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JOINT

(57) **Abrégé/Abstract:**

The present invention relates to vesicular formulations for use in the treatment of pain and/or reduced mobility associated with a loss of lubrication and/or structural integrity and/or swelling of a collagen structure. It also relates to a method of treating pain such as joint pain or tendonitis comprising topically administering a vesicular formulation according to the invention.

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(54) Title: VESICULAR FORMULATIONS FOR USE IN THE TREATMENT OF PAIN OR REDUCED MOBILITY OF A JOINT

(57) Abstract: The present invention relates to vesicular formulations for use in the treatment of pain and/or reduced mobility associated with a loss of lubrication and/or structural integrity and/or swelling of a collagen structure. It also relates to a method of treating pain such as joint pain or tendonitis comprising topically administering a vesicular formulation according to the invention.

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## VESICULAR FORMULATIONS FOR USE IN THE TREATMENT OF PAIN OR REDUCED MOBILITY OF A JOINT

The present invention relates to vesicular formulations for use in the treatment of pain and/or reduced mobility associated with a loss of lubrication and/or structural integrity and/or swelling of a collagen structure. It also relates to a method of treating pain such as joint pain or tendonitis comprising topically administering a vesicular formulation according to the invention.

In healthy joints (such as the knee), the bones are not directly connected but are joined by connective tissue that forms a capsule around the ends of the bones. A layer of cartilage (which comprises collagen as the major tissue, as well as proteoglycans and elastins) covers the ends of the bones within the joint. The area between the bones, within the capsule, is filled with synovial fluid. The surface of the cartilage layer found on the end of each bone is coated with a layer of phospholipids which are anchored within the cartilage structure. The hydrophilic head of each molecule is orientated into the synovial space and each is highly hydrated, which allows free movement of the joint without pain or discomfort. As the phospholipids on the cartilage surface deplete, in a healthy joint they are replenished by phospholipids present in the synovial fluid. Articular cartilage as well as other collagenous material such as ligaments and tendons, consist of a matrix of structural molecules and cellular material. One integral structural molecule is proteoglycan which is highly hydrated due to the molecule being highly sulphated. The hydroscopic nature of these structural subunits gives cartilage and other collagenous materials high tensile strength.

In a damaged joint, for example due to osteoarthritis, overuse, excess weight etc, inflammation occurs. One factor of the inflammation cascade is an increase in phospholipases. These enzymes break down the phospholipids on the cartilage surface and in the synovial fluid, leading to the cleavage of the lipids and thus degradation of the protective layer between the layers of cartilage. This loss of protection leads to further progressive damage of the cartilage by way of erosion and friction, which leads to pain and/or reduced mobility of the joint. In osteoarthritis (OA), for example, the proteoglycan molecules (a key factor of the structural



integrity) break down and become fragmented. This degradation reduces the water capacity and therefore water content of the collagenous components of the joint thereby losing structural support. Through this mechanism the structural integrity of various joint structures including articular cartilage, ligaments, joint membranes etc is compromised causing various OA symptoms, including pain, loss of mobility and swelling. Joint degradation causes pain via the release of pain mediators from destroyed cells. Loss of structural integrity contributes to further cellular destruction and, macroscopically, joint degradation, both factors increasing the release of pain mediators.

Currently, either the pain is managed by way of analgesics and/or anti-inflammatory medication. Alternatively, hyaluronic acid, a polymer can be injected on a regular basis in to the synovial capsule to provide enhanced temporary lubrication of the joint. However, the procedure is painful in itself, involving the injection of the polymer using a large bore needle directly into the joint. The procedure must be repeated on a monthly or up to three monthly basis and can only be carried out by a specialised physician or other medical professional. Clearly this is far from ideal in managing joint pain on a long term basis.

Joint pain and/or reduced mobility may also be due to a loss of lubrication and/or structural integrity and/or swelling due to the degradation or lack of a protein called lubricin, a proteoglycan thought to assist in the free movement of joints, which is found in the synovial fluid. The vesicular formulation used in accordance with the invention can provide lubrication and thus pain relief, when lubricin is depleted. The formulation of the invention can provide an increase in joint mobility.

Carpal tunnel syndrome can be caused by inflammation in the wrist, causing pressure to build up around the nerves. The present invention can provide lubrication around the tendons and ligaments of the wrist, allowing the inflammation to subside and reduce the pressure on the nerves, and thus reducing pain, and increasing mobility.

It is also known that specialised liposomes can provide a lubricating film to mitigate the effects of inflammatory damage. (Sivan, S. *et al* (2010) Longmuir, 26(2), 1107-1116). However, these would require intra-articular injection to deliver the liposomes to the site of damage. This would be a monthly procedure in all likelihood. The procedure is the same as described above, and therefore both inconvenient and painful for the patient. The liposomes, in order to be effective, must also be highly hydrated, highly compressible and very stable. Vesicles lacking any one of these properties will not provide suitable lubrication to overcome the lack of natural lubrication in the inflamed joint.

Therefore, present invention provides a vesicular formulation comprising a phospholipid and a surfactant for use in the treatment of pain and/or reduced mobility associated with a loss of lubrication as well as a loss of structural integrity and/or swelling of a collagen structure in an animal by topically applying the vesicular formulation to the skin surrounding the collagen structure. The collagen structure may be cartilage within an articulated joint or it may be a tendon or a ligament.

The vesicular formulation for use according to the invention may be useful in the prevention or treatment of joint locking or joint freezing (i.e. immobility of a joint) associated with a loss of lubrication and/or structural integrity and/or swelling of a collagen structure in an animal.

It is appreciated by the skilled person that pain due to a loss of lubrication and/or structural integrity and/or swelling may be treated independently of reduced mobility due to a loss of lubrication and/or structural integrity and/or swelling. The present invention is concerned with the treatment of pain, of reduced mobility or a combination of pain and reduced mobility. Improving the level of pain may result in increased mobility and vice versa. A loss of lubrication and/or structural integrity and/or swelling may result in a loss of mobility but little pain or vice versa. Thus, the present invention is useful for the treatment of pain and/or reduced mobility.



5 The vesicular formulation for use according to the invention may treat pain and/or reduced mobility due to a loss of lubrication or due to a loss of structural integrity or a combination of loss of lubrication and structural integrity. In other words, the pain and/or reduced mobility may be treated by addressing the loss of lubrication or by addressing the loss of structural integrity with the use of the vesicular formulation.

