, small encapsulant size and adequate protective encapsulation is something which can be difficult to achieve using other known encapsulation techniques.

- The ready, and often inexpensive, availability of spore exines, together with their natural origin, also make them highly suitable candidates for active substance delivery vehicles.

Further advantages associated with the use of spore-derived exine shells may be as described in WO-2005/000280, for example at pages 3 and 4 and in the paragraph spanning pages 5 and 6.

Where the exine shell is used in a formulation containing an active substance, the formulation may be suitable and/or adapted and/or intended for delivery in any manner. For example where the active substance is for systemic use (such as a pharmaceutically or nutraceutically active substance, a foodstuff or any other active substance intended to be taken into a living body, whether plant or animal), the formulation may be suitable and/or adapted and/or intended for oral, intravenous, pulmonary, nasal, transdermal, subcutaneous, buccal, intraperitoneal or any other suitable form of delivery.

The formulation may be for suitable and/or adapted and/or intended for topical delivery of an active substance to a surface, in which case the surface may be a living surface (again, either plant or animal) or an inanimate surface. The ability of the exine shell to act as an antioxidant, and not merely as a physical barrier protecting an encapsulated active substance, can be of particular significance in this context, since on release of the active substance onto a surface, the substance will then be exposed on the outside of the exine shell, yet can continue to benefit from a degree of oxidative protection.

In the context of the present invention a "topical" formulation may be suitable and/or adapted and/or intended for topical application to areas of a living body such as the skin or other epithelia, the hair, the nails or the teeth, in particular to the skin. A living surface may be either plant or animal, in particular animal, and in the case of an animal surface may either be human or non-human, in particular human.

The formulation in which the exine shell is used may have any suitable physical form. Exine shells may be present in suspension in a suitable liquid (the term "suspension" including emulsions and other multi-phase dispersions), or as a solid such as a powder or tablet. A formulation suitable for topical delivery may take the form of a lotion, cream, ointment, paste, gel, foam or any other physical form known for topical administration, including for instance a formulation which is, or may be, applied to a carrier such as a sponge, swab, brush, tissue, skin patch, dressing or dental fibre to facilitate its topical administration. It may take the form of a nasal spray or of eye or ear drops. Alternatively a topical formulation may take the form of a powder, for example when the active substance is a makeup product such as a blusher, eye shadow or foundation colour, or when it is intended for use in a dusting powder. Exine shells can be extremely efficient at absorbing liquids, in particular lipids, to result in an effectively dry product with all of the liquid encapsulated within the shells, as demonstrated in Example 11 below. Other active substances, for example food supplements or ingredients, or pharmaceutically or nutraceutically active substances, may also be formulated as powders.

Other suitable pharmaceutical and dietetic dosage forms are those disclosed in WO-2005/000280, for instance at pages 3 and 6 to 9.

An active substance may be any substance capable of producing an effect at the site of application. It may for example be selected from pharmaceutically and nutraceutically active substances, foods and food ingredients, food supplements, herbicides, pesticides and pest control agents, plant treatment agents such as growth regulators, antimicrobially active substances, cosmetics (including fragrances), toiletries, disinfectants, detergents and other cleaning agents, adhesives, diagnostic agents, dyes and inks, fuels, explosives, propellants and photographic materials. In general, the present invention may be used to stabilise any active substance, including for example oligomeric or polymeric active substances.

In one embodiment of the invention, the active substance is a cosmetic substance. A cosmetic substance may for example be selected from makeup products (for example foundations, powders, blushers, eye shadows, eye and lip liners, lipsticks, other skin colourings and skin paints), skin care products (for example cleansers, moisturisers,

emollients, skin tonics and fresheners, exfoliating agents and rough skin removers), fragrances, perfume products, sunscreens and other UV protective agents, self tanning agents, after-sun agents, anti-ageing agents and anti-wrinkle agents, skin lightening agents, topical insect repellants, hair removing agents, hair restoring agents and nail care products such as nail polishes or polish removers. A perfume product may comprise more than one fragrance.

In another embodiment of the invention, the active substance may be for use in a toiletry product. It may therefore be selected from soaps; detergents and other surfactants; deodorants and anti-perspirants; lubricants; fragrances; perfume products; dusting powders and talcum powders; hair care products such as shampoos, conditioners and hair dyes; and oral and dental care products such as toothpastes, mouth washes and breath fresheners.

In yet another embodiment of the invention, the active substance is for use in a household product. It may for example be selected from disinfectants and other antimicrobial agents, fragrances, perfume products, air fresheners, insect and other pest repellants, pesticides, laundry products (eg, washing and conditioning agents), fabric treatment agents (including dyes), cleaning agents, UV protective agents, paints and varnishes.

In a further embodiment of the invention, the active substance is a pharmaceutically or nutraceutically active substance, which includes substances for veterinary use. Pharmaceutically active substances suitable for topical delivery may for example be selected from substances for use in treating skin or skin structure conditions (for example acne, psoriasis or eczema), wound or burn healing agents, anti-inflammatory agents, anti-irritants, antimicrobial agents (which can include antifungal and antibacterial agents), vitamins, vasodilators, topically effective antibiotics and antiseptics.

A pharmaceutically or nutraceutically active substance may be suitable and/or intended for either therapeutic or prophylactic use.

In yet another embodiment of the invention, the active substance is a foodstuff, which includes food ingredients. Food ingredients may include for example food supplements

(such as vitamins and minerals, folic acid, omega-3 oils or fibre), flavourings, fragrances, colourings, preservatives, stabilisers, emulsifiers or agents for altering the texture or consistency of a food product.

In particular the active substance may be selected from pharmaceutically and nutraceutically active substances, foodstuffs and cosmetic and toiletry substances.

In one embodiment of the invention, the active substance may be intended and/or adapted and/or suitable for topical delivery, in which case it is preferably not a substance which is intended for and/or capable of systemic use (in particular by transdermal delivery). Suitably such a substance is not intended and/or adapted and/or suitable for ingestion, in particular by humans.

In certain cases it may be suitable for the active substance to be a substance other than an essential oil, or at least for it not to be an essential oil which is intended and/or suitable for systemic use in a living body.

