



Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E.

S.

D.

REF: Expediente 11-163-902

TÍTULO SOLICITADO: Composición farmacéutica estable y métodos para utilizar la misma.

PUBLICACIÓN: Gaceta 647 de 29/06/2012, N° de publicación 838.

PRIORIDAD: US 61/173.763 de 29/04/2009

SOLICITANTE: AMARIN PHARMA, INC

APODERADO: LUZ CLEMENCIA DE PAEZ.

TRÁMITE: SUSTENTACIÓN DE OPOSICIÓN

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUIMICAS S.A.**, en ejercicio de lo dispuesto por el artículo 42, inciso 2° de la Decisión 486 de 2000, por medio del presente escrito, procedo a sustentar y complementar la oposición presentada el 27 de Septiembre de 2012, en los siguientes términos:

I. RATIFICACIÓN

Comedidamente me permito insistir en los argumentos planteados en la oposición respectiva, los cuales, como se dijo en su momento, demuestran que la solicitud de la referencia incumple los requisitos de patentabilidad establecidos por los

artículos 14 y 18 de la Decisión 486 de 2000, y que por ello no merece recibir el beneficio patentario.

La mencionada solicitud colombiana se refiere a una composición farmacéutica que comprende por lo menos 95% de ácido eicosapentaenóico (EPA) encerrado en una cubierta de cápsula, en donde la composición tiene un valor inicial de peróxido no mayor de 5Meq/kg y después de almacenamiento a 25°C y 60% de HR durante un periodo de 6 meses la composición tiene un segundo valor de peróxido no mayor de 8Meq/kg, y que además comprende un método de tratamiento o prevención de una enfermedad cardiovascular.

Como se observa, la materia de dicha reclamación se encuentra definida mediante una propiedad de la composición la cual es una función dependiente del tiempo, la temperatura, la humedad relativa, etc. Sin embargo, estas propiedades si bien son un criterio de calidad de un determinado producto, no definen estructuralmente el mismo por lo que la solicitud no tiene fundamento inventivo o novedoso.

En el mismo sentido, la composición reclamada mediante dichas propiedades únicamente se refieren a resultados a alcanzar de la composición en la que está hecha la cápsula, pero donde dicha composición de la cápsula no se divulga ni en sus componentes ni en su formulación, enseñando únicamente que se trata de EPA, lo que no se considera una característica técnica esencial para llegar a este resultado.

Así entonces las características únicamente se reducen a la presencia de EPA en una cápsula, para lo cual cualquier técnico medio en la materia que buscara identificar una composición estable de la misma, llegaría fácilmente a un material determinado que lograra el efecto de peróxido deseado a las condiciones mencionadas en las reivindicaciones de la solicitud.

Adicionalmente a lo anterior, en mi memorial de oposición se presentaron argumentos que impiden la concesión de la presente solicitud, tal es el caso de las reivindicaciones 44 a 46, las cuales se encuentran dirigidas a métodos de tratamiento.

Por otro lado, todo el capítulo reivindicatorio carece de claridad dado que no se define correctamente la materia, empleando parámetros, resultados a alcanzar, propiedades de la cápsula y de la solución después de un tiempo de almacenamiento, lo cual no puede ser aceptable ya que no permitiría su comparación precisa con el estado de la técnica o con cualquier otro medicamento de EPA en el mercado.

Ahora bien, en el documento de oposición presentado se citaron los siguientes documentos del estado de la técnica:

- La Publicación Americana US 2006/0134178 “Composition containing organic substance having double bond with improved oxidative stability” en comparación con lo revelado en la solicitud en trámite, permite observar:

CARACTERÍSTICAS DE LA REIVINDICACION 1 CO 11-163-902	US 2006/0134178
Composición	Pág 8 Reivindicación 4
Comprende EPA	Pág 8 [0119] Pág 8 Reivindicación 4
En una cápsula	Pág 5 [0054]
Aceptable Estabilidad en el tiempo	Abstract Pág 2 [0016]

Lo anterior demuestra que lo contenido en la solicitud no cumple con los criterios de novedad ya que la composición se revelaba previamente en la publicación US 2006/0134178.

Fueron citados adicionalmente al anterior documento, otras publicaciones donde se muestran los ejemplos de formulaciones que contienen EPA y/o DHA, con sus proporciones y cantidades a usar, que afectan la novedad de la presente solicitud tales como:

- US 2003/0100610 “Use of polyunsaturated fatty acids for the primary prevention of major cardiovascular events”, en donde se evidenciaramente que la práctica de elaborar composiciones farmacéuticas con ácidos grasos poliinsaturados, EPA y/o DHA como ingredientes activos en eventos cardiovasculares tal y como lo reivindica la solicitud colombiana en cuestión, era ampliamente reconocida en el estado de la técnica y además permitió identificar

las formulaciones que pueden realizarse incluso con antioxidantes como el tocoferol dentro de las mismas (ver tabla de la página 3):

[0026]

	A (mg)	B (mg)	C (mg)
EPA ¹	525	—	>400
DHA ¹	315	—	>340
EPA + DHA		850	>800
Total ω-3 ¹		—	>900
d-α-tocopherol	4 I.U.	—	4 I.U.
d,I-α-tocopherol		0.3	
gelatin	246	—	246
gelatin succinate		233	
glycerol	118	67	118
OFR	2.27	—	2.27
OFG	1.27		1.27
SOB		1.09	
SPOB		0.54	

1-ethyl esters; OFR: red iron oxide OFG: yellow iron oxide; SOB: sodium p-oxybenzoate;
SPOB: sodium propyl p-oxybenzoate; I.U.: international units.

Se tiene como prueba entonces que en el estado de la técnica ya se conocían composiciones de EPA en gelatina y tocoferol, lo que demuestra en consecuencia, una composición con la misma estructura química de la composición reclamada por la solicitud en trámite y con los mismos fundamentos de estabilidad al incorporar el antioxidante tocoferol, lo cual, como ya se ha visto carece de novedad y de altura inventiva dado que cualquier técnico medio emplearía dicho compuesto para evitar la oxidación de cualquier composición en una capsula.

Se demostró además, que utilizar materiales que forman películas y agentes plastificantes en el material de encapsulado (ver reivindicación 15 y dependientes de la solicitud) es una práctica común, tal y como se expone en el documento del estado de la técnica US 2003/0104048 “**Pharmaceutical dosage forms for highly hydrophilic materials**”,el cual señala con exactitud los plastificantes higroscópicos y los no higroscópicos que pueden emplearse y que la solicitud en estudio también acoge para definir las características de sus reivindicaciones. Así entonces, los materiales, glicerol, gelatina, maltitol, lactitol, xilitol, hidrolizado de almidón hidrogenado y jarabe de glucosa ya habían sido divulgados en la misma composición.

II. COMPLEMENTO DE INFORMACION

Adicionalmente a lo ya expuesto, el documento ya mencionado US 2006/0134178, divulga incluso los valores base de peróxido determinados (no mayores de 5Meq/kg) (ver párrafo 0040)

[0040] The antioxidant used in the present invention does not reduce substances that have been already oxidized. It is therefore necessary that oxides be removed from the organic substance having a double bond before adding the antioxidant. For a polyunsaturated fatty acid or its salt or ester, it is also necessary to remove oxides by degumming, deacidification, decolorization, deodorization, or the like. Preferably, the organic substance is refined to a PV of 3.0 meq/kg or less and an AV of 1.0 or less, and has no odor in terms of sensory testing.

Con lo cual también queda claro que el método para obtener condiciones de almacenamiento apropiadas estaba contemplado en dicha anterioridad, por tanto, así las reivindicaciones no mencionen las características técnicas de la composición, el problema técnico había sido abordado por el documento del estado de la técnica y su solución había sido efectiva en el mismo modo que la solicitud en estudio al reportar la medición del sustrato oxidativo en menos del 3.0 Meq/kg.