10 The loss of lubrication and/or structural integrity and/or swelling may be due to degradation of phospholipids upon the surface of cartilage within an articulated joint or due to the depletion of phospholipids within the synovial fluid in an articulated joint.

15 In the case of a tendon or a ligament, the loss of lubrication and/or structural integrity and/or swelling may be due to depletion of phospholipids between the tendon or ligament and a surface over which it moves.

In the present invention, the pain and/or reduced mobility is treated by visco-supplementation.

20 The invention provides a vesicular formulation comprising a phospholipid and a surfactant for use in the treatment of joint pain and/or reduced mobility. The joint pain and/or reduced mobility may be associated with a reduction in phospholipids in the synovial fluid and/or with osteoarthritis or rheumatoid arthritis. The joint may be a knee, hip, shoulder, elbow, wrist, ankle, hand, finger, toe, foot or other point of articulation, for example between vertebrae and the intervertebral discs.

25 The invention also provides a vesicular formulation comprising a phospholipid and a surfactant for use in the treatment of tendonitis or for the treatment of carpal tunnel syndrome.

30 The animal to be treated may be a human, a companion animal or an agricultural animal. Anecdotal evidence suggests that arthritis in cats and dogs may be successfully treated in accordance with the invention.

When the animal to be treated is a human, the invention may be useful for those patients aged 45 to 85 years old, 50 to 80 years old, 55 to 75 years old or 60 to 65 years old. The human patient may be male or female.

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As a second aspect, a method of treating pain and/or reduced mobility associated with a loss of lubrication and/or structural integrity and/or swelling of a collagen structure comprising topically applying a vesicular formulation comprising a phospholipid and a surfactant to the skin surrounding the collagen structure is provided.

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The invention also relates to a method of treating osteoarthritis, carpal tunnel syndrome or tendonitis comprising topically applying a vesicular formulation comprising a phospholipid and a surfactant to the skin surrounding the collagen structure.

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All features of the first aspect apply to the second and third aspects of the invention, *mutatis mutandis*.

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US Patent No. 6,165,500 describes a preparation for the application of agents which are provided with membrane-like structures consisting of one or several layers of amphiphilic molecules, or an amphiphilic carrier substance, in particular for transporting the agent into and through natural barriers such as skin and similar materials. These Transfersome™ formulations consist of one or several components, most commonly a mixture of basic substances, one or several edge-active substances, and agents.

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US Patent Application Publication No. US 2004/0071767 describes formulations of nonsteroidal anti-inflammatory drugs (NSAIDs) based on complex aggregates with at least three amphiphatic components suspended in a pharmaceutically acceptable medium.

US Patent Application Publication No. US 2004/0105881 describes extended surface



aggregates, suspendable in a suitable liquid medium and comprising at least three amphiphats (amphiphatic components) and being capable to improve the transport of actives through semi-permeable barriers, such as the skin, especially for the non-invasive drug application *in vivo* by means of barrier penetration by such aggregates.

5 WO 2010/140061 describes the use of “empty” vesicular formulations for the treatment of deep tissue pain. WO 2011/022707 describes the use of the same “empty” vesicles for treating disorders relating to fatty acid deficiencies and *inter alia* disorders related to inflammation.

10 None of these documents disclose or teach the use of vesicular formulations for the treatment of pain and/or reduced mobility caused by a loss of lubrication and/or structural integrity and/or swelling of a collagen structure.

Citation of any reference in this section of the application is not an admission that the  
15 reference is prior art to the application. The above noted publications are hereby incorporated by reference in their entirety.

The present invention relates to a vesicular formulation comprising a phospholipid and a surfactant for use in the treatment of pain and/or reduced mobility associated  
20 with a loss of lubrication and/or structural integrity and/or swelling of a collagen structure. Vesicular formulations are described in WO2011/022707 and WO2010/140061 and throughout this application. The formulation may be a cream, lotion, ointment, gel, solution, spray, lacquer or film forming solution.

25 The vesicular formulation does not need to contain any known pharmaceutically active ingredient. The formulation may not contain any known pharmaceutically active ingredient acknowledged in the prevention or treatment of pain, reduced mobility or inflammation.

30 The present invention also provides a method of treating pain and/or reduced mobility due to a loss of lubrication and/or structural integrity and/or swelling of a collagen structure, the method comprising topically administering a formulation according to



the invention to the skin surrounding the collagen structure in a patient in need thereof.

5 The invention encompasses vesicular formulations comprising one or more phospholipids and one or more surfactants that are effective for the delivery of fatty acids and/or phospholipids in the treatment of pain and/or reduced mobility caused by a loss of lubrication and/or structural integrity and/or swelling of a collagen structure. These vesicular formulations are suitable for topical administration.

10 The formulations of the invention are preferably formulated in the absence of any pharmaceutically active agent, i.e., any non-lipid non- surfactant pharmaceutically active agent. However, it may be possible to include an active agent within the structural wall or lumen of the vesicles of the formulation. Such agents may include an analgesic, an anti-inflammatory, a steroid or therapeutic proteins.

15 A pharmaceutically active agent is here defined as an agent that has pharmacological, metabolic or immunological activity.

20 Despite the lack of a recognized pharmaceutically active agent, the vesicles elicit a therapeutic effect, namely the treatment of pain and/or reduced mobility associated with a loss of lubrication and/or structural integrity and/or swelling of a collagen structure. Without being bound by any theory, the applicant believes that the vesicle components themselves are responsible for this effect.

25 The vesicular formulation for use in the invention may consist essentially of one or more phospholipids and one or more surfactants and a pharmaceutically acceptable carrier. The vesicular formulation for use according to the invention may optionally contain one or more of the following ingredients: co-solvents, chelators, buffers, antioxidants, preservatives, microbicides, emollients, humectants, lubricants and  
30 thickeners.

The vesicular formulation of the invention is able (without wishing to be bound by theory) to achieve its function through the unique properties of multi-layer vesicles, bilayer vesicles, micelles or aggregates composed of surfactant and lipid (“vesicles”), such as soy phosphatidylcholine. The uniqueness of the vesicles derives from the inclusion in the formulation of a specific amount of surfactant, which modifies the phospholipid membrane to such an extent that the resulting vesicles are in a permanent liquid crystalline state and, since the surfactant also confers membrane stability, the vesicles are ultra hydrated, deformable and stable (have reduced rigidity without breaking). The surfactant may be nonionic.