In some cases it may be suitable for the active substance to be a substance other than a drug (at least a drug which is intended and/or suitable for systemic delivery) or a dietetic substance.

In some cases it may be suitable for the active substance to be a substance other than a vitamin, a mineral, an essential oil, a food flavouring and/or a nutraceutical.

The active substance may comprise a volatile substance, in particular a fragrance. The present invention can be particularly suitable for formulations containing such substances as the exine shell can help to inhibit release of any volatile components prior to use. This is also not necessarily predictable, bearing in mind that exine shells of naturally occurring spores are known to be porous. Nevertheless, they can in cases be capable of encapsulating volatile actives and reducing their loss to the atmosphere, as shown in Example 10 below.

The active substance may be a lipid or lipid-like substance (for example, an oil, fat or wax), and/or it may be lipophilic. It may be present in a secondary fluid vehicle such as a liquid vehicle, in particular a non-aqueous (or essentially or at least partially non-aqueous) vehicle, more particularly a non-aqueous vehicle and yet more particularly a

lipid vehicle, such as an oil. The active substance may therefore be present in the form of a solution or suspension, the term "suspension" including emulsions and other multi-phase dispersions. A secondary vehicle may for example be a water-in-oil or oil-in-water-in-oil emulsion.

The active substance may itself be a naturally occurring substance or derived from a natural source, in particular a plant source.

In some cases the active substance may be non-polar.

The active substance may be sensitive to one or more external influences such as heat, light, oxygen or water. In particular it may be susceptible to oxidation, in particular from atmospheric or dissolved oxygen. It may be susceptible to UV-induced oxidation (ie, photochemical oxidation), more particularly under ambient conditions.

The formulation containing the active substance may be suitable and/or adapted and/or intended for storage and/or use in a fluid environment which contains oxygen, for instance in air or in a liquid such as water which contains dissolved oxygen. It may be suitable and/or adapted and/or intended for storage and/or use in an environment which is or may be exposed to UV radiation, in particular from sunlight. In these contexts the present invention can be particularly effective in protecting the active substance against oxidation and therefore in increasing the stability of the overall formulation.

A formulation in which the present invention is used may contain more than one active substance. Two or more such substances may for example be co-encapsulated in the same exine shell. Instead or in addition, a formulation prepared according to the invention may comprise two or more populations of active substance-containing exine shells, each chemically or physically bound to, or encapsulating, a different active substance.

Thus for example, a cosmetic formulation prepared according to the invention might contain both a sunscreen and an insect repellant, or a sunscreen and a moisturiser, or a foundation or other skin colouring agent and a sunscreen. Two or more active substances may therefore benefit from the antioxidant protection afforded by the exine shell(s).

This can also enable two or more active substances to be kept separate prior to use – of value for example if they are incompatible with one another or would interact in an undesirable manner – and then released together *in situ* at the intended point of use.

A formulation prepared according to the invention may be contained in a product, which may for example be selected from cosmetic products; toiletries (eg, bath products, soaps and personal care products); hair care products; nail care products; dental products such as toothpastes, mouth washes and dental flosses; household products (whether for internal or external use) such as surface cleaners, disinfectants, air fresheners, pest repellants and laundry and fabric treatment products; paints, inks, dyes and other colouring products; adhesive products; pharmaceutical and nutraceutical products; food products, including food additives and food ingredients; agricultural and horticultural products; fuels; explosives; propellants; and photographic materials.

Such a product may be suitable and/or adapted and/or intended for delivery by any suitable route, including for instance by topical application.

In particular such a product may be selected from cosmetic products (which includes skin care products), toiletries, hair and nail care products and dental products.

In another embodiment of the invention, the product is a pharmaceutical or nutraceutical product, which in both contexts includes products for veterinary use.

In yet another embodiment, the product is a food product.

In a formulation prepared according to the invention, the active substance may be chemically or physically bound to, or encapsulated within, the exine shell. Suitably it is either physically bound to or encapsulated within the exine shell. More suitably it is at least partially encapsulated within the shell.

Suitable ways in which a substance may be chemically bound to an exine shell are described in WO-2005/000280, for example in the paragraph spanning pages 4 and 5, and at pages 14 to 22 and 24 to 32. They may involve chemical derivatisation of the exine shell so as to facilitate its chemical binding to the substance in question. Chemical binding may encompass covalent or other forms of chemical bond, for example hydrogen bonds, sulphide linkages, Van der Waals bonds or dative bonds.

Physical binding of an active substance to an exine shell may include for example adsorption (eg, involving hydrophobic/hydrophilic interactions) of the substance onto a surface (whether internal or external) of the shell.

Encapsulation of an active substance means that the substance is retained within the cavities that are inherently present in the exine shell wall and/or within the central cavity defined by the exine shell.

An active substance may be attached to an exine shell by more than one of the above described means; for example, it may be encapsulated within the shell and also chemically bound to it, or a portion of the substance may be adsorbed onto the outer surface of the shell whilst another portion is contained inside the shell.

In a formulation prepared according to the invention, the exine shell may have a diameter (which may be determined by scanning electron microscopy) of from 1 to 300 μm, suitably from 1 to 250 μm or from 3 to 50 μm or from 15 to 40 μm. Grass pollen-derived exines, and other exine shells of approximately 20 μm diameter, might also be expected to be suitable.

The preferred exine particle size may depend on the intended mode of administration of the relevant active substance or formulation. For example, for pulmonary delivery relatively small particles (for instance of diameter 10 μm or less, or 8 or 5 μm or less for nasal delivery or less than 5 μm for delivery into the lungs) may be preferred. For oral delivery, particle sizes of less than 25 μm may be suitable. However, if the active substance is intended for delivery to the gut (for example certain nutraceuticals, such as probiotics) then particle sizes of 40 μm or greater may be suitable. In general, for active substances intended for systemic use, particle sizes of 25 μm or less may be preferred because of their ready ability to pass into the bloodstream.