Este documento también revela que en la composición es necesario adicionar tocoferol como antioxidante:

[0052] In addition to the antioxidative sesame component and the ascorbic acid or ascorbyl fatty acid ester, a tocopherol may be added. The tocopherol may be selected from among α -, β -, γ -, and δ -tocopherols and mixed tocopherol, but preferably δ -tocopherol is used. Tocopherol is often added to commercially available polyunsaturated fatty acids and their salts and esters, such as refined fish oil, in the stage of production. Hence, use of these raw materials naturally results in a product containing tocopherol. Whether tocopherol is present or absent does not affect the synergistic effect of the antioxidative sesame component and the ascorbic acid or ascorbyl fatty acid ester.

Dando como resultado que cualquier modalidad particular del objeto de la solicitud ha sido abordada por el documento y anticipada en sus características técnicas, demostrando una vez más que el objeto no cumple con los requisitos de novedad o nivel inventivo.

Adicional a los dos documentos mencionados anteriormente, se pone de presente los documentos:

- La publicación americana US 2007/0105954 (Puri) publicado el 10 de mayo de 2007, que divulga una formulación que comprende ácido eicosapentaenoico EPA donde en una de las modalidades preferidas no incluyen ácido docosahexaenóico o DHA

[0027] Preferably the formulation comprises substantially no docosahexaenoic acid. Preferably the formulation comprises no docosahexaenoic acid.

En este sentido, el documento anticipa la solicitud en estudio dado que en las reivindicaciones 5 y 6 se reclama la composición de etil-EPA que contiene menos de 03% de DHA o no lo contiene.

Ya que los documentos observados en el presente escrito mencionan todas las características técnicas esenciales de las composiciones de EPA en una cápsula cubierta, incluso con los mismos parámetros de medición de estabilidad a la oxidación y componentes para tal fin, la solicitud no cumple con los criterios de

Novedad y en vista de la falta de demostración de un efecto sorprendente, el Nivel inventivo de modalidades particulares no puede ser reconocido.

III. ANEXOS

- La Publicación Americana US 20060134178 "Composition containing organic substance having double bond with improved oxidative stability" págs. 2, 5, 8 y abstract.
- La Publicación Americana US 20030100610 "use of polyunsaturated fatty acids for the primary prevention of major cardiovascular events" págs. 2 y3.
- La Publicación Americana US 20030104048 "Pharmaceutical dosage forms for highly hydrophilic materials" pág 3, abstract.
- La publicación Americana US 2007/0105954 "Formulation containing a carboxylic acid or an ester thereof" publicado el 10 de mayo de 2007. Portada, página 2 y reivindicaciones.

Señor Superintendente,



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US 20060134178A1

(19) **United States**

(12) **Patent Application Publication**

Doisaki et al.

(10) **Pub. No.: US 2006/0134178 A1**

(43) **Pub. Date: Jun. 22, 2006**

(54) **COMPOSITION CONTAINING ORGANIC SUBSTANCE HAVING DOUBLE BOND WITH IMPROVED OXIDATIVE STABILITY**

(30) **Foreign Application Priority Data**

Nov. 22, 2002 (JP) 2002-339906

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Publication Classification

(51) **Int. Cl.**

A61K 47/00 (2006.01)

A61K 35/60 (2006.01)

(52) **U.S. Cl.** 424/439; 424/523

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(21) Appl. No.: 10/535,413

(22) PCT Filed: Nov. 21, 2003

(86) PCT No.: PCT/JP03/14877

(57) **ABSTRACT**

To an organic substance having a double bond such as a polyunsaturated fatty acid was added an antioxidative component containing an antioxidative sesame component and ascorbic acid or an ascorbyl fatty acid ester. The above method provides a composition containing an organic substance having a double bond exhibiting enhanced oxidative stability. Particularly, it extremely improves oxidative stability of fat and oil which contains polyunsaturated fatty acid. General-purpose refined fish oil which is easy to handle can be provided for food, medicine or feed uses.

pp. 291-295 (1992), and 45 (3) pp. 285-290 (1992). Although some of the antioxidants use sesamol and an ascorbic acid ester in combination, they do not produce effects particularly superior to other antioxidants. These literatures discuss effects in oxidation of vegetable oils (having 3 or less unsaturated bonds) at high temperatures, but not in oxidation of polyunsaturated fatty acids (having at least three unsaturated bonds) during storage at room temperature; hence different objects are used under different conditions. This is probably because the combination of sesamol and an ascorbic acid ester does not produce superior effects. In addition, the thermal instability of the antioxidants may affect antioxidant properties.

[0014] Demand for oil or fat containing an unsaturated fatty acid is increasingly growing. Accordingly, it is highly desired to solve the problem of oxidative stability, including how to handle the oil or fat, in a strict sense.

DISCLOSURE OF INVENTION

[0015] The object of the present invention is to provide an organic substance, particularly a polyunsaturated fatty acid or its ester, having a double bond with extremely improved oxidative stability.

[0016] The inventors of the present invention have conducted intensive research in order to enhance the oxidative stability of oils and fats (oils and fats containing a high proportion of polyunsaturated fatty acid, such as oils of fish and aquatic animals). As a result, the inventors found that the oxidative stability of oils and fats can be extremely enhanced by adding an antioxidative component of sesame, such as sesamol, an ascorbic acid or ascorbyl fatty acid ester in combination. In addition, the present inventors found that the antioxidant properties of antioxidative sesame component other than sesamol are also extremely enhanced by combination with ascorbic acid or ascorbyl fatty acid ester, and thus accomplished the present invention.

[0017] The main points of the present invention are the composition described in the following (1) to (13).

(1) A composition having oxidative stability comprising: an organic substance having a double bond which contains an antioxidant comprising an antioxidative sesame component and ascorbic acid or an ascorbyl fatty acid ester.

(2) A composition according to (1), wherein the double bond has active methylene, or located at the end of the organic substance.

(3) A composition according to (1), wherein the organic substance having the double bond is a polyunsaturated fatty acid or its salt or ester.

(4) A composition according to (3), wherein the polyunsaturated fatty acid contains at least one of eicosapentaenoic acid and docosahexaenoic acid.

(5) A composition according to (3) or (4), wherein the ester of the polyunsaturated fatty acid is a triglyceride containing the polyunsaturated fatty acid as a constituent, or a lower alcohol ester of the polyunsaturated fatty acid.

(6) A composition according to (3) or (4), wherein the ester of the polyunsaturated fatty acid is added in a form of refined fish oil.

[0018] (7) A composition according to any one of (1) to (6), wherein the antioxidative sesame component is at least one of the substances represented by peaks detected by high-performance liquid chromatography using an electrochemical detector at elution times of about 2.66, 3.40, 3.84, 4.57, 4.98, 5.82, 7.00, 8.67, 9.84, 11.24, 12.29, 12.49, 13.36, 14.04, 14.32, 14.74, 15.22, 15.60, 15.82, 16.34, 16.98, 18.10, 18.43, and 34.91 minutes.

(8) A composition according to any one of (1) to (6), wherein the antioxidative sesame component is extracted from sesame, sesame oil, or sesame residue, using a solvent, a lipid, or an emulsifier singly or in combination.

[0019] (9) A composition according to any one of (1) to (6), wherein the antioxidative sesame component is at least one selected from the group consisting of sesamol, sesaminol, episesaminol, pinorelinol, epihinorelinol, syringaresinol, samine, sesamolinal, and 2,3-di(4'-hydroxy-3'-methoxybenzyl)-2-buten-4-olide.

(10) A composition according to any one of (1) to (9), wherein the ascorbyl fatty acid ester contains ascorbyl palmitate or ascorbyl stearate.

(11) A composition according to any one of (1) to (10), wherein the ascorbic acid or the ascorbyl fatty acid ester is contained in an excessive amount more than the amount soluble in the polyunsaturated fatty acid or its salt or ester.

(12) A composition according to (11), wherein the excessive amount of the ascorbic acid is in a powder or solid form.