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The vesicular formulation contains vesicles suspended in an aqueous buffer that is applied topically. The vesicles are highly hydrophilic and this property, together with their ultra deformability, is key to their ability to be transported across the skin and into the tissue to lubricate collagen structures. When the formulation of the invention is applied to the skin and allowed to dry, the rehydration driving force of the vesicles combined with their deformability gives rise to movement of the vesicles to areas of higher water content on and below the skin permeability barrier. This drives their movement through skin pores and intracellular gaps. The specific ratio of lipid to surfactant facilitates transdermal delivery of vesicles.

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Once they pass through the skin, the vesicles of the invention (sometimes referred to as “Deformasomes”) eventually present as intact vesicles. Efficient clearance of vesicles does not occur via the cutaneous blood microvasculature (capillaries) owing to their relatively large size, but they are hypothesised to be transported with the interstitial fluid into other and/or deeper tissues below the site of dermal application. A number of clinical studies conducted with vesicles of the invention labelled with a marker molecule (terbinafine) showed that the vesicles did not enter the vasculature because, following topical application, high concentrations of the marker molecule were observed locally with minimal systemic absorption (at or below the level of detection). Due to highly deformable nature of the vesicles, they are able to penetrate the tissue from the skin through to the synovial capsule or other collagen containing structures. Here, the vesicles deform into a flattened lamellar-like structure to present

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the highly hydrophilic and hydrated head groups of the phospholipids to the surface of the collagen structure, be that within a joint in the form of cartilage or a tendon or ligament, on the surface of or within bones, (as in carpal tunnel syndrome).

5 Again, without wishing to be bound by theory, the loss of lubrication and/or structural integrity may be addressed by the replacement of lost membrane components. The vesicles of the invention may be able to repair synovial or protective membranes around a collagen structure (such as cartilage or tendons/ligaments, respectively) by providing structural support to prevent further loss of lubricating, structural or  
10 protective joint constituents or tendon/ligament associated factors.

The vesicles of the vesicular formulation for use in the invention are highly hydrated and are able to penetrate into the porous collagenous structures of the joints. Once occupying the extracellular matrix the vesicles can provide hydration and support to  
15 the collagenous material thus restoring the joint integrity lost as a consequence of, for example, OA.

Restoration of structural integrity of cartilage and other collagenous joint structures may reduce symptoms such as pain while restoring the integrity of boundary  
20 structures such as the synovial membrane may reduce effusion and subsequently joint swelling.

Furthermore, the vesicles of the invention may penetrate a collagen matrix (e.g. the cartilaginous cap of a long bone) to provide a nano-scaffold or nano-support to such a  
25 collagen structure that has lost its structural integrity.

In one embodiment, the invention provides a pharmaceutical package or kit comprising one or more containers filled with the formulation of the invention, and instructions for administration of the formulation to a patient or subject in need  
30 thereof for the treatment of pain and/or reduced mobility associated with a lack of lubrication. In various embodiments, the container comprises a formulation formulated as a suspension, emulsion, gel, cream, lotion, spray, film forming solution

or lacquer. The invention provides packages or kits that can be used in any of the above- described methods or uses.

5 In one embodiment, the invention comprises a method for the treatment of pain and/or reduced mobility associated with a lack of lubrication of collagen structures, wherein the vesicular formulations of the invention are topically administered over a period of one or more weeks, for example for at least five weeks, six weeks, seven weeks, eight weeks, nine weeks, ten weeks, eleven weeks, or twelve weeks, sixteen weeks, twenty  
10 four weeks, four months, six months, eight months, ten months, one year, two or more years, or indefinitely. The formulation may be administered once, twice, three times or more per day. Alternatively the formulation may be administered on alternate days, two or three times per week, once per week or less frequently as needed.

In one embodiment, a 0.1 to 10 gram dose of the formulation of the invention is  
15 administered to the patient. The dose may be 1 to 10 gram, or 1 to 5 gram or about 1 gram, 2 gram, 3 gram, 4 gram, 5 gram, 6 gram, 7 gram, 8 gram, 9 gram or 10 gram. In some embodiments, the dose is measured as the total weight of the Deformasome. In some embodiments, the dose is measured as the total weight of the lipid(s) and surfactant(s) in the Deformasome. The dose may be administered once or twice daily  
20 for the treatment of pain associated with loss of lubrication and/or structural integrity and/or swelling. The dose may be administered once, twice, three, four, five, six, or seven times per week in accordance with the invention. The dose may be administered every day, every other day, or two to three times a week in accordance with the invention.

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The formulation is topically applied to the skin surrounding collagen structure where pain is felt by the patient. For example where the pain is joint pain and the reduced mobility is joint stiffness, the skin surrounding the knee joint, wrist, shoulder, ankle, hip, elbow or back. Alternatively the formulation can be applied to the skin of the  
30 shin, thigh, lower or upper arm, back etc in the case of pain due to tendonitis. The formulation may be applied to any external skin surface.



In some embodiments, the lipid in the pharmaceutical composition is a phospholipid. In some embodiments, the second lipid is a lysophospholipid. In some embodiments, the surfactant is a nonionic surfactant.

5 In some embodiments, the compositions of the invention form vesicles or other extended surface aggregates (ESAs), wherein the vesicular preparations have improved permeation capability through the semi-permeable barriers, such as skin. The adaptability and deformability of the vesicles allow the vesicles to penetrate beneath the skin to the muscle and the joint itself, however, the size of the vesicle  
10 prevents penetration into the vasculature and as a result prevents systemic delivery. While not to be limited to any mechanism of action, the formulations of the invention are able to form vesicles characterized by their deformability and/or adaptability. The adaptability or deformability of the vesicles may be determined by the ability of the vesicles to penetrate a barrier with pores having an average pore diameter that is  
15 smaller than the average vesicle diameter before the penetration. The pore diameter may be at least 50% smaller than the average vesicle diameter.

Generally, the nomenclature used herein and the laboratory procedures in organic chemistry, medicinal chemistry, and pharmacology described herein are those well  
20 known and commonly employed in the art. Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

The invention can be used on any animal which suffers from pain or reduced mobility  
25 caused by a lack of lubrication of a collagen structure. For example, the invention is useful for the treatment of humans, or a companion animal (e.g. a cat, a dog or a horse) or an agricultural animal.