In some cases larger exine shells, for example of 30 or 40 μm diameter or greater, may be particularly suited as topical delivery agents as they are less likely to be persorbed into the bloodstream. If however a topically delivered active substance is required to penetrate the hair follicles, then smaller exine shells – for example of 7 μm or less, or 5 or 3 μm or less, or ideally 2 μm or less such as from 1 to 2 μm – may be suitable.

Larger exine shells may have the advantage of allowing higher active substance loadings, but may compromise the texture and/or appearance of the overall formulation, which for oral or topical delivery may be of significance. Thus in such contexts, in particular for oral delivery of for example pharmaceutical or nutraceutical substances or foods, it may be suitable for the exine shells to have a diameter of 10 μm or less. Moreover when using larger shells, an associated active substance may be less homogeneously distributed throughout a formulation than when associated with a larger number of smaller shells. In general a minimum diameter of 4 μm might be preferable so as to be able to achieve reasonable active substance loadings. However there may be cases where the minimum diameter is suitably 60 μm or even more.

In one embodiment of the invention, the exine shell may additionally contain all or part of the cellulose intine layer from the naturally occurring spore. This can be achieved if the spore is subjected to treatment with only organic solvent and alkali, and not with acid. Such base hydrolysis, for instance using potassium hydroxide, can ensure that proteinaceous components of the spore are removed, yet can allow at least a proportion of the original cellulosic intine to survive.

In one embodiment of the invention, the exine shell may be intact or substantially so. In other words, apart from the micro- or nanopores which are naturally present in the surfaces of such shells, it will provide a continuous outer wall defining an inner cavity into which an active substance can be loaded. The exine shell may however be broken or damaged in parts; the invention thus embraces the use of a fragment of a spore-derived exine shell, in particular in the case where an active substance is chemically or physically bound to the exine shell. Suitably however the exine shell is continuous over at least 50 %, suitably at least 75 or 80 or 90 %, of the surface area which an exine shell from the relevant species would have if intact. Thus in many cases, the present invention relates to the use of an exine shell of a naturally occurring spore rather than to a fragment of such a shell.

The exine shell may be chemically modified, either to alter its properties (for example its solubility) or to target it to an intended site of administration (for example, to render it more surface-active), or to facilitate its attachment to an active substance. Suitable such chemical modifications, and methods for achieving them, are described in WO-

2005/000280, in particular in the paragraph spanning pages 4 and 5 and at pages 14 to 22 and 24 to 32. The outside of the exine shell may for instance be modified by the (typically chemical) attachment of functional groups such as cationic and/or anionic groups (see WO-2005/000280 and also G. Shaw, M. Sykes, R.W. Humble, G. Mackenzie, D. Marsdan & E. Phelivan, *Reactive Polymers*, 1988, 9, 211-217), and/or functional groups which increase the affinity of the shell for a surface to which it is intended to be applied.

An active substance may be attached to, or encapsulated within, an exine shell using known techniques, again suitably as described in WO-2005/000280. In particular an exine shell may be impregnated with an active substance by immersing the shell in the active substance or a solution or suspension thereof. One or more penetration enhancing agents may be used, again as described in WO-2005/000280, to aid impregnation of the shell by the active substance. A reduced or increased pressure (with respect to atmospheric pressure) may instead or in addition be used to facilitate impregnation.

The exine shell may be loaded with any suitable quantity of the active substance, depending on the context of intended use. A formulation prepared according to the invention may for example contain the active substance and exine shells at an active substance:exine shell weight ratio of from 0.01:1 to 35:1 or 33:1, such as from 0.01 to 20:1 or from 0.1:1 to 12:1 or 8:1 or 5:1, or from 0.5:1 to 5:1, or from 1:1 to 5:1 or 8:1. The loading achievable may depend on the size of the exine shell, if at least partial encapsulation of the active substance is intended.

In accordance with the invention, a formulation may contain a proportion of an active substance which is not encapsulated within the inner cavity of the exine shell, since the exine shell may still contribute an antioxidant effect even when the substance is outside the shell. Thus relatively high active substance concentrations may be used in the formulation, for example active substance:exine shell weight ratios in excess of 5:1 or 10:1 or even 20:1 or 30:1. Again the maximum suitable loading may depend on the size of the exine shell.

In cases, the weight ratio of the active substance to the exine shell in the formulation is such that the exine shell is filled with the active substance (or with an active substance-

containing solution or suspension) and in addition there is a coating of 10 μm thickness or less of the active substance/solution/suspension around the outer surface of the shell. This situation may represent a suitable maximum for the active substance to exine shell weight ratio, allowing a reasonable level of antioxidant effect from the exine shell.

The exine shell may be coated with a barrier layer for further protection of an associated active substance against atmospheric effects. This may be of particular use for the delivery of volatile active substances, and/or oxygen sensitive substances. Suitable coatings are solid or semi-solid under the normal storage conditions for the formulation (typically at room temperature) but may melt at a higher temperature (for instance, skin temperature) at which they are intended to be topically applied. Lipid coatings may be suitable for use in this way, examples including butters and other solid fats (eg, cocoa butter or hardened palm kernel oil), oils (eg, cod liver oil) and waxes (eg, carnuba wax or beeswax). In particular if an active substance is intended for topical delivery, the coating may be a material which melts at or around skin temperature (cocoa butter is an example of such a material), and can therefore allow release of the active substance on topical application to the skin. Other potential coatings may be materials which can rupture on application of manual pressure, for example brittle solids such as shellac, or other materials which melt, break or otherwise change on administration (eg, topically) so as to allow release of the active substance. Gelatin may for example be a suitable coating material.

Other known coating excipients may be chosen depending on the desired delivery route and intended site of action (for example, coatings may be used to delay, target or otherwise control release of an active substance). Various natural or synthetic coating excipients, including oligomers and polymers, may be used to protect the active substance in a formulation prepared according to the invention. Vegetable-derived coating materials may be preferred.

Coatings may be applied to exine shells in known fashion, for instance by spraying, rolling, panning or dipping. Coatings do not necessarily have to be continuous around the entire outer surfaces of the shells.