(13) A composition according to any one of (1) to (12), further comprising tocopherol.

(14) A food containing the composition as set forth in any one of (1) to (13).

(15) A powdered oil or fat containing the composition as set forth in any one of (1) to (13).

(16) A powdered baby milk containing the composition as set forth in any one of (1) to (13).

(17) A health food containing the composition as set forth in any one of (1) to (13).

ADVANTAGES

[0020] The present invention can extremely enhance the oxidative stability of a composition containing an organic substance with low oxidative stability which has a double bond having active methylene, or a double bond at its end, particularly containing a polyunsaturated fatty acid. The present invention can make easy the addition of a composition containing a polyunsaturated fatty acid to medical drugs, cosmetic preparations, food, and the like, which has been conventionally limited. Also, the content of such a composition can be increased. Since the antioxidative sesame component and ascorbic acid or an ascorbyl fatty acid ester used in the present invention have been ingested as food for a long time, a safe antioxidant can be provided for food as well.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1 shows amounts of oxygen absorbed by oil or fat in Example 1.

fatty acid ester is used under conditions not allowing natural diffusion, preferably, it is finely powdered and uniformly dispersed.

[0052] In addition to the antioxidative sesame component and the ascorbic acid or ascorbyl fatty acid ester, a tocopherol may be added. The tocopherol may be selected from among α -, β -, γ -, and δ -tocopherols and mixed tocopherol, but preferably δ -tocopherol is used. Tocopherol is often added to commercially available polyunsaturated fatty acids and their salts and esters, such as refined fish oil, in the stage of production. Hence, use of these raw materials naturally results in a product containing tocopherol. Whether tocopherol is present or absent does not affect the synergistic effect of the antioxidative sesame component and the ascorbic acid or ascorbyl fatty acid ester.

[0053] The oil or fat whose oxidative stability has been enhanced by adding an antioxidative sesame component and ascorbic acid or an ascorbyl fatty acid ester can exhibit satisfactory oxidative stability alone, but it may be used in combination with another antioxidant. Also, it may be mixed with another oil or fat having superior oxidative stability (for example, vegetable oil). Other antioxidants include erythorbic acid, sodium erythorbate, isopropyl citrate, dibutylhydroxytoluene (BHT), and butylhydroxyanisole (BHA). Antioxidants used in combination in food include: food additives, such as tocopherols, hollyhock flower extract, Azuki extract, *aspergillus terreus* extract, calcium disodium ethylenediaminetetraacetate, disodium ethylenediaminetetraacetate, ellagic acid, erythorbic acid, sodium erythorbate, enju extract, γ -oryzanol, catechin, licorice oil extract, guajac resin, quercetin, isopropyl citrate, clove extract, enzymatically modified isoquercitrin, enzymatically modified rutin (extract), enzymatically decomposed apple extract, sesame seed oil unsaponifiable matter, rice bran oil extract, enzymatically decomposed rice bran, L-cysteine hydrochloride, dibutylhydroxytoluene, Queensland arrowroot extract, essential oil-removed fennel extract, horseradish extract, sesamol, sage extract, dropwort extract, buckwheat extract, amino acid-sugar reaction product, tea extract, tempeh extract, dokudami extract, rape seed oil extract, coffee bean extract, nordihydroguajaretic acid, sunflower seed extract, pimento extract, ferulic acid, butylhydroxyanisole, grape seed extract, blueberry leaf extract, propolis extract, hego-ginkgo leaf extract, hesperetin, pepper extract, garden balsam extract, gallic acid, propyl gallate, melaleuca oil, morin, chinese bayberry extract, eucalyptus leaf extract, gentian root extract, enzymatically decomposed rutin, rutin (extract), and rosemary extract; and antioxidants approved in other countries, such as thioldipropionic acid, distearyl thioldipropionate, octyl gallate, dodecyl gallate, and tert-butylhydroquinone.

[0054] Since the composition of the present invention has superior oxidative stability, it can be added to various types of food. For example, skimmed milk, milk casein, milk protein, lactose, oligosaccharide, cane sugar, or dextrin is dissolved in hot water to mix, and then vitamins and minerals are dissolved in the water phase. The oil or fat of the present invention is added to the water phase and mixed with a homo-mixer or the like, followed by homogenizing with a homogenizer. The resulting emulsion is sterilized, concentrated, or spray-dried in the usual manner to yield modified powder milk. Also, the composition of the present invention may be powdered oil using various types of

powder base material in the same manner. If the composition is added to food, it is determined whether the composition is in oil or fat form or powdered oil form, depending on the characteristics of the food. The composition may be added to general food, or encapsulated or tableted to prepare health food or a supplement.

EXAMPLES

[0055] The present invention will be further described with reference to the following examples, but the invention is not limited to the examples.

[0056] In the examples, the following materials were used as the refined fish oil, sesamol, ascorbyl palmitate, ascorbic acid, δ -tocopherol.

[0057] Refined fish oil (containing 0.5% by weight of δ -tocopherol): DD Oil Type 3 (refined fish oil produced by refining tuna oil to a peroxide value of 5 meq/kg or less, an acid value of 1 or less, and a color Gardner of 3 or less by degumming, deacidification, deodorization, or other process), produced by Nippon Suisan Kaisha, Ltd.

[0058] Refined fish oil (not containing δ -tocopherol): taken as a sample before adding δ -tocopherol in the process of DD Oil Type 3 preparation, produced by Nippon Suisan Kaisha, Ltd.

[0059] Refined fish oil (sardine oil): DD Oil Type 2 (refined fish oil produced by refining sardine oil to a peroxide value of 5 meq/kg or less, an acid value of 1 or less, and a color Gardner of 3 or less by degumming, deacidification, deodorization, or other process; containing 28% by weight of EPA, 12% by weight of DHA, and 0.5% by weight of δ -tocopherol), produced by Nippon Suisan Kaisha, Ltd.

[0060] Sesamol: sesamol (purity: 98%) produced by Nacalai Tesque, Inc.

[0061] Ascorbyl palmitate: ascorbyl palmitate (purity: 95% or more) produced by Sankyo Foods Co. Ltd.

[0062] Ascorbic acid: L(+)-ascorbic acid (purity: 99.5%) produced by Nacalai Tesque, Inc.

[0063] δ -Tocopherol: D- δ -tocopherol (purity: 90%) produced by Wako Pure Chemical Industries Eicosapentaenoic acid ethyl ester: prepared by ethanolysis of sardine oil in the presence of metallic sodium to prepare sardine oil ethyl ester and purifying the ester by distillation and HPLC to a purity of 99%.

[0064] α -Tocopherol: ($\pm$)- α -tocopherol (purity: 98%) produced by Wako Pure Chemical Industries

Example 1

<Test for Effect of Sesamol+Ascorbyl Palmitate+ δ -Tocopherol>

[0065] The following antioxidant preparations were added separately to refined fish oil (containing 0.5% by weight of δ -tocopherol) to prepare samples.

[0066] sesamol (1.0% by weight)+ascorbyl palmitate (0.01% by weight)

[0067] sesamol (1.0% by weight) alone

[0068] ascorbyl palmitate (0.01% by weight) alone

[0107] sesamol (0.5%)+ascorbyl palmitate (0.1%)

[0108] sesamol (1.0%)+ascorbyl palmitate (0.1%)

[0109] After 4 mL of the samples were placed separately in respective 30 mL brown bottles and hermetically sealed with septums, the samples were stored at 60° C. The concentration of oxygen during storage was measured by gas chromatography, and thus the amount of oxygen reacted with (absorbed by) the oil was calculated. Also, the remaining antioxidant contents were analyzed by HPLC with an electrochemical detector.