As used herein, a "sufficient amount," "amount effective to" or an "amount sufficient  
30 to" achieve a particular result refers to an amount of the formulation of the invention is effective to produce a desired effect, which is optionally a therapeutic effect (i.e., by administration of a therapeutically effective amount). Alternatively stated, a

"therapeutically effective" amount is an amount that provides some alleviation, mitigation, and/or decrease in at least one clinical symptom. Clinical symptoms associated with the disorder that can be treated by the methods of the invention are well-known to those skilled in the art. Further, those skilled in the art will appreciate  
5 that the therapeutic effects need not be complete or curative, as long as some benefit is provided to the subject. For example, a "sufficient amount" or "an amount sufficient to" can be an amount that is effective to treat the symptoms of pain associated with a lack of lubrication of a collagen structure.

10 As used herein, the term "reduced mobility" refers to reduced joint mobility as compared to a healthy joint, when the collagen structure is within an articulated joint. This can also be referred to as "joint stiffness". The invention allows for improvement in mobility and stiffness such that it is easier to move the joint affected by a loss of lubrication and/or structural integrity and/or swelling, upon administration  
15 of the vesicular formulation. Thus, physical function of a joint is improved. The vesicular formulation for use according to the invention can prevent joint locking or joint freezing, which may occur if the reduced mobility is left untreated. Joint locking or freezing, as used herein, means the inability to move or bend the joint in question. Where the collagen structure is a tendon or ligament, reduced mobility may also be  
20 due to pain or discomfort associated with tendonitis, or a ligament wherein the pain is not localised to or associated with a specific joint.

As used herein, pain or reduced mobility due to a loss of lubrication means that the pain or reduced mobility is associated with friction within a joint due to a degradation  
25 of cartilage of the joint, or the friction caused by degradation of collagen or protective elements/membranes of a tendon or ligament.

As used herein, the term "structural integrity" means the integrity of articular cartilage, or synovial membranes, or membranes surrounding tendons or ligaments. A  
30 loss of structural integrity is used herein to mean that the collagen structure to which this term relates has been compromised, for example, by the loss or degradation of one or more structural proteins (such as proteoglycan) or the loss or degradation of



one or more membrane components (such as phospholipids). When referring to joints, loss of integrity may be associated with the destruction of cartilage, beginning with breakdown and release of proteoglycan, leading to destruction of collagen type II and complete or partial loss of the cartilage matrix. Due to the slow growing nature of cartilage, degradation usually happens at a greater rate, leading to the net reduction of cartilage structures in affected joints. The vesicular formulation for use according to the present invention therefore allows the body's natural healing process to "catch up" by providing support to cartilage matrix, preventing further degradation.

Swelling as used in the context of the invention refers to swelling via effusion (fluid leak into the joint), which can occur when the structural integrity of the synovial membrane is compromised. Increasing the fluid content of the joint increases the hydrostatic pressure further progressing damage to the joint.

As used herein, the terms "treat", "treating" or "treatment of mean that the severity of a subject's condition is reduced or at least partially improved or ameliorated and/or that some alleviation, mitigation or decrease in at least one clinical symptom is achieved and/or there is an inhibition or delay in the progression of the condition and/or delay in the progression of the onset of disease or illness. The terms "treat", "treating" or "treatment of also means managing the disease state.

As used herein, the term "pharmaceutically acceptable" when used in reference to the formulations of the invention denotes that a formulation does not result in an unacceptable level of irritation in the subject to whom the formulation is administered. Preferably such level will be sufficiently low to provide a formulation suitable for approval by regulatory authorities.

As used herein with respect to numerical values, the term "about" means a range surrounding a particular numeral value which includes that which would be expected to result from normal experimental error in making a measurement. For example, in certain embodiments, the term "about" when used in connection with a particular

numerical value means  $\pm 20\%$ , unless specifically stated to be  $\pm 1\%$ ,  $\pm 2\%$ ,  $\pm 3\%$ ,  $\pm 4\%$ ,  $\pm 5\%$ ,  $\pm 10\%$ ,  $\pm 15\%$ , or  $\pm 20\%$  of the numerical value.

5 The term "alkyl" refers to a linear or branched saturated monovalent hydrocarbon radical, wherein the alkyl may optionally be substituted with one or more substituents Q as described herein. The term "alkyl" also encompasses both linear and branched alkyl, unless otherwise specified. In certain embodiments, the alkyl is a linear saturated monovalent hydrocarbon radical that has 1 to 20 ( $C_{1-20}$ ), 1 to 15 ( $C_{1-15}$ ), 1 to 12 ( $C_{1-12}$ ), 1 to 10 ( $C_{1-10}$ ), or 1 to 6 ( $C_{1-6}$ ) carbon atoms, or a branched saturated  
10 monovalent hydrocarbon radical of 3 to 20 ( $C_{3-20}$ ), 3 to 15 ( $C_{3-15}$ ), 3 to 12 ( $C_{3-12}$ ), 3 to 10 ( $C_{3-10}$ ), or 3 to 6 ( $C_{3-6}$ ) carbon atoms. As used herein, linear  $C_{1-6}$  and branched  $C_{3-6}$  alkyl groups are also referred to as "lower alkyl". Examples of alkyl groups include, but are not limited to, methyl, ethyl, propyl (including all isomeric forms), n-propyl, isopropyl, butyl (including all isomeric forms), n-butyl, isobutyl, sec-butyl, t-butyl,  
15 pentyl (including all isomeric forms), and hexyl (including all isomeric forms). For example,  $C_{1-6}$  alkyl refers to a linear saturated monovalent hydrocarbon radical of 1 to 6 carbon atoms or a branched saturated monovalent hydrocarbon radical of 3 to 6 carbon atoms. It is understood in the chemical arts, that the use of the longer chains described herein may be appropriate, or appropriate only in limited amounts, within a  
20 molecule so that the properties of the resulting molecule (such as solubility) are appropriate for the use. Thus, while those in the art may use the above longer length alkyl substituents they will be used only when appropriate to provide the desired function.

25 The term "aryl" refers to a monocyclic aromatic group and/or polycyclic monovalent aromatic group that contain at least one aromatic hydrocarbon ring. In certain embodiments, the aryl has from 6 to 20 ( $C_{6-20}$ ), from 6 to 15 ( $C_{6-15}$ ), or from 6 to 10 ( $C_{6-10}$ ) ring atoms. Examples of aryl groups include, but are not limited to, phenyl, naphthyl, fluorenyl, azulenyl, anthryl, phenanthryl, pyrenyl, biphenyl, and terphenyl.  
30 Aryl also refers to bicyclic or tricyclic carbon rings, where one of the rings is aromatic and the others of which may be saturated, partially unsaturated, or aromatic, for example, dihydronaphthyl, indenyl, indanyl, or tetrahydronaphthyl (tetralinyl). In



certain embodiments, aryl may also be optionally substituted with one or more substituents Q as described herein.