A second aspect of the present invention provides the use of an exine shell of a naturally occurring spore, or a fragment thereof, for the purpose of removing a previously formed oxidation product from a substance or composition. In other words, it has been found possible to "clean up" a substance or composition which has already undergone a degree of oxidation, removing at least some of the products of that oxidation and thereby improving the effective stability of the substance or composition. For example, where the substance is a lipid such as an oil, and has already turned at least partially rancid, an exine shell of a spore may be added to that substance, and/or may be loaded with that substance, in order to reduce its rancidity.

The second aspect of the invention therefore embraces a method for reducing rancidity, or other oxidative degradation, of a substance or composition, the method involving encapsulating the substance or composition in, or chemically or physically binding it to, or in cases mixing it with, an exine shell of a naturally occurring spore or a fragment thereof.

Thus the second aspect of the invention may be carried out for example by encapsulating an at least partially oxidised active substance or composition in an exine shell of a naturally occurring spore, or chemically or physically binding the active substance or composition to the exine shell or fragment. On subsequent separation of the substance or composition from the exine shell, for example on delivery to an intended site of action, it may then be found to be less oxidised than prior to being associated with the exine shell. The degree of oxidation in such cases may be measured in any suitable manner, for instance by assessing the level of by-products of oxidation reactions (peroxides, for example, and/or acids) in the active substance or composition. In the case of a lipid active substance, the degree of oxidation will typically equate to the level of rancidity.

In an embodiment of the second aspect of the invention, it may be preferred for the exine shell or fragment to contain all or part of the cellulose intine layer from the naturally occurring spore. This can be achieved, as described above, if the spore is subjected to treatment with only organic solvent and alkali, and not with acid.

It may be suitable, in accordance with the second aspect of the invention, for the exine shell to have a particle diameter of from 20 to 60 μm or from 20 to 50 or 30 to 50 μm, such as around 40 μm. The exine shell may be derived from *Lycopodium clavatum* spores.

A third aspect of the invention provides a method for protecting an active substance from oxidation, and/or for increasing the stability of the active substance or of a composition containing it, the method involving formulating the active substance with an exine shell of a naturally occurring spore or a fragment thereof. Suitably the active substance is encapsulated within, or chemically or physically bound to, the exine shell or fragment.

A fourth aspect of the invention provides a method for formulating an active substance, the method involving (a) preparing or providing an exine shell of a naturally occurring spore or a fragment thereof; and (b) encapsulating the active substance in the shell, or chemically or physically binding the active substance to the shell or fragment, for the purpose of providing an antioxidant effect in the resultant formulation.

A fifth aspect of the invention provides an exine shell of a naturally occurring spore, or a fragment thereof, for use as a pharmaceutically active antioxidant.

A sixth aspect provides the use of an exine shell of a naturally occurring spore, or a fragment thereof, in the manufacture of a medicament for delivering an antioxidant to a human or animal body.

A seventh aspect provides a method of treatment of a human or animal patient in need of an antioxidant, the method involving administering to the patient a therapeutically (which includes prophylactically) effective amount of an exine shell of a naturally occurring spore or a fragment thereof.

In accordance with the fifth to the seventh aspects of the invention, the exine shell or fragment may be administered together with another pharmaceutically active substance, for example a lipid lowering agent such as a statin. It may be used to treat any condition which is caused or exacerbated by oxidative stress, for example cardiovascular disease. It may be used to treat any condition which is caused or exacerbated by the generation in

the body of free radicals and/or other oxidants, for example from ingested materials; it may thus be used for instance to reduce inflammation and/or to treat cancers such as bowel cancer.

Since a spore-derived exine shell may be resistant to high levels of moisture, acid, alkali and heat, it will not typically dissolve or decompose in the mouth. It is also tasteless. It can therefore be used to mask the flavour of an active substance, for example a pharmaceutical or nutraceutical, a foodstuff or a food supplement.

Thus an eighth aspect of the invention provides the use of an exine shell of a naturally occurring spore, or a fragment thereof, as a taste masking agent. Again the exine shell or fragment will suitably be used as part of a formulation containing an active substance, in order to mask, at least partially, the flavour of the active substance. The formulation may be suitable and/or adapted and/or intended for oral delivery, or for delivery by any other route which might result in a perception of flavour for a patient to whom it was administered.

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", mean "including but not limited to", and do not exclude other moieties, additives, components, integers or steps.

Throughout the description and claims of this specification, the singular encompasses the plural unless the context otherwise requires. In particular, where the indefinite article is used, the specification is to be understood as contemplating plurality as well as singularity, unless the context requires otherwise.

Preferred features of each aspect of the invention may be as described in connection with any of the other aspects.

Other features of the present invention will become apparent from the following examples. Generally speaking the invention extends to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims and drawings). Thus features, integers, characteristics, compounds, chemical moieties or groups described in conjunction with a particular aspect, embodiment or

example of the invention are to be understood to be applicable to any other aspect, embodiment or example described herein unless incompatible therewith.

Moreover unless stated otherwise, any feature disclosed herein may be replaced by an alternative feature serving the same or a similar purpose.

The present invention will now be described by means of the following non-limiting examples.

Examples

The following experiments demonstrate the ability of spore-derived exine shells to act as natural antioxidants, in particular to reduce the oxidation rates of oils. This in turn shows their suitability for use as delivery vehicles for oxygen-sensitive active substances, in particular lipids, and for increasing the stability of such substances prior to, during and after administration.

The exine shells used were extracted from the spores of *Lycopodium clavatum* L. (common club moss), which can be purchased for example from Unikem, Post Apple Scientific, Fluka and Tibrewala International. Both 25 and 40 μm spores were tested, the 40 μm being derived from a sub-species or genetic variant of the plant. The former have a reticulated outer surface whilst the latter appear smoother and rounder. Both are believed to have an exine shell approximately 1.5 μm thick.

The exine shells were isolated from other components present in the spores (in particular the proteinaceous components) using the extraction procedures described below. Samples designated "AHS" were subjected to acid hydrolysis with phosphoric acid following base hydrolysis with potassium hydroxide, whereas those designated "BHS" were subjected only to base hydrolysis with potassium hydroxide. The BHS samples therefore comprised not only the exine shell but also a proportion of the cellulosic intine layer.