[0110] The results are shown in FIGS. 10 and 11. The figures show that, in either case, ascorbyl palmitate was consumed (oxidized) to be lost first, and then the refined fish oil, δ -tocopherol, and sesamol simultaneously started oxidizing. It is therefore assumed that ascorbyl palmitate plays an important role when the antioxidant preparation contains δ -tocopherol, sesamol, and ascorbyl palmitate. In order to confirm this assumption, 0.1% of ascorbyl palmitate was further added to the same system as in FIG. 11 on day four. The results are shown in FIG. 12. FIG. 11 shows that the oil was not oxidized until ascorbyl palmitate was consumed (around day ten); FIG. 12, in which ascorbyl palmitate was added on day four, shows that oxidation of the oil was continuously suppressed even after day ten.

[0111] Thus, it has been shown that it is important that ascorbyl palmitate is present in the antioxidant system of the present invention.

Example 12

<Tests for Effect on Eicosapentaenoic Acid Ethyl Ester of the Present Invention, and for Effect of Excessive Amount of Ascorbyl Palmitate>

[0112] Samples were prepared by adding the following antioxidant preparations separately to eicosapentaenoic acid ethyl ester with a purity of 99% (containing 0.2% of α -tocopherol)

[0113] sesamol (1.0%)+ascorbyl palmitate (0.1%)

[0114] sesamol (1.0%)+ascorbyl palmitate (0.5%)

[0115] Each sample was stored at 60° C., and the peroxide value (PV) was measured.

[0116] The results are shown in FIG. 13. The antioxidant property of the antioxidant preparation containing an excessive amount of ascorbyl palmitate (0.5%) was maintained in comparison with that of the antioxidant preparation containing a soluble amount of ascorbyl palmitate (0.1%).

Example 13

<Comparison with Antioxidant (t-Butylhydroxytoluene (BHT)) Generally Used in External Preparations>

[0117] The antioxidant preparation of the present invention was compared with t-butylhydroxytoluene (BHT), which is an antioxidant generally used. Samples were prepared by adding 0.5%, 1.5%, or 10.0% of BHT, or 1.0% of sesamol and 0.5% of ascorbyl palmitate to refined fish oil (sardine oil) containing 0.5% of δ -tocopherol. To 30 mL brown bottles, 4 mL of the samples were placed separately. The bottles were hermetically sealed with septums and stored at 37° C. The oxygen concentration in the headspace

was measured with time by gas chromatography, and thus the amount of oxygen reacted with (absorbed by) the oil was calculated.

Results

[0118] FIG. 14 shows the comparison of the antioxidant properties between BHT and the antioxidant preparation of the present invention. The results show that the antioxidant preparation of the present invention exhibited higher antioxidant property than the same amount (1.5%) of BHT as the total amounts of the antioxidants in the antioxidant preparation of the present invention, and also than a much larger amount (10.0%) of BHT.

INDUSTRIAL APPLICABILITY

[0119] The present invention can provide oils and fats having oxidative stability far superior to known oils and fats containing a polyunsaturated fatty acid. Consequently, when a polyunsaturated fatty acid is added to food, medical drugs, and the like for health promotion, such products can be easily produced and preserved. Also, the types of food to which the composition is added and the polyunsaturated fatty acid content can be easily increased. Specifically, general-purpose refined fish oil containing EPA and DHA can be provided for health food or the like.

1. A composition having oxidative stability comprising: an organic substance having a double bond which contains an antioxidant comprising an antioxidative sesame component which is purified form sesame or synthesized and ascorbic acid or an ascorbyl fatty acid ester.

2. A composition according to claim 1, wherein the double bond has active methylene, or located at the end of the organic substance.

3. A composition according to claim 1, wherein the organic substance having the double bond is a polyunsaturated fatty acid or its salt or ester.

4. A composition according to claim 3, wherein the polyunsaturated fatty acid contains at least one of eicosapentaenoic acid and docosahexaenoic acid.

5. A composition according to claim 3, wherein the ester of the polyunsaturated fatty acid is a triglyceride containing the polyunsaturated fatty acid as a constituent, or a lower alcohol ester of the polyunsaturated fatty acid.

6. A composition according to claim 3, wherein the ester of the polyunsaturated fatty acid is added in a form of refined fish oil.

7. A composition according to claim 1, wherein the antioxidative sesame component is at least one of the substances represented by peaks detected by high-performance liquid chromatography using an electrochemical detector at elution times of about 2.66, 3.40, 3.84, 4.57, 4.98, 5.82, 7.00, 8.67, 9.84, 11.24, 12.29, 12.49, 13.36, 14.04, 14.32, 14.74, 15.22, 15.60, 15.82, 16.34, 16.98, 18.10, 18.43, and 34.91 minutes.

8. A composition according to claim 1, wherein the antioxidative sesame component is extracted from sesame, sesame oil, or sesame residue, using a solvent, a lipid, or an emulsifier singly or in combination.

9. A composition according to claim 1, wherein the antioxidative sesame component is at least one selected from the group consisting of sesamol, sesaminol, episaminol,

12

pinoresinol, epipinoresinol, syringaresinol, samine, sesamol, and 2,3-di(4'-hydroxy-3'-methoxybenzyl)-2-buten-4-olide.

10. A composition according to claim 1, wherein the antioxidative sesame component is sesamol.

11. A composition according to claim 1, wherein the antioxidative sesame component is extracted from sesame residue.

12. A composition according to claim 11, wherein the antioxidative sesame component extracted from sesame residue is extraction using a solvent, a lipid, or an emulsifier singly or in combination.

13. A composition according to claim 1, wherein the ascorbyl fatty acid ester contains ascorbyl palmitate or ascorbyl stearate.

14. A composition according to claim 1, wherein the ascorbic acid or the ascorbyl fatty acid ester is contained in

an excessive amount more than the amount soluble in the poly unsaturated fatty acid or its salt or ester.

15. A composition according to claim 14, wherein the excessive amount of the ascorbic acid is in a powder or solid form.

16. A composition according to claim 1, further comprising tocopherol.

17. A food containing the composition as set forth in claim 1.

18. A powdered oil or fat containing the composition as set forth in claim 1.

19. A powdered baby milk containing the composition as set forth in claim 1.

20. A health food containing the composition as set forth in claim 1.

* * * * *



US 20030100610A1

13

(19) **United States**

(12) **Patent Application Publication**
Shibuya

(10) **Pub. No.: US 2003/0100610 A1**

(43) **Pub. Date: May 29, 2003**

(54) **USE OF POLYUNSATURATED FATTY ACIDS
FOR THE PRIMARY PREVENTION OF
MAJOR CARDIOVASCULAR EVENTS**

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(21) **Appl. No.: 10/281,208**

(22) **Filed: Oct. 28, 2002**

(30) **Foreign Application Priority Data**

Nov. 12, 2001 (IT) MI2001 A 002384

Publication Classification
(51) **Int. Cl.⁷ A61K 31/202**
(52) **U.S. Cl. 514/560**

(57) **ABSTRACT**

The use of polyunsaturated fatty acids of the ω-3 series such as eicosapentaenoic acid (EPA, C_{20:5} ω-3), docosahexaenoic acid (DHA, C_{22:6} ω-3), or their pharmaceutically acceptable derivatives is described for the primary prevention of major cardiovascular events in subjects who have not undergone previous infarct episodes.

other defects of mechanical type (cardiac insufficiency, decompensation), possibly aggravated by concomitant pathologies such as diabetes.

[0015] The use of polyunsaturated fatty acids of the ω -3 series according to the invention is particularly indicated if the occurrence of a major event is predicted, such as an infarct, in particular of the myocardium, death from a cardiological cause, or sudden death, and where such an occurrence takes place in cardiopathic subjects affected, for example, by coronary ischemia, arrhythmia, atrial and/or ventricular fibrillation, electrical hyperexcitability of the myocardium cells, disorder of the diffusion of electrical excitement or of electrical conduction of the myocardium, or cardiac disorders of mechanical type, for example cardiac insufficiency or cardiac decompensation, possibly affected by diabetic pathology concomitant with the cardiopathy.