5 The term "heteroaryl" refers to a monocyclic aromatic group and/or multicyclic aromatic group that contain at least one aromatic ring, wherein at least one aromatic ring contains one or more heteroatoms independently selected from O, S, and N. Each ring of a heteroaryl group can contain one or two O atoms, one or two S atoms, and/or one to four N atoms, provided that the total number of heteroatoms in each ring is four or less and each ring contains at least one carbon atom. The heteroaryl may be  
10 attached to the main structure at any heteroatom or carbon atom which results in the creation of a stable compound. In certain embodiments, the heteroaryl has from 5 to 20, from 5 to 15, or from 5 to 10 ring atoms. Examples of monocyclic heteroaryl groups include, but are not limited to, pyrrolyl, pyrazolyl, pyrazolinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thiadiazolyl, isothiazolyl, furanyl, thienyl, oxadiazolyl,  
15 pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, and triazinyl. Examples of bicyclic heteroaryl groups include, but are not limited to, indolyl, benzothiazolyl, benzoxazolyl, benzothienyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyranyl, indoliziny, benzofuranyl, isobenzofuranyl, chromonyl, coumarinyl, cinnolinyl, quinoxalinyl, indazolyl, purinyl, pyrrolopyridinyl,  
20 furopyridinyl, thienopyridinyl, dihydroisoindolyl, and tetrahydroquinolinyl. Examples of tricyclic heteroaryl groups include, but are not limited to carbazolyl, benzindolyl, phenanthroline, acridinyl, phenanthridinyl, and xanthenyl. In certain embodiments, heteroaryl may also be optionally substituted with one or more substituents Z as described herein.

25

The term "alkenoyl" as used herein refers to -C(O)-alkenyl. The term "alkenyl" refers to a linear or branched monovalent hydrocarbon radical, which contains one or more, in one embodiment, one to five, carbon-carbon double bonds. The alkenyl may be optionally substituted with one or more substituents Z as described herein. The term  
30 "alkenyl" also embraces radicals having "*cis*" and "*trans*" configurations, or alternatively, "Z" and "E" configurations, as appreciated by those of ordinary skill in the art. As used herein, the term "alkenyl" encompasses both linear and branched

alkenyl, unless otherwise specified. For example, C<sub>2-6</sub> alkenyl refers to a linear unsaturated monovalent hydrocarbon radical of 2 to 6 carbon atoms or a branched unsaturated monovalent hydrocarbon radical of 3 to 6 carbon atoms. In certain embodiments, the alkenyl is a linear monovalent hydrocarbon radical of 2 to 30 (C<sub>2-30</sub>), 2 to 24 (C<sub>2-24</sub>), 2 to 20 (C<sub>2-20</sub>), 2 to 15 (C<sub>2-15</sub>), 2 to 12 (C<sub>2-12</sub>), 2 to 10 (C<sub>2-10</sub>), or 2 to 6 (C<sub>2-6</sub>) carbon atoms, or a branched monovalent hydrocarbon radical of 3 to 30 (C<sub>3-30</sub>), 3 to 24 (C<sub>3-24</sub>), 3 to 20 (C<sub>3-20</sub>), 3 to 15 (C<sub>3-15</sub>), 3 to 12 (C<sub>3-12</sub>), 3 to 10 (C<sub>3-10</sub>), or 3 to 6 (C<sub>3-6</sub>) carbon atoms. Examples of alkenyl groups include, but are not limited to, ethenyl, propen-1-yl, propen-2-yl, allyl, butenyl, and 4-methylbutenyl. In certain embodiments, the alkenoyl is mono-alkenoyl, which contains one carbon-carbon double bond. In certain embodiments, the alkenoyl is di-alkenoyl, which contains two carbon-carbon double bonds. In certain embodiments, the alkenoyl is poly-alkenoyl, which contains more than two carbon-carbon double bonds.

The term “heterocyclyl” or “heterocyclic” refers to a monocyclic non-aromatic ring system and/or multicyclic ring system that contains at least one non-aromatic ring, wherein one or more of the non-aromatic ring atoms are heteroatoms independently selected from O, S, or N; and the remaining ring atoms are carbon atoms. In certain embodiments, the heterocyclyl or heterocyclic group has from 3 to 20, from 3 to 15, from 3 to 10, from 3 to 8, from 4 to 7, or from 5 to 6 ring atoms. In certain embodiments, the heterocyclyl is a monocyclic, bicyclic, tricyclic, or tetracyclic ring system, which may include a fused or bridged ring system, and in which the nitrogen or sulfur atoms may be optionally oxidized, the nitrogen atoms may be optionally quaternized, and some rings may be partially or fully saturated, or aromatic. The heterocyclyl may be attached to the main structure at any heteroatom or carbon atom which results in the creation of a stable compound. Examples of such heterocyclic radicals include, but are not limited to, acridinyl, azepinyl, benzimidazolyl, benzindolyl, benzoisoxazolyl, benzisoxazinyl, benzodioxanyl, benzodioxolyl, benzofuranonyl, benzofuranyl, benzonaphthofuranyl, benzopyranonyl, benzopyranyl, benzotetrahydrofuranyl, benzotetrahydrothienyl, benzothiadiazolyl, benzothiazolyl, benzothiophenyl, benzotriazolyl, benzothiopyranyl, benzoxazinyl, benzoxazolyl, benzothiazolyl, [beta]-carbolinyl, carbazolyl, chromanyl, chromonyl, cinnolinyl,