Firstly, the raw spores were suspended in acetone and stirred under reflux for 4 hours. For this, 250 g of the spores were dissolved in 750 ml of acetone, and refluxed for 4 hours in a 2 litre round bottomed flask fitted with two double surface Liebigs condensers

(20 cm – 4 cm). The resultant defatted spores (DFS) were then filtered (porosity grade 3) and dried overnight in air.

To produce the base-hydrolysed (BHS) exines, the defatted spores (DFS) were suspended in 6 % w/v aqueous potassium hydroxide and stirred under reflux (conditions as described above) for 6 hours. After filtration (porosity grade 3), this operation was repeated with a fresh sample of the potassium hydroxide solution. Again the suspension was filtered (grade 3) and the resultant solid washed with hot water (three times) and hot ethanol (twice). It was then refluxed in ethanol (conditions as described above) for 2 hours, filtered (grade 3) and dried overnight in air. Subsequently it was thoroughly dried in an oven at 60 °C.

To produce the acid-hydrolysed (AHS) exines, the defatted spores were suspended in 85 % v/v *ortho*-phosphoric acid (750 ml), and stirred under reflux (conditions as described above) for 7 days. The solid was then filtered (porosity grade 3), washed with water (5 times, 250 ml), acetone (5 times, 250 ml), ethanol (once, 250 ml), 2M sodium hydroxide (once, 250 ml), water (5 times, 250 ml), acetone (once, 300 ml) and ethanol (once, 300 ml). It was then dried in an oven at 60 °C.

Both the BHS and the AHS products contained essentially no nitrogen (assessed by combustion elemental analysis and by IR spectroscopy), indicating removal of proteins and nucleic acids and hence potentially allergenic components of the original spores. They were observed by scanning electron microscope and confocal electron microscopy to be essentially hollow capsules, free of the original inner sporoplasm.

Unless otherwise stated, the exine shells were loaded with oil using the following procedure. The oil was heated to between 40 and 60 °C and mixed with a few drops of ethanol. The relevant exine shells were then added to the resulting emulsion to form a homogeneous mixture. This was subjected to vacuum (30 hPa) for 1 to 2 hours.

Example 1 – Stability to UV light (1)

This example used 25 μm AHS exine shells loaded with either sunflower, rapeseed or soybean oil at an oil:exine weight ratio of 1:1.

The exine shells were loaded with the relevant oil using the procedure outlined above. Each sample was then spread out on a sheet of paper and irradiated with UV light for 2 hours, using a Philips™ Original Home Solaria type HB 171/A, 220-230 volt, 50 Hz, 75 watts, with four Philips™ CLEO 15 W UV type 30 bulbs. The lamp was held at a distance of 13 cm from the samples.

As controls, unloaded exine samples were subjected to the same treatment.

Following irradiation, the peroxide value (PV) of each sample was determined by titration. For this, the sample was dissolved by stirring in chloroform (10 ml), and acetic acid (15 ml) was added together with a saturated aqueous potassium iodide solution (1 ml). This mixture was shaken in a stoppered flask for 1 minute and set aside, away from the light, for exactly 5 minutes at room temperature. It was then diluted with 75 ml of distilled water and titrated against aqueous sodium thiosulphate (0.01 N), using starch solution as indicator. From this the peroxide value, which is a measure of the amount of active oxygen contained in the sample, could be calculated – degradation of the fat by oxygen generates peroxides, which when treated as described above yield molecular iodine, which is detectable by its reaction with starch to generate colourless sodium iodide. PVs were therefore determined using a standard procedure (IUPAC method 2.500).

The peroxide value of a lipid sample provides an indication of the extent to which the lipid has been degraded to peroxides, and hence of its rancidity. The higher the peroxide value, the more rancid the lipid, and thus the greater the degree of oxidation which it has undergone.

The results are shown in Table 1 below.

Table 1

Oil	***Loaded/unloaded exine sample***	***Exposure to UV (hours)***	***Peroxide value (PV) (meq/kg)***
Sunflower	Unloaded	0	25.3
	Unloaded	2	31.2
	Loaded	2	24.9
	Loaded	2	27.7

Rapeseed	Unloaded	0	5.4
	Unloaded	0	5.0
	Unloaded	2	36.4
	Loaded	0	8.2
	Loaded	2	5.7
Soybean	Unloaded	0	10.2
	Unloaded	2	20.6
	Loaded	2	12.2

The Table 1 results show that encapsulation of the oils in the exine shells significantly reduces their oxidation rate on exposure to UV light. This makes the exine shells highly suitable for use as vehicles for oxygen- and/or UV-sensitive substances, in particular lipids, which can then be protected against oxidation during their storage prior to use.

Example 2 – Stability to UV light (2)

Duplicate samples were prepared in which echium oil (0.5 g) was added to 25 μm AHS exine shells (0.125 g) to form a homogeneous mixture with an oil:exine weight ratio of 4:1. Unlike in Example 1, the mixture was not subjected to vacuum in order to impregnate the shells with the oil; the oil and exine shells were therefore present as a simply physical mixture, with the majority of the oil outside of the shells.

The samples were irradiated with UV light, and their peroxide values determined both before and after irradiation, as described in Example 1. Again, neat echium oil was used as a control.

The results are shown in Table 2.

Table 2

Oil:exine weight ratio	***Exposure to UV (hours)***	***Peroxide value (meq/kg)***
1:0	0	9.0
1:0	0	8.5
1:0	2	110.1
1:0	2	130.3
4:1	2	10.1
4:1	2	12.5

Within experimental error, these data show that the exine shells protect the echium oil to a very significant extent against UV light. This illustrates the natural antioxidant properties of the shells, since in this case most of the oil is likely to be surrounding the exine shells rather than encapsulated within them.