[0016] Preferably, the content of EPA and/or DHA and/or of the at least one derivative thereof is between 50% and 100%, in particular between 75% and 95%, and more preferably about 85% by weight on the total fatty acid weight. The preferred EPA and/or DHA derivatives are selected from the corresponding C_1 - C_3 alkyl esters and/or from their salts with pharmaceutically acceptable bases such as sodium hydroxide, lysine, arginine or aminoalcohols such as choline. The ethylesters of EPA and DHA, in particular mixed together in any concentration and percentage, are the most preferred.

[0017] The drug is administered preferably orally, in particular in the form of soft gelatin capsules. For oral use, the unit dose generally comprises 100-1000 mg of polyunsaturated fatty acids of the ω -3 series, preferably 500-1000 mg or 300-500 mg, the total dose being usually around 0.1-3.0 g per day or per alternate day, according to the case concerned, and preferably 0.3-2.0 g per day and in particular 1.0 g per day. The effective dose of the drug suitable for the use of the invention is 1.0-60.0 mg/kg of body weight/day.

[0018] Other types of formulation for oral administration are also suitable for the purposes of the invention; for example hard capsules or tablets, in which the polyunsaturated fatty acids are adsorbed on solid supports. It is also possible to use emulsions, granulates in dispersing excipients, syrups, droplets, etc., and other forms of administration able to ensure systemic absorption of the drug, such as sterile solutions or emulsions and the like, suitable for parenteral use and the like, as evaluated by the expert of the art, on the basis of the severity of the pathology.

[0019] Those compositions illustrated in the European Pharmacopea 2000 (EuPh. 2000), containing quantities greater than or equal to 80 wt % of mixtures of EPA and DHA ethylesters and a total of ω -3 polyunsaturated fatty acid ethylesters greater than or equal to 90 wt % are also suitable for the purposes of the present invention.

[0020] The aforesaid compositions and the drugs suitable for the use of the invention can be prepared by methods known to the expert of the art, such as those described in U.S. Pat. No. 5,130,061, WO 89/11521, IT 1235879, JP 02/25447, which are incorporated into the present description with regard to the method of preparation.

[0021] The drug suitable for use according to the present invention can also comprise other active principles and/or drugs, in association, possessing activity complementary to or synergic with that of the drug suitable for use according to the invention, and also at least one pharmaceutically acceptable vehicle and/or one diluent and/or one surfactant and/or one thickener and/or one binder and/or one lubricant and/or one aromatizer and/or one colorant and/or one stabilizer and the like, which can easily be selected by the expert of the art. Of the stabilizers, antioxidants such as vitamin E (tocopherol), ascorbyl palmitate, ascorbic acid, hydroxytoluene and the like, which can be easily selected by the expert of the art, are particularly preferred.

[0022] According to another aspect, the invention relates to a method for the primary prevention of a major cardiovascular event in subjects who have not undergone previous infarct episodes, comprising the administration of an effective dose of a drug comprising polyunsaturated fatty acids of the ω -3 series as hereinbefore described. In particular, the method of the invention is indicated whenever the occurrence of a major cardiac event is predicted such as an infarct, in particular of the myocardium, death from a cardiological cause or sudden death.

[0023] The following examples illustrate the invention but without limiting it.

[0024] The compositions illustrated in the following table were prepared by the methods described in U.S. Pat. No. 5,130,061 (compositions A, C, D, F), IT 1235879 (composition B), JP 02/25447 (composition E) and WO 89/11521 (compositions G-I).

[0025] All the quantities indicated in the following table express percentages by weight on the total weight of polyunsaturated fatty acids of the ω -3 series.

	A ¹	B ¹	C ¹	D ¹	E ¹	F ¹	G ¹	H ²	I ³
EPA	>40	>44	>40	>25	>80	>20	<15	>40	>40
DHA	>34	>30	>34	>20	<10	>25	>80	>30	>30
EPA + DHA	>85	>80	>80	>50	>80	>50	>85	>80	>80
esters ⁴		>3	—						
tot. esters ⁵			>90						
α -tocopherol	0.03	0.03	0.1	0.3	0.1	0.3	0.03	0.1	0.1

¹-ethyl esters; ²-acids; ³-sodium salts; ⁴-ethyl esters of other (C_{20} , C_{21} , C_{22}) ω -3 acids;
⁵-total ethyl esters of ω -3 acids.

EXAMPLE 2

[0026] The compositions illustrated in the following table, relative to soft gelatin capsules containing 1 g of polyunsaturated fatty acid ethyl esters, were prepared by methods known in the art.

	A (mg)	B (mg)	C (mg)
EPA ¹	525	—	>400
DHA ¹	315	—	>340
EPA + DHA		850	>800
Total ω-3 ¹		—	>900
d-α-tocopherol	4 I.U.	—	4 I.U.
d,l-α-tocopherol		0.3	
gelatin	246	—	246
gelatin succinate		233	
glycerol	118	67	118
OFR	2.27	—	2.27
OFG	1.27	—	1.27
SOB		1.09	
SPOB		0.54	

¹-ethyl esters; OFR: red iron oxide OFG: yellow iron oxide; SOB: sodium p-oxybenzoate; SPOB: sodium propyl p-oxybenzoate; I.U.: international units.

[0027] Pharmacological Activity

[0028] The pharmacological activity of the compositions of the invention was evaluated on the basis of tests carried out on small laboratory animals (mouse, guinea pig, rat); this experimental model was chosen because of the ability to make rapid and highly reproducible verifications and to use a sufficiently large number of animals, such as to enable a statistically accurate evaluation of the results to be made without exposing the patient to risk, with evident ethical implications.

[0029] During the course of these tests, groups of animals were pretreated repeatedly with the formulations of Examples 1 and 2 and then, in comparison with untreated groups, were subjected to the action of cardiotoxic or respiration-depressive substances, then visually measuring protection against death, or—by means of electrocardiographic recording—measuring the delay in the start of initial cardiac arrhythmia or of ventricular tachycardia and above all the delay in or the prevention of animal death due to sudden cardiac and/or respiratory arrest.

[0030] Using an analogous experimental model, cardiolo- gical pathology, coronary ischemia and a state of infarct were induced by coronary ligation instead of by cardiotoxic agents.

[0031] Test 1

[0032] The experimental sudden death model was obtained by cardiac arrest induced by intravenous (i.v.) administration of a cardiotoxic agent (ouabain). In preliminary tests, various doses of ouabain were administered to non-anesthetized guinea pigs of both sexes of weight 300-380 g, in order to determine the minimum lethal dose for 100% of the animals within 15 minutes from i.v. injection (240 mg/kg, intravenously administered over 3 minutes).

[0033] Two groups of 20 guinea pigs were then treated with 50 and 100 mg/kg of a composition containing 85% of EPA and DHA ethylesters (Ex. 1, composition A) for 10 days. After 2 hours from the last administration the two

groups of guinea pigs and a further untreated group, used as control, were treated with 240 mg/kg i.v. of ouabain, recording mortality within the subsequent 15 minutes.

[0034] Results expressed as survivors after 15 minutes:

Controls	00/20
50 mg/kg	11/20
100 mg/kg	16/20

[0035] Test 2

[0036] 3 groups of 15 male mice, initial weight 25-32 g, were treated orally for 15 days with physiological solution (control group) and with 50 or 100 mg/kg of a composition containing 85% of EPA and DHA ethylesters (Ex. 1, composition A).

[0037] 60 minutes after the end of the last treatment, the animals of all the groups were treated with sodium pentobarbital i.p. (50 mg/kg) and then with aconitine i.v. (0.25 mg/kg). The times of appearance of cardiac arrhythmia (deviation >5 seconds from the normal sinus rhythm), of ventricular fibrillation and of cardiac arrest were determined by electrocardiograph recording. Results expressed as mean±standard deviation (seconds) on the positive animals.