coumarinyl, decahydroisoquinolinyl, dibenzofuranyl, dihydrobenzothiazinyl, dihydrobenzoxazinyl, dihydrofuryl, dihydropyranal, dioxolanyl, dihydropyrazinyl, dihydropyridinyl, dihydropyrazolyl, dihydropyrimidinyl, dihydropyrrolyl, dioxolanyl, 1,4-dithianyl, furanonyl, furanyl, imidazolidinyl, imidazolinyl, imidazolyl, 5 imidazopyridinyl, imidazothiazolyl, indazolyl, indolinyl, indoliziny, indolyl, isobenzotetrahydro furanyl, isobenzotetrahydrothienyl, isobenzothienyl, isochroman, isocoumarinyl, isoindolinyl, isoindolyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isoxazolidinyl, isoxazolyl, morpholinyl, naphthyridinyl, octahydroindolyl, octahydroisoindolyl, oxadiazolyl, oxazolidinonyl, oxazolidinyl, 10 oxazolopyridinyl, oxazolyl, oxiranyl, perimidinyl, phenanthridinyl, phenathrolinyl, phenarsazinyl, phenazinyl, phenothiazinyl, phenoxazinyl, phthalazinyl, piperazinyl, piperidinyl, 4-piperidonyl, pteridinyl, purinyl, pyrazinyl, pyrazolidinyl, pyrazolyl, pyridazinyl, pyridinyl, pyridopyridinyl, pyrimidinyl, pyrrolidinyl, pyrrolinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, quinuclidinyl, tetrahydrofuryl, tetrahydro 15 furanyl, tetrahydroisoquinolinyl, tetrahydropyranal, tetrahydrothienyl, tetrazolyl, thiadiazolopyrimidinyl, thiadiazolyl, thiamorpholinyl, thiazolidinyl, thiazolyl, thienyl, triazinyl, triazolyl, and 1,3,5-trithianyl. In certain embodiments, heterocyclic may also be optionally substituted with one or more substituents Z as described herein. The term "halogen", "halide" or "halo" refers to fluorine, chlorine, bromine, and/or iodine.

20

The term "optionally substituted" is intended to mean that a group, including alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl, and heterocycl, may be substituted with one or more substituents Z, in one embodiment, one, two, three or four substituents Z, where each Z is independently selected from the group consisting of cyano, halo, OXO, nitro, C<sub>1-6</sub> alkyl, halo-C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>3-7</sub> 25 cycloalkyl, C<sub>6-14</sub> aryl, C<sub>7-14</sub> aralkyl, heteroaryl, heterocycl, -C(O)R<sup>e</sup>, -C(O)OR<sup>e</sup>, -C(O)NR<sup>f</sup>R<sup>g</sup>, -C(NR<sup>e</sup>)NR<sup>f</sup>R<sup>g</sup>, -OR<sup>e</sup>, -OC(O)R<sup>e</sup>, -OC(O)OR<sup>e</sup>, -OC(O)NR<sup>f</sup>R<sup>g</sup>, -OC(=NR<sup>e</sup>)NR<sup>f</sup>R<sup>g</sup>, -OS(O)R<sup>e</sup>, -OS(O)<sub>2</sub>R<sup>e</sup>, -OS(O)NR<sup>f</sup>R<sup>g</sup>, -OS(O)<sub>2</sub>NR<sup>f</sup>R<sup>g</sup>, -NR<sup>f</sup>R<sup>g</sup>, -NR<sup>e</sup>C(O)R<sup>f</sup>, -NR<sup>e</sup>C(O)OR<sup>f</sup>, -NR<sup>e</sup>C(O)NR<sup>f</sup>R<sup>g</sup>, -NR<sup>e</sup>C(=NR<sup>h</sup>)NR<sup>f</sup>R<sup>g</sup>, -NR<sup>e</sup>S(O)R<sup>f</sup>, 30 -NR<sup>e</sup>S(O)<sub>2</sub>R<sup>f</sup>, -NR<sup>e</sup>S(O)NR<sup>f</sup>R<sup>g</sup>, -NR<sup>e</sup>S(O)<sub>2</sub>NR<sup>f</sup>R<sup>g</sup>, -SR<sup>e</sup>, -S(O)R<sup>e</sup>, and -S(O)<sub>2</sub>R<sup>e</sup>, and -S(O)<sub>2</sub>NR<sup>f</sup>R<sup>g</sup>, wherein each R<sup>e</sup>, R<sup>f</sup>, R<sup>g</sup>, and R<sup>h</sup> is independently hydrogen, C<sub>1-6</sub> alkyl, C<sub>2-6</sub> alkenyl, C<sub>2-6</sub> alkynyl, C<sub>3-7</sub> cycloalkyl, C<sub>6-14</sub> aryl, C<sub>7-14</sub> aralkyl, heteroaryl, or

heterocyclyl; or R<sup>f</sup> and R<sup>g</sup> together with the N atom to which they are attached form heterocyclyl.

5 The term "solvate" refers to a compound provided herein or a salt thereof, which further includes a stoichiometric or non-stoichiometric amount of solvent bound by non- covalent intermolecular forces. Where the solvent is water, the solvate is a hydrate.

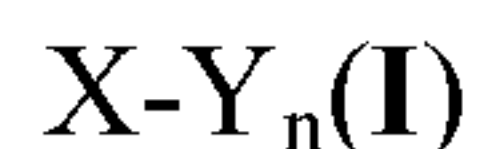
10 In accordance with this disclosure, the term "comprising" is inclusive or open- ended and does not exclude additional, unrecited elements or method steps; the term "consisting of" excludes any element, step, or ingredient not specified; and the term "consisting essentially of" excludes any element, step, or ingredient that materially changes a basic characteristic of the invention.

15 In some embodiments, the formulation of the invention provided herein comprise at least one lipid, preferably a phospho or sulpholipid, at least one surfactant, which may be a nonionic surfactant, optionally suspended in a pharmaceutically acceptable medium, preferably an aqueous solution, preferably having a pH ranging from 3.5 to 9.0, preferably from 4 to 7.5. The formulation of the invention may optionally contain  
20 buffers, antioxidants, preservatives, microbicides. antimicrobials, emollients, co-solvents, and/or thickeners. In some embodiments, the formulation of the invention comprises a mixture of more than one lipid, preferably more than one phospholipids. In some embodiments, the formulation of the invention consists essentially of at least one lipid, preferably a phospholipid, at least one surfactant, which may be a nonionic  
25 surfactant, a pharmaceutically acceptable carrier, and optionally buffers, antioxidants, preservatives, microbicides, antimicrobials, emollients, co- solvents, and/or thickeners. In some embodiments, the formulation of the invention consists of at least one lipid, preferably a phospholipid, at least one surfactant, which may be a nonionic surfactant, a pharmaceutically acceptable carrier, and one or more of the following:  
30 buffers, antioxidants, preservatives, microbicides, antimicrobials, emollients, co-solvents, and thickeners.