Example 3 – Stability against aerial oxidation (1)

This experiment evaluated the protective properties of exine shells against aerial oxidation. Oxidative induction times (OITs), as a measure of the effect of ambient oxygen on oil rancidity, were determined using a Metrohm™ 743 Rancimat machine, version 1.0 SRI, with an air flow rate of 20 l/hour and an operating temperature of 50 °C. The Rancimat determines the oxidative stability of in particular edible oils and fats, according to the AOCS Air Oxidation Method (AOM-AOCS Cd 12b-92).

All materials – including oils, fats, fatty acid amides and other fatty acid derivatives – have a degree of innate resistance to oxidation. The level of this natural antioxidancy depends on the material itself and any additives it contains, as well as on its prior treatment. Oxidation tends to proceed slowly until the innate resistance is overcome, at which point it accelerates rapidly. The OIT is the length of time before the onset of such acceleration. It is the time limit after which the material under test is generally considered to be rancid.

Using a Rancimat, a stream of filtered and dried air is passed through a sample which is held in a heating block at a predetermined temperature. The effluent air leaving the sample is then bubbled through deionised water, the electrical conductivity of which is constantly measured via a conductivity measuring cell. The sample as it oxidises produces volatile organic compounds including carboxylic acids, predominantly formic acid; the presence of such species in the effluent air produces a corresponding change in conductivity of the initially deionised water. A graph is produced showing the change in conductivity with time, from which the OIT (defined as the point of maximum change in the oxidation rate) can be automatically derived by the Rancimat by reference to the maximum in the second derivative of the conductivity with respect to time.

Three samples were prepared, each in duplicate: fresh echium oil, mixed into glass wool; empty exine shells (obtained as described above) mixed into glass wool; and echium oil

loaded into 40 μm AHS exine shells. The oil:exine weight ratio in the latter case was 0.5:1. Confocal electron microscopy showed that in the third sample, the oil was encapsulated by the exine shells.

Air was blown below a loose dispersion of each sample, so as to ensure a large contact surface area. The samples were then assessed using the Rancimat machine, as described above. The results are shown in Table 3.

Table 3

Tube	*Glass wool (g)*	*Product (g)*		*Oil (g)*	*Oil:exine weight ratio*	*Induction time (hours)*
1	1.5	Empty exines	0.5	0.0	0:1	>190
2	1.5		0.5	0.0	0:1	>190
3	1.5	Oil loaded exines	1.5	0.5	0.5:1	>190
4	1.5		1.5	0.5	0.5:1	>190
5	1.5	Echium oil	0.5	0.5	1:0	45
6	1.5		0.5	0.5	1:0	50

The Table 3 data show that the exine-encapsulated oil is significantly more resistant to aerial oxidation, and hence significantly more stable. This implies a protective effect due to the exine shell. The protection is likely to be more than simply the shell acting as a physical barrier to the ingress of oxygen, as spore-derived exine shells are known to be at least partially porous.

Example 4 – Stability against aerial oxidation (2)

Example 3 was repeated, but using 25 μm AHS exine shells and replacing the encapsulated oil sample with a physical mixture of echium oil and exine shells. The physical mixture contained an oil:exine weight ratio of 5:1 (0.5 g of oil to 0.1 g of the exine shells).

The results are shown in Table 4 below.

Table 4

Tube	*Glass wool (g)*	*Product (g)*	*Oil (g)*	*Oil:exine weight ratio*	*Induction time (hours)*

1	1.033	Empty exines	0.350	0.000	0:1	>190
2	1.446		0.408	0.000	0:1	>190
3	2.00	Oil mixed with exines	0.600	0.500	5:1	>190
4	2.00		0.600	0.500	5:1	>190
5	0.000	Echium oil	2.432	2.432	1:0	56
6	0.000		3.648	3.648	1:0	57
7	1.802	Echium oil	8.246	8.246	1:0	46
8	2.171		7.440	7.440	1:0	41

Again the echium oil was found to be protected against aerial oxidation by air for at least 190 hours when mixed in excess (5:1) with the exine shells. Since a substantial amount of the oil in this case must be on the outside of the exine shells, this indicates that the shells are themselves acting as antioxidants rather than providing a purely physical barrier to oxygen.

Examples 1 and 3 show that when an oil is encapsulated within an exine shell (ie, housed within the internal cavity of the exine microcapsule with the minimum or no oil on the outside surface – as observed by confocal microscopy), good protection can be observed against UV-induced and aerial oxidation. However when an excess of oil is present, as in this and Example 2, such that there is a significant amount on the outsides of the exine shells and the oil is therefore readily exposed to both air and ambient UV light, we have found that the exine shells themselves act to inhibit oxidation of the oil.

Example 5 – Stability to UV light (3)

Exine shells were loaded with either echium oil or cod liver oil, using the procedure outlined above. The oil:exine weight ratio in each case was 1:1. Both 25 and 40 μm shells were tested, and both AHS (exine alone) and BHS (exine + intine) versions.

Each sample was spread out on a watch glass and irradiated with UV light as described in Example 1. As controls, unencapsulated oil samples were subjected to the same treatment.

The peroxide value (PV) of each sample was determined both before and after irradiation, again as described in Example 1.

The results are shown in Tables 5 to 8 below, for the various types of exine shells tested.

Table 5 - 25 μm AHS

Oil	***Loaded/unloaded exine sample***	***Exposure to UV (hours)***	***Peroxide value (PV) (meq/kg)***
Echium	Unloaded	0	13.5
	Unloaded	2	67.9
	Loaded	0	17.1
	Loaded	2	38.6
Cod liver	Unloaded	0	4.5
	Unloaded	2	18.3
	Loaded	0	8.5
	Loaded	2	9.1

Table 6 - 40 μm AHS

Oil	***Loaded/unloaded exine sample***	***Exposure to UV (hours)***	***Peroxide value (PV) (meq/kg)***
Echium	Unloaded	0	13.5
	Unloaded	2	67.9
	Loaded	0	20.9
	Loaded	2	36.7
Cod liver	Unloaded	0	4.5
	Unloaded	2	18.3
	Loaded	0	8.5
	Loaded	2	8.7

Table 7 - 25 μm BHS

Oil	***Loaded/unloaded exine sample***	***Exposure to UV (hours)***	***Peroxide value (PV) (meq/kg)***
Echium	Unloaded	0	13.5
	Unloaded	2	67.9
	Loaded	0	13.0
	Loaded	2	17.5
Cod liver	Unloaded	0	4.5
	Unloaded	2	18.3

	Loaded	0	4.5
	Loaded	2	7.3

Table 8 - 40 μm BHS

Oil	***Loaded/unloaded exine sample***	***Exposure to UV (hours)***	***Peroxide value (PV) (meq/kg)***
Echium	Unloaded	0	13.5
	Unloaded	2	67.9
	Loaded	0	0.0
	Loaded	2	0.0
Cod liver	Unloaded	0	4.5
	Unloaded	2	18.3
	Loaded	0	0.0
	Loaded	2	0.0

These data confirm that encapsulation of the oils into exine shells can significantly reduce their oxidation rate on exposure to UV light.