T	t ₁ (sec)	t ₂ (sec)	t ₃ (sec)	S
C	123 ± 12 (15/15)	174 ± 7 (15/15)	214 ± 32 (15/15)	00/15
50 mg/kg	168 ± 8 (08/15)	235 ± 16 (06/15)	350 ± 26 (06/15)	09/15
100 mg/kg	195 ± 15 (05/15)	284 ± 18 (03/15)	378 ± 35 (03/15)	12/15

T-treatment; t₁-time of appearance of arrhythmia (number of animals); t₂-time of appearance of fibrillation (number of animals); t₃-time of cardiac arrest (number of animals); S-survivors, after 15 minutes; C-control (with physiological solution).

[0038] Test 3

[0039] 2 groups of 20 male rats, initial weight 310-350 g, were treated orally for 15 days with physiological solution (control group) and with 100 mg/kg of a composition containing >80% of EPA and DHA ethylesters (Ex. 1, composition B). The rats of the 2 groups were then anesthetized with sodium pentobarbital i.p. (50 mg/kg), then subjected to ligation of the left anterior descending coronary artery, which allows blood flow to the left ventricle, so inducing an acute ischemic state of the myocardium. During the subsequent 15 minutes the duration of ventricular fibrillation was recorded by ECG, this either resolving itself spontaneously or concluding with sudden death.

[0040] Results

T	F (sec)	Mortality	S (%)
C	190 ± 24 (18/20)	16/20	20
100 mg/kg	55 ± 5 (04/20)	01/20	95

T-treatment; F-duration of fibrillation; S-survivals after 15 minutes; C-control (with physiological solution).



US 20030104048A1

16

(19) **United States**

(12) **Patent Application Publication**

Patel et al.

(10) **Pub. No.: US 2003/0104048 A1**

(43) **Pub. Date: Jun. 5, 2003**

(54) **PHARMACEUTICAL DOSAGE FORMS FOR
HIGHLY HYDROPHILIC MATERIALS**

Jun. 8, 2001, which is a continuation-in-part of appli-
cation No. 09/345,615, filed on Jun. 30, 1999, now
Pat. No. 6,267,985.

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Publication Classification

(51) **Int. Cl.⁷** **A61K 9/00; A61K 9/48**
(52) **U.S. Cl.** **424/451; 424/400**

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(57) **ABSTRACT**

(73) Assignee: **LIPOCINE, INC.**

(21) Appl. No.: **10/158,206**

(22) Filed: **May 29, 2002**

Related U.S. Application Data

(63) Continuation-in-part of application No. 09/898,553,
filed on Jul. 2, 2001, now Pat. No. 6,451,339, which
is a continuation of application No. 09/258,654, filed
on Feb. 26, 1999, now Pat. No. 6,294,192. Continu-
ation-in-part of application No. 09/877,541, filed on

Pharmaceutical dosage forms having a highly hydrophilic
fill material and a shell encapsulating the fill material are
disclosed and described. Generally, the shell has at least one
plasticizing agent therein in order to provide the shell with
an effective plasticity. In one aspect, the shell may have
included therein an amount of plasticizing agent that is
sufficient to provide the shell with an effective plasticity
upon migration of a portion of the plasticizing agent into the
fill material. In another aspect, the plasticizing agent may
have a solubility in the fill material of less than about 10%
w/w. In yet another aspect, a combination of a plasticizing
agent, and a plasticizing agent having a solubility in the fill
material of less than about 10% w/w, may be presented in a
total amount sufficient to provide the shell with an effective
plasticity upon migration of plasticizing agent into the fill
material.

17

the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited.

[0029] As an illustration, a concentration range of "about 4% w/w to about 60% w/w" should be interpreted to include not only the explicitly recited concentration of about 4% w/w to about 60% w/w, but also include individual concentrations and the sub-ranges within the indicated range. Thus, included in this numerical range are individual concentrations such as 4% w/w, 10% w/w, 23% w/w, and 46% w/w, and sub-ranges such as from 10% w/w to 50% w/w, from 20% w/w to 40% w/w, from 25% w/w to 35% w/w, from 15% w/w to 20% w/w, etc.

[0030] This same principle applies to ranges reciting only one numerical value. Furthermore, such an interpretation should apply regardless of the breadth of the range or the characteristics being described.

[0031] Invention

[0032] Applicants have discovered pharmaceutical dosage forms having a shell that retains an effective plasticity while encapsulating a highly hydrophilic fill material. Such dosage forms present a number of advantages including increased freedom in formulating, processing and manufacturing specific dosage forms, increased absorption and/or efficacy of the active ingredient, more consistent performance of the dosage form with respect to disintegration of the dosage and dissolution/ solubilization of the active ingredient and improved storage stability.

A. Shell Formulations

[0033] The shell of the present invention may be either a hard or soft capsule shell, and includes a number of fundamental constituents as will be recognized by those of ordinary skill in the art, namely a matrix forming material, and at least one plasticizing agent. A wide variety of matrix forming materials are suitable for use in the dosage forms of the present invention, and the selection of specific materials may be based, at least in part, on factors such as the specific results to be achieved. Examples of specific materials include without limitation, gelatins, including type A gelatins, such as the gelatin derived from acid-treated pigskins, and type B gelatins, such as those derived from alkali-treated bovine bones and hides, hydroxypropyl methylcellulose (HPMC), starches, and gum acacia. Other specific matrix forming materials that may be particularly desired in view of a given overall dosage form can be determined by those of ordinary skill in the art.

[0034] The specific amount of matrix forming material used in the shell formulation may be determined in part by a variety of factors, including the type of shell to be formed (i.e. hard or soft), and by the amount and type of other constituents or additives that are to be included in the shell. However, in one aspect, the amount of matrix forming material may be from about 20% w/w to about 70% w/w of the shell. In another aspect, the amount may be from about 30% w/w to about 50% w/w of the shell.

[0035] Many plasticizing agents are known, and may also be used in the shell of the present dosage form. One basis for selecting a particular plasticizing agent may be the solubility of that agent in a specific hydrophilic fill material to be used. In one aspect, the plasticizing agent may have a solubility of

less than about 10% w/w in the fill material. In another aspect, the solubility of the plasticizing agent in the fill material may be less than about 5% w/w. In yet another aspect, the solubility may be less than about 1% w/w. In a further aspect, the solubility of the plasticizing agent may be less than about 0.5% w/w. Lowered solubility in the specific hydrophilic fill material substantially impedes the migration of the plasticizing agent out of the shell and into the fill material. Examples of specific plasticizing agents displaying such limited solubilities in many hydrophilic surfactant materials include without limitation: sorbitol, sorbitanes, xylitol, maltitol, maltitol syrup, partially dehydrated hydrogenated glucose syrups, hydrogenated starch hydrolysate, polyhydric alcohols having an equilibrium relative humidity of greater than or equal to 80%, carrageenan, polyglycerol, non-crystallizing solutions of sorbitol, glucose, fructose, glucose syrups, and mixtures and equivalents thereof.

[0036] Whether the plasticizing agent selected and used is one that has a low solubility in the fill material or not, in accordance with one aspect of the invention, the plasticizing agent may be presented in an amount that is sufficient to maintain an effective shell plasticity upon migration of a portion of the plasticizing agent from the shell and into the fill and/or may be present in a sufficient amount to maintain a desirable dissolution/disintegration profile with respect to the rate and the extent release and/or dispersing of the encapsulated active agent in a specific dissolution medium or upon administration inside the GI tract. The exact amount of plasticizing agent required to compensate for the plasticizing agent anticipated to be lost may depend on a variety of factors, such as the specific fill material and solubility of the plasticizing agent therein. However, those of ordinary skill in the art will be able to readily determine approximate amounts required to maintain effective shell plasticity based on the known characteristics presented by a given dosage form, and will further be able to identify specific amounts through routine experimentation with the dosage form. In one aspect of the invention, such an amount of plasticizing agent may be from about 4% w/w to about 60% w/w of the shell. In another aspect, the amount may be from about 10% w/w to about 35% w/w.