In the sense of this disclosure, a "lipid" is any substance, which has properties like or similar to those of a fat. As a rule, it has an extended apolar group (the "chain", X) and generally also a water-soluble, polar hydrophilic part, the "head" group (Y) and has the basic Formula I:

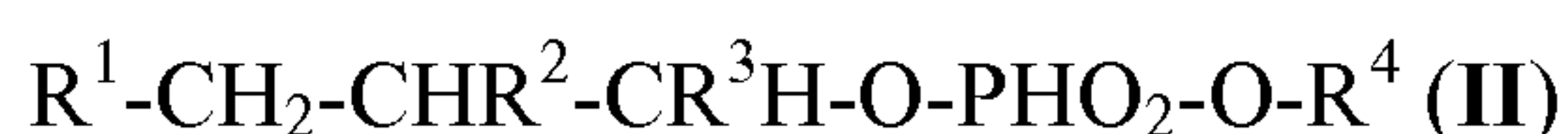
5



wherein n is equal to or larger than zero.

10 Lipids with n=0 are referred to as apolar lipids and lipids with n>1 are referred to as polar lipids. In this sense, all amphophilic substances, including, but not limited to glycerides, glyccrophospholipids, glycerophosphinolipids, glycerophosphonolipids, sulfolipids, sphingolipids, isoprenoid lipids, steroids or sterols and carbohydrate-containing lipids can generally be referred to as lipids, and are included as such in this  
15 disclosure. A list of relevant lipids and lipid related definitions is provided in EP 0 475 160 A1 (see, e.g. p. 4, 1. 8 to p. 6, 1. 3) and U.S. Patent No. 6,165,500 (see, e.g., col. 6, 1. 10 to col. 7, 1. 58), each incorporated herein by reference in their entirety.

A phospholipid in various embodiments may contain (I) a moiety derived from  
20 glycerol or a sphingosine, (2) a phosphate group, and/or (3) simple organic molecule such as choline. A phospholipid as used herein may, for example, be a compound of Formula II:



25

wherein R<sup>1</sup> and R<sup>2</sup> are hydrogen, OH, an alkyl group, an aliphatic chain, an aliphatic chain derived from a fatty acid or a fatty alcohol: provided however that R<sup>1</sup> and R<sup>2</sup> cannot both be hydrogen, OH or a C1-C3 alkyl group,; In some embodiments R<sup>1</sup> and R<sup>2</sup> are independently, an aliphatic chain, most often derived from a fatty acid or a  
30 fatty alcohol; R<sup>3</sup> generally is a hydrogen.

The OH-group of the phosphate is a hydroxyl radical or hydro xyl anion

(i.e. hydroxide) form, dependent on degree of the group ionization. Furthermore, R<sup>4</sup> may be a proton or a short-chain alkyl group, substituted by a tri-short-chain alkylammonium group, such as a trimethylammonium group, or an amino -substituted short-chain alkyl group, such as 2-trimethylammonium ethyl group (choliny) or 2-dimethylammonium short alkyl group.

A sphingophospholipid is, for example, a compound of Formula IIB:



wherein R<sup>1</sup> is a fatty-acid attached via an amide bond to the nitrogen of the sphingosine and R<sup>4</sup> has the meanings given under Formula II.

A lipid preferably is a substance of formulae II or HB, wherein R<sup>1</sup> and/or R<sup>2</sup> are acyl or alkyl, n-hydroxyacyl or n-hydroxyalkyl, but may also be branched, with one or more methyl groups attached at almost any point of the chain; usually, the methyl group is near the end of the chain (iso or anteiso). The radicals R<sup>1</sup> and R<sup>2</sup> may moreover either be saturated or unsaturated (mono-, di- or poly-unsaturated). R<sup>3</sup> is hydrogen and R<sup>4</sup> is 2-trimethylammonium ethyl (the latter corresponds to the phosphatidyl choline head group), 2-dimethylammonium ethyl, 2-methylammonium ethyl or 2-aminoethyl (corresponding to the phosphatidyl ethanolamine head group). R<sup>4</sup> may also be a proton (giving phosphatidic acid), a serine (giving phosphatidylserine), a glycerol (giving phosphatidylglycerol), an inositol (giving phosphatidylinositol), or an alkylamine group (giving phosphatidylethanolamine in case of an ethylamine), if one chooses to use a naturally occurring glycerophospholipid. Otherwise, any other sufficiently polar phosphate ester, such that will form a lipid bilayer, may be considered as well for making the formulations of the disclosure.

A phospholipid is, for example, a compound of Formula IIC as described in WO2011/022707, wherein R<sup>1</sup> and R<sup>2</sup> are independently an acyl group, alkyl group, n-hydroxyacyl group, or n- hydroxyalkyl group, most often derived from a fatty acid or



a fatty alcohol, wherein  $R^1$  and  $R^2$  may also be branched, with one or more methyl groups attached at almost any point of the chain: usually, the methyl group is near the end of the chain (iso or anteiso). wherein  $R^1$  and  $R^2$  cannot both be hydrogen, OH or a  $C_1$ - $C_3$  alkyl group. The radicals  $R^1$  and  $R^2$  may moreover either be saturated or unsaturated (mono-, di- or poly-unsaturated).  $R^3$  generally is a hydrogen. The OH-group of the phosphate is a hydroxyl radical or hydroxyl anion (i.e. hydroxide) form, dependent on degree of the group ionization. Furthermore, R may be a proton or a short-chain alkyl group, substituted by a tri- short-chain alkylammonium group, such as a trimethylammonium group, or an amino-substituted short-chain alkyl group, such as 2-trimethylammonium ethyl group (choliny) or 2-dimethylammonium short alkyl group.  $R^4$  may be 2-trimethylammonium ethyl (the latter corresponds to the phosphatidyl choline head group), 2-dimethylammonium ethyl, 2-methylammonium ethyl or 2-aminoethyl (corresponding to the phosphatidyl ethanolamine head group).  $R^4$  may also be a proton (giving phosphatidic acid), a serine (giving phosphatidylserine), a glycerol (giving phosphatidylglycerol), an inositol (giving phosphatidylinositol), or an alkylamine group (giving phosphatidylethanolamine in case of an ethylamine), if one chooses to use a naturally occurring glycerophospholipid. Otherwise, any other sufficiently polar phosphate ester, such that will form a lipid bilayer may be considered as well for making the formulations of the disclosure.

Table 1 lists preferred phospholipids in accordance with one embodiment of the disclosure.