The results are particularly marked for the 40 μm BHS, which appears to completely protect both oils from oxidation. Moreover, the exine shells in this case appear to "clean up" the oils, reducing their peroxide values even before UV irradiation: this suggests that this BHS is contributing a significant antioxidant effect irrespective of its ability to screen the oil from applied UV light, and that it may even in certain circumstances be capable of removing any previously accrued rancidity.

Example 6 – "Clean up" of rancid oils (1)

Example 5 was repeated using cod liver oil, 40 μm exine shells (both AHS and BHS) and an exine:oil weight ratio of 0.5:1, ie, a much higher oil loading. The results are shown in Table 9 below.

Table 9

Sample	***PV (meq/kg) before irradiation***	***PV (meq/kg) after irradiation***
Neat cod liver oil	4.5	18

40 μm AHS + oil	10	13
40 μm BHS + oil	0	0

Again this demonstrates the ability of the BHS (exine + intine) shells to "clean up" rancidity, the peroxide value for the (exine + oil) sample being lower even than that for the original oil sample.

Example 7 – Clean up of rancid oils (2)

Example 6 was repeated, but using an echium oil that already had a peroxide value of 20.5 meq/kg, ie, which was already turning rancid.

The results, prior to irradiation, are shown in Table 10.

Table 10

Sample	***Exine:oil weight ratio***	***PV (meq/kg) before irradiation***
Neat echium oil	0:1	20.5
40 μm AHS + oil	1:1	25.5
40 μm AHS + oil	0.5:1	26.5
40 μm BHS + oil	1:1	3
40 μm BHS + oil	0.5:1	8.5

Again these data demonstrate the surprising ability of the 40 μm BHS (ie, exine/intine combination) to "clean up" an already rancid oil. The peroxide value of the original oil sample is significantly reduced after encapsulation in the exine shells. The higher the proportion of exine shells, the greater the effect.

Example 8 – Stability against aerial oxidation (3)

Example 3 was repeated but using cod liver oil.

40 μm exine shells (both AHS and BHS) were used for these tests, and were loaded with cod liver oil at oil:exine weight ratios of 1:1, 3:1 and 5:1. Each sample was wedged into the middle of a sample tube between two glass wool wads. A capillary tube was passed

through the resulting plug, ensuring that no oil ran down the bottom of the tube. These tubes were then inserted into the heating blocks of the Rancimat machine and air flow commenced.

The results are shown in Table 11 below.

Table 11

Sample	***Oil:exine ratio (w/w)***	***OIT (hours)***
Cod liver oil	1:0	56
Oil:BHS	5:1	59
Oil: BHS	3:1	>120
Oil:BHS	1:1	>120
Oil:AHS	5:1	73
Oil:AHS	3:1	>120
Oil:AHS	1:1	>120

The Table 11 data again show that the exine-encapsulated oil is significantly more resistant to aerial oxidation, and hence significantly more stable.

The higher the oil loading, the lower the protective effect. This may be because more of the oil is outside of the exine shells and/or only loosely associated with them (encapsulated oil benefits from the natural antioxidancy of the exine shells and may also benefit from some physical protection from the air).

Example 9 – Exine shells as taste masking agents

Exine shells were prepared as described above. They were loaded with cod liver oil (The Boots Company PLC), at an oil:exine weight ratio of 2:1, by subjecting a homogeneous mixture of both components to a vacuum for 1.5 hours.

The resultant material was then tasted by three people. All found it to have a smooth texture and to be free from any oily taste and texture.

This demonstrates the potential for exine shells to be used as taste masking agents in for example pharmaceutical or nutraceutical products, foods and food supplements. Food

supplements such as omega-3 oils have for instance what many regard as an unpleasant taste; formulating them with a plant-derived exine shell could therefore serve not only to protect them against oxidation but also to mask their flavour and/or smell, of particular use when they are intended to be added to food or nutraceutical products.

Example 10 – Protection of volatile actives

This experiment assessed the evaporation rate of a volatile active substance from within spore-derived exine shells.

Exine shells (AHS, 40 μm diameter) were prepared as described above, and loaded with butanol. Alcohols are not only volatile substances, but are also commonly used as diluents in topical formulations such as cosmetics. Impregnation was achieved by "passive contact", ie, by mixing the alcohol with the exine shells at room temperature and pressure and allowing the fluid to permeate into the shells.

Sample A contained 2 ml of neat butanol, as a control; sample B contained 2 ml of butanol encapsulated in 1 g of exine shells.

Each sample was spread on a Petri dish and weighed at 5 minute intervals in order to measure the time taken for all of the encapsulated alcohol to evaporate. All experiments were conducted in triplicate.

The results of these tests are shown in Table 12 below. The half life quoted in each case is a theoretical, calculated indication of the time taken for half the amount of encapsulated alcohol to evaporate.

Table 12

Sample	***Evaporation time (min)***	***Half life (min)***
A	200	61
B	300	115

Table 12 shows that encapsulation of a volatile alcohol within an exine shell can considerably inhibit its release by evaporation. A protective coating, for example a lipid

coating layer, could be applied to the shells in order to slow evaporative loss yet further and thus to protect volatile active substances in formulations prepared according to the invention.