[0037] An additional option for maintaining effective shell plasticity and/or a desirable dissolution/disintegration profile of the encapsulated active agent in view of the highly hydrophilic fill material is to include a combination of plasticizing agents in the shell in a total amount sufficient to maintain effective shell plasticity upon migration of a portion of either or both agents into the fill material. In one aspect of the invention, such a combination may include a first plasticizing agent, and a second plasticizing agent having a limited solubility in the fill material as recited above. The total amounts and ratios of each ingredient required to maintain an effective plasticity may be determined by one of ordinary skill in the art in the manners already indicated. While a variety of ratios and amounts are contemplated, in one aspect, the total amount of combined plasticizing agent may be within the ranges already established for plasticizing agents herein.

[0038] In addition to the components of a matrix forming material and the at least one plasticizing agent, the shells used in the dosage forms of the present invention may include additional additives as required, in order to achieve a specifically desired formulation or result. Examples of



US 20070105954A1

128

(19) **United States**
(12) **Patent Application Publication** (10) **Pub. No.: US 2007/0105954 A1**
Puri (43) **Pub. Date: May 10, 2007**

(54) **FORMULATION CONTAINING A
CARBOXYLIC ACID OR AN ESTER
THEREOF**

(86) PCT No.: **PCT/GB04/05461**
§ 371(c)(1),
(2), (4) Date: **Jan. 19, 2007**

(75) Inventor: **Basant Kumar Puri**, Cambridge (GB)

(30) **Foreign Application Priority Data**
Dec. 31, 2003 (GB)..... 0330206.4
Jul. 6, 2004 (GB)..... 0415097.5

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Publication Classification

(51) **Int. Cl.**
A61K 31/215 (2006.01)
A61K 31/202 (2006.01)
(52) **U.S. Cl.** **514/529; 514/559; 514/560**

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(57) **ABSTRACT**
A formulation comprising eicosapentaenoic acid, or an ester thereof, and a triterpene, or an ester thereof, and the use of such a formulation.

(21) Appl. No.: **10/585,026**

(22) PCT Filed: **Dec. 31, 2004**

powerful anti-inflammatory and immuno-modulating effects and thus can be used to treat the above plethora of medical conditions.

[0016] The formulation comprising eicosapentaenoic acid or an ester thereof, and a triterpene or an ester thereof, may be administered orally.

[0017] The formulation may be administered orally as a liquid, a paste, a tablet or a capsule.

[0018] Preferably a capsule for oral administration contains a formulation comprising between about 260 mg and about 300 mg of eicosapentaenoic acid and between about 80 mg and about 120 mg of virgin evening primrose oil, and preferably substantially none, or no, docosahexaenoic acid. More preferably a capsule contains a formulation comprising about 280 mg of eicosapentaenoic acid, about 100 mg of virgin evening primrose oil and preferably substantially none, or no, docosahexaenoic acid. The capsule may also comprise between about 3 mg and about 100 mg of conjugated linoleic acid, more preferably between about 5 mg and about 80 mg of conjugated linoleic acid, preferably about 60 mg of conjugated linoleic acid.

[0019] The oral formulation may be prepared as an inert porous matrix tablet which is obtained by mixing the eicosapentaenoic acid or an ester thereof, and a triterpene or an ester thereof, with waxes or water insoluble polymers and with fillers and binders. Paraffin, polyvinylchloride, ethylcellulose, stearic alcohol, cetyl alcohol, carnauba wax, polyethylene, polyvinyl acetate, polymethyl methacrylate could be used as suitable diffusion retarding compounds. Other excipients used in the preparation of such tablets may include lactose, mannitol, calcium phosphate, magnesium stearate, hydroxypropyl methylcellulose, methyl cellulose, polyvinylpyrrolidone, aluminium silicate, sodium carbonate, potassium phosphate or other suitable materials.

[0020] Alternatively, the formulation comprising eicosapentaenoic acid or an ester thereof, and a triterpene or an ester thereof, may be administered topically. The formulation to be applied topically may also comprise one or more of occlusive agent, a surfactant system, a solvent and water.

[0021] One or more various solvents that may be present in the topical formulation comprise various short-chain alcohols including, but not limited to, ethyl alcohol, propylene alcohol, triacetin, hexylen glycol and combinations thereof. The solvent may be present in an amount ranging from about 5.0 to about 30.0 w/w %.

[0022] Suitable occlusive agents that may be present in the topical formulation include, but are not limited to, petrolatum, microcrystalline wax, dimethicone, beeswax, mineral oil, squalane, liquid paraffin, shea butter, carnauba wax, SEPIGELO™ (a blend of isoparaffin/polyacrylamide/

[0023] laureth-7), and combinations thereof. The occlusive agent may be present in an amount of at least about 10.1 w/w %.

[0024] Suitable surfactant systems comprise at least one surfactant and exhibit a HLB value in a range from about 7.0 to about 10.9. The surfactant system may be present in the formulation in an amount ranging from about 0.25 to about 10.0 w/w%. Suitable surfactants include, but are not limited to, CETOMACROGOL™ 1000 (Croder, Inc.), glycerol monostearate, glycerol distearate, glyceryl stearate, poly-

oxyethylene stearate, a blend of glyceryl stearate and PEG-100 stearate (as ARLACEL™ 165), polysorbate 40, polysorbate 60, polysorbate 80, CETETH™-200, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, and combinations thereof.

[0025] The topical formulation may also include a carrier, a skin conditioner, a preservative, a buffer, a fragrance, water or combinations thereof.

[0026] According to another aspect the invention provides a method for the treatment of various physiological and disease states including rheumatoid arthritis, osteoarthritis, back-ache, psoriasis, pre-menstrual syndrome, bacterial infections, viral infections, fatigue, such as chronic fatigue syndrome, insomnia, anxiety, obesity, influenza, diabetes mellitus, alcoholism, cancer, neurological disorders such as multiple sclerosis, epilepsy, tardive dyskinesia and choreiform disorders such as Huntington's disease, psychiatric disorders such as depression and attention-deficit/hyperactivity disorder, cardiovascular disorders such as hyperlipidemia and high blood pressure, dermatological disorders such as eczema and atopic dermatitis, respiratory disorders, learning disability and ageing, in a subject comprising administering to the subject an effective amount of a formulation comprising eicosapentaenoic acid or an ester thereof, and a triterpene or an ester thereof.

[0027] Preferably the formulation comprises substantially no docosahexaenoic acid. Preferably the formulation comprises no docosahexaenoic acid.

[0028] According to a further aspect the invention provides a formulation comprising eicosapentaenoic acid or an ester thereof, and a triterpene or an ester thereof for use in a method of treatment of a human or animal body by surgery or therapy or of diagnosis practised on the human or animal body.

[0029] In a further aspect the invention provides the use of eicosapentaenoic acid or an ester thereof, and a triterpene or an ester thereof, in the manufacture or preparation of a medicament for the treatment of various physiological and disease states including rheumatoid arthritis, osteoarthritis, back-ache, psoriasis, pre-menstrual syndrome, bacterial infections, viral infections, fatigue, such as chronic fatigue syndrome, insomnia, anxiety, obesity, influenza, diabetes mellitus, alcoholism, cancer, neurological disorders such as multiple sclerosis, epilepsy, tardive dyskinesia and choreiform disorders such as Huntington's disease, psychiatric disorders such as depression and attention-deficit/hyperactivity disorder, cardiovascular disorders such as hyperlipidemia and high blood pressure, dermatological disorders such as eczema and atopic dermatitis, respiratory disorders, learning disabilities and ageing.

[0030] Preferably the medicament comprises substantially no docosahexaenoic acid. Preferably the medicament comprises no docosahexaenoic acid.

[0031] Formulations comprising eicosapentaenoic acid or an ester thereof, and a triterpene or an ester thereof, may be used in cosmetic treatments. The cosmetic treatment may have an anti-ageing effect or reverse the process of ageing.

[0032] Preferably the formulation comprises eicosapentaenoic acid or an ester thereof, and a triterpene or an ester thereof in a cosmetically acceptable form.

described Method 2, to her hands. The improvements observed included relief from the pain, for the first time, and a decrease in the size of tophi (swellings).