Table 1:

Bechen(o)yl
Eruca(o)yl
Arachin(o)yl
Gadolen(o)yl
Arachindon(o)yl
Ole(o)yl

Stear(o)yl
Linol(o)yl
Linole(n/o)yl
Palmitole(o)yl
Palmit(o)yl
Myrist(o)yl
Laur(o)yl
Capr(o)yl

The preferred lipids in the context of this disclosure are uncharged and form stable, well hydrated bilayers; phosphatidylcholines, phosphatidylethanolamine, and sphingomyelins are the most prominent representatives of such lipids. Any of those  
5 can have chains as listed in the Table 1; the ones forming fluid phase bilayers, in which lipid chains are in disordered state, being preferred.

Different negatively charged, i.e., anionic, lipids can also be incorporated into vesicular lipid bilayers. Attractive examples of such charged lipids are  
10 phosphatidylglycerols, phosphatidylinositols and, somewhat less preferred, phosphatidic acid (and its alkyl ester) or phosphatidylserine. It will be realized by anyone skilled in the art that it is less commendable to make vesicles just from the charged lipids than to use them in a combination with electro- neutral bilayer component(s). In case of using charged lipids, buffer composition and/or pH care  
15 must selected so as to ensure the desired degree of lipid head-group ionization and/or the desired degree of electrostatic interaction between the, oppositely, charged drug and lipid molecules. Moreover, as with neutral lipids, the charged bilayer lipid components can in principle have any of the chains of the phospholipids as listed in the Table 1. The chains forming fluid phase lipid bilayers are clearly preferred,  
20 however, both due to vesicle adaptability increasing role of increasing fatty chain fluidity and due to better ability of lipids in fluid phase to mix with each other.

The fatty acid- or fatty alcohol-derived chain of a lipid is typically selected amongst the basic aliphatic chain types below:



Dodecanoic	cis-9-Tetradecanoic	10-cis,13-cis-Hexadecadienoic
Tridecanoic	cis-7-Hexadecanoic	7-cis,10-cis-Hexadecandienoic
Tetradecanoic	cis-9-Hexadecanoic	7-cis,10-cis,13-cis-Hexadecatrienoic
Pentadecanoic	cis-9-Octadecanoic	12-cis, 15-cis-Octadecadienoic
Hexadecanoic	cis-11-Octadecanoic	trans-10, trans-12-Octadecadienoic
Heptadecanoic	cis-11-Eicosanoic	9-cis,12-cis,15-cis-Octadecatrienoic
Octadecanoic	cis-14-Eicosanoic	6-cis,9-cis,12-cis-Octadecatrienoic
Nonadecanoic	cis-13-Docosanoic	9-cis,11-trans,13-trans-Octadecatrienoic
Eicosanoic	cis-15-Tetracosanoic	8-trans,10-trans,12-cis-Octadecatrienoic
Heneicosanoic	trans-3-Hexadecanoic	6,9,12,15-Octadecatetraenoic
Docosanoic	tans-9-Octadecanoic	3,6,9,12-Octadecatetraenoic
Tricosanoic	trans-11-Octadecanoic	3,6,9,12,15-Octadecapentaenoic
Tetracosanoic		14-cis,17-cis-Eicosadienoic
		11-cis,14-cis-Eicosadienoic
		8-cis,11-cis-14-cis-Eicosadienoic
		8-cis,11-cis-14-cis-Eicosadienoic
		5,8,11all-cis-Eicosatrienoic
		5,8,11;14-all-cis-Eicosatrienoic
		8,11,14,17-all-cis-Eicosatetraenoic
		5,8,11,14,17-all-cis-Eicosatetraenoic
		13,16-Docosadienoic
		13,16,19-Docosadienoic
		10,13,16-Docosadienoic
		7,10,13,16-Docosadienoic
		4,7,10,13,16-Docosadienoic
		4,7,10,13,16,19-Docosadienoic

Other double bond combinations or positions are possible as well.

5 Suitable fatty residues can furthermore be branched, for example, can contain a

5 methyl group in an iso or anteiso position of the fatty acid chain, or else closer to the chain middle, as in 10-R-methyloctadecanoic acid or tuberculostearic chain. Relatively important amongst branched fatty acids are also isoprenoids, many of which are derived from 3,7,11,15-tetramethylhexadec-trans-2-en-1-ol, the aliphatic alcohol moiety of chlorophyll. Examples include 5,9,13,17-tetramethyloctadecanoic acid and especially 3,7,11,15-tetramethylhexadecanoic (phytanic) and 2,6,10,14-tetramethylpentadecanoic (pristanic) acids. A good source of 4,8,12-trimethyltridecanoic acid are marine organisms. Combination of double bonds and side chains on a fatty residue are also possible.

10

Alternatively, suitable fatty residues may carry one or a few oxy- or cyclic groups, especially in the middle or towards the end of a chain. The most prominent amongst the later, alicyclic fatty acids, are those comprising a cyclopropane (and sometimes cyclopropene) ring, but cyclohexyl and cycloheptyl rings can also be found and might be useful for purposes of this disclosure. 2-(D)-Hydroxy fatty acids are more ubiquitous than alicyclic fatty acids, and are also important constituents of sphingolipids. Also interesting are 15-hydroxy-hexadecanoic and 17-hydroxy-octadecanoic acids, and maybe 9-hydroxy-octadeca-trans-10,trans-12-dienoic (dimorphecolic) and 13-hydroxy-octadeca-cis-9,trans-11-dienoic (coriolic) acid. Arguably the most prominent hydroxyl-fatty acid in current pharmaceutical use is ricinoleic acid, (D-(-)-12-hydroxy-octadec-cis-9 enoic acid, which comprises up to 90% of castor oil, which is also often used in hydrogenated form. Epoxy-, methoxy-, and furanoid-fatty acids are of only limited practical interest in the context of this disclosure.

25

Generally speaking, unsaturation, branching or any other kind of derivatization of a fatty acid is best compatible with the intention of present disclosure of the site of such modification is in the middle or terminal part of a fatty acid chain. The cis-unsaturated fatty acids are also more preferable than trans-unsaturated fatty acids and the fatty radicals with fewer double bonds are preferred over those with multiple double bonds, due to oxidation sensitivity of the latter. Moreover, symmetric chain lipids are generally better suited than asymmetric chain lipids.

30



A preferred lipid of the Formula II is, for example, a natural phosphatidylcholine, which used to be called lecithin. It can be obtained from egg (rich in palmitic, C16