Example 11 – High active substance loadings

Oil was stirred with 25 μm AHS exine shells to form a homogeneous mixture which was then subjected to vacuum (30 kPa) for 2 hours in order to impregnate the shells with the oil. The oils used were soybean oil, sunflower oil, echium oil and rapeseed oil, each up to 3g per gram of exine shells and in the case of the cod liver oil up to 3.5g per gram of exine shells.

It was found that even at these relatively high loadings, the oil-loaded exine shells behaved as powders, confirming effective encapsulation of the oils. This was further confirmed by confocal microscopy. It demonstrates one of the advantages of using spore-derived exine shells as delivery vehicles for active substances. It also shows the suitability of the shells as vehicles in powder formulations, for instance for topical delivery of cosmetic substances, cleaning products or laundry products, or for delivery of pharmaceutically or nutraceutically active substances, food supplements and the like.

At loading levels at and above 5g of oil per gram of exine shells, the samples behaved more as pastes, indicating that a significant proportion of the oil was then outside of the exine shells. Such formulations might be suitable for application as a cream or ointment, for example, or might have application in certain types of food product. At loading levels at and below 2g of oil per gram of exine shells, the powders were fine, free flowing powders and reasonably dry to the touch.

Claims

1. Use of an exine shell of a naturally occurring spore (which term embraces a pollen grain and an endospore of a micro-organism), or a fragment thereof, as an antioxidant.

2. Use according to claim 1, which is for the purpose of protecting a substance or composition against aerial and/or UV-induced oxidation.

3. Use according to claim 1 or claim 2, wherein the exine shell or fragment is used as an antioxidant in a formulation containing an active substance.

4. Use according to claim 3, wherein the active substance is encapsulated within, or chemically or physically bound to, the exine shell or fragment.

5. Use according to claim 4, wherein the active substance is at least partially encapsulated within the shell.

6. Use according to any one of claims 3 to 5, wherein the active substance is for systemic use.

7. Use according to any one of claims 3 to 6, wherein the formulation is for topical delivery of an active substance.

8. Use according to any one of claims 3 to 7, wherein the active substance is selected from pharmaceutically and nutraceutically active substances, foods and food ingredients, food supplements, herbicides, pesticides and pest control agents, plant treatment agents such as growth regulators, antimicrobially active substances, cosmetics (including fragrances), toiletries, disinfectants, detergents and other cleaning agents, adhesives, diagnostic agents, dyes and inks, fuels, explosives, propellants and photographic materials.

9. Use according to claim 8, wherein the active substance is a cosmetic substance.

10. Use according to claim 8, wherein the active substance is for use in a toiletry product.

11. Use according to claim 8, wherein the active substance is for use in a household product.

12. Use according to claim 8, wherein the active substance is a pharmaceutically or nutraceutically active substance.

13. Use according to claim 8, wherein the active substance is a foodstuff.

14. Use according to any one of claims 3 to 13, wherein the active substance comprises a volatile substance.

15. Use according to claim 14, wherein the active substance comprises a fragrance.

16. Use according to any one of claims 3 to 15, wherein the active substance is a lipid or lipid-like substance.

17. Use according to any one of claims 3 to 16, wherein the active substance is sensitive to one or more external influences selected from heat, light, oxygen and water.

18. Use according to claim 17, wherein the active substance is susceptible to oxidation.

19. Use according to claim 18, wherein the active substance is susceptible to UV-induced oxidation.

20. Use according to any one of claims 3 to 19, wherein the formulation is for storage and/or use in a fluid environment which contains oxygen.

21. Use according to any one of claims 3 to 20, wherein the formulation is for storage and/or use in an environment which is or may be exposed to UV radiation.

22. Use according to any one of claims 3 to 21, wherein the formulation contains more than one active substance.

23. Use according to claim 22, wherein the formulation comprises two or more populations of active substance-containing exine shells or fragments, each chemically or physically bound to, or encapsulating, a different active substance.

24. Use according to any one of claims 3 to 23, wherein the formulation is contained in a product selected from cosmetic products; toiletries; hair care products; nail care products; dental products; household products; paints, inks, dyes and other colouring products; adhesive products; pharmaceutical and nutraceutical products; food products, including food additives and food ingredients; agricultural and horticultural products; fuels; explosives; propellants; and photographic materials.

25. Use according to claim 24, wherein the product is selected from cosmetic products (which includes skin care products), toiletries, hair and nail care products and dental products.

26. Use according to claim 24, wherein the product is a pharmaceutical or nutraceutical product.

27. Use according to claim 24, wherein the product is a food product.

28. Use according to any one of the preceding claims, wherein the exine shell or fragment has a diameter of from 1 to 300 μm.

29. Use according to claim 28, wherein the exine shell or fragment has a diameter of from 3 to 50 μm.

30. Use according to any one of the preceding claims, wherein the exine shell or fragment has a diameter of 10 μm or less.

31. Use according to any one of the preceding claims, wherein the exine shell or fragment additionally contains all or part of the cellulose intine layer from the naturally occurring spore.

32. Use according to any one of claims 3 to 31, wherein the weight ratio of the active substance to the exine shell or fragment in the formulation is from 0.01:1 to 35:1.

33. Use according to claim 32, wherein the weight ratio of the active substance to the exine shell or fragment in the formulation is from 0.1:1 to 5:1.

34. Use according to any one of claims 3 to 33, wherein the weight ratio of the active substance to the exine shell or fragment in the formulation is 5:1 or less.

35. Use according to any one of claims 3 to 34, wherein the exine shell or fragment is coated with a barrier layer.

36. Use according to claim 35, wherein the barrier layer comprises a lipid coating material.

37. Use according to any one of the preceding claims, wherein the outside of the exine shell or fragment is modified by the attachment of a functional group which increases the affinity of the shell or fragment for a surface to which the formulation is intended to be applied.

38. Use of an exine shell of a naturally occurring spore (which term embraces a pollen grain and an endospore of a micro-organism), or a fragment thereof, for the purpose of removing a previously formed oxidation product from a substance or composition.

39. Use according to any one of the preceding claims, which is substantially as herein described.