Therapeutic/Cosmetic Effect—Skin Sores

[0094] A 69-year-old female subject with severe rheumatoid arthritis (see above) also noticed that her skin sores on her hands were much better seven to eight days after beginning use of the cream made by the above-described Method 2. They had previously failed to respond to medical treatment and had had to be bandaged.

Therapeutic Effect—Psoriasis

[0095] Subject 1—A female aged 17 with severe intractable treatment-resistant psoriasis started to improve after two to three days following topical application of cream, made by the above-described Method 2, to her arms and legs.

[0096] Subject 2—A female aged 31 with severe psoriasis affecting her upper limbs responded after six to seven days when applying the cream, made by the above-described Method 2, to her upper limbs; she had previously tried a wide range of medical and 'alternative' treatments, to no avail.

Therapeutic/Cosmetic Effect—Eczema

[0097] A 52-year-old female subject with severe eczema responded within one week to the topical application of cream, made by the above-described Method 2, again where conventional medical treatment had previously failed.

Oral Administration—Learning Difficulties

[0098] An 11-year-old boy with learning difficulties started taking 1.5 g daily of the oral formulation discussed above with reference to Method 3. Within four weeks he started to show signs of improvement according to his parents and teachers. This improvement was in several domains, including cognitive functioning, reading and understanding his school work. The improvement continued and he reached a new, higher, level of intellectual functioning after three months, which has continued to be sustained for 6 months.

Oral Administration with Capsules According to Method 4

[0099] In the following examples all patients were administered capsules containing a formulation comprising 280 mg pure eicosapentaenoic acid, 100 mg virgin evening primrose oil, 5 mg conjugated linoleic acid and no docosahexaenoic acid.

Chronic Fatigue Syndrome (Myalgic Encephalomyelitis)

[0100] By the end of August 2004, a total of 109 patients meeting the 1994 CDC Revised Diagnostic Criteria for chronic fatigue syndrome had been treated with capsules according to Method 4. The first eight were treated with a dose of between four and six capsules daily; five of them showed significant clinical improvement within three months. The remaining 101 patients were treated with eight capsules daily; 81 showed significant clinical improvements in 4 months. Improvements were particularly noticeable in the following areas: reduced fatigue; increased energy levels; improved quantity and quality of sleep; and reduced myalgia. These 81 patients no longer fulfil the 1994 CDC Revised Diagnostic Criteria for chronic fatigue syndrome.

Depression

[0101] By the end of August 2004, a total of 86 patients meeting the American Psychiatric Association DSM-IV-TR criteria for major depressive disorder had been treated with capsules according to Method 4 at an average dose of eight capsules daily. Seventy-two of had shown significant clinical improvement in 4 months. Improvements were particularly noticeable in the following areas: improved mood; improved quantity and quality of sleep; reduced fatigue; increased energy levels; reduced social phobia; reduced tearfulness; and increased libido. Suicidal feelings were amongst the first symptoms to improve—in fact, in all cases they cleared up completely. These 72 patients no longer fulfil the DSM-IV-TR criteria for major depressive disorder, and have shown marked improvements in their depression ratings as measured by the Hamilton Depression Rating Scale and the Montgomery and Asberg Depression Rating Scale.

Huntington's Disease

[0102] Two patients with Huntington's disease took capsules according to Method 4, at a dose of eight capsules daily. Within three months they both started to notice improvements, particularly in respect of their movement disorder and mood.

Skin Disorders

[0103] One-hundred-and-ninety patients with eczema or psoriasis had been treated with capsules according to Method 4 by the end of August 2004, at an average dose of four capsules daily. One-hundred-and-seventy-six had shown significant clinical improvements in their skin condition within 4 months. This has been evident on examination of the skin, and also in terms of reduced pruritus.

Rheumatoid Arthritis and Osteoarthritis

[0104] One-hundred-and-forty-seven patients with arthritis had been treated with capsules according to Method 4 at a dose of between six and eight capsules daily by the end of August 2004. One-hundred-and-twenty-three showed significant clinical improvements within 4 months, including improvements in the range of joint movement and reduced joint pain.

1-46. (canceled)

47. A formulation comprising:

- (a) eicosapentaenoic acid or an ester thereof,
- (b) a triterpene or an ester thereof, and
- (c) substantially no docosahexaenoic acid.

48. A formulation according to claim 47 comprising no docosahexaenoic acid.

49. A formulation according to claim 47 in which the eicosapentaenoic acid or ester thereof is selected from the group comprising natural eicosapentaenoic acids, synthetic eicosapentaenoic acids, naturally occurring esters of eicosapentaenoic acids, synthetic esters of eicosapentaenoic acids, and combinations thereof.

50. A formulation according to claim 47 in which the eicosapentaenoic acid or ester thereof is isolated from fish oil.

51. A formulation according to claim 47 in which the eicosapentaenoic acid or ester thereof is pure.

52. A formulation according to claim 47 in which the triterpene or ester thereof is selected from the group comprising naturally occurring triterpenes, synthetic triterpenes, naturally occurring esters of a triterpene, and synthetic esters of a triterpene, and combinations thereof.

53. A formulation according to claim 52 in which the triterpene is selected from the group comprising 3-O-trans caffeoyl derivatives of betulinic acid, morolic acid or ole-anolic acid, faradiol-O-laurate, faradiol-O-palmitate and faradiol-O-myristate.

54. A formulation according to claim 47 in which the triterpene is isolated from the flower heads of marigolds (*Calendula officinalis*), *Zygophyllum eichwaldii*, *Carthamus lanatus*, *Oenothera bienni* (evening primrose) or *Pyrus comminus*.

55. A formulation according to claim 54 in which the triterpene is provided in the form of evening primrose oil.

56. A formulation according to claim 55 in which the evening primrose oil is virgin evening primrose oil.

57. A formulation according to claim 47 comprising up to 99% w/w of eicosapentaenoic acid or an ester thereof.

58. A formulation according to claim 47 comprising up to 99% w/w of triterpene or an ester thereof.

59. A formulation according to claim 57 comprising up to 70% w/w of eicosapentaenoic acid or an ester thereof and from 1 to 30% w/w of a triterpene or an ester thereof.

60. A formulation according to claim 47 in a pharmaceu-tically or cosmetically acceptable form.

61. A formulation according to claim 47 comprising a pharmaceutical or cosmetic carrier, diluent or excipient.

62. A formulation according to claim 47 comprising one or more compounds for co-administration.

63. A formulation according to claim 62 comprising gamma-linolenic acid.

64. A formulation according to claim 62 comprising conjugated linoleic acid.

65. A liquid, a paste, a tablet or a capsule for oral administration, or a compound for topical administration, wherein the liquid, paste, tablet capsule or compound com-prises:

- (a) eicosapentaenoic acid or an ester thereof;
- (b) a triterpene or an ester thereof; and
- (c) substantially no docosahexaenoic acid.

66. A method of cosmetic treatment, or a method for the treatment of physiological or disease states including rheu-matoid arthritis, osteoarthritis, back-ache, psoriasis, pre-menstrual syndrome, bacterial infections, viral infections, fatigue, insomnia, anxiety, obesity, influenza, diabetes mel-litus, alcoholism, cancer, neurological disorders, epilepsy, tardive dyskinesia and choreiform disorders, psychiatric disorders, cardiovascular disorders, dermatological disor-ders, respiratory disorders, learning disability and ageing, in a subject, the method comprising administering to the sub-ject an effective amount of a formulation comprising:

- (a) eicosapentaenoic acid or an ester thereof;
- (b) a triterpene or an ester thereof; and
- (c) substantially no docosahexaenoic acid.

67. The method of claim 66, wherein the eicosapentaenoic acid, or an ester thereof, and the triterpene, or an ester thereof, are administered simultaneously, either in the same or different formulations, or sequentially.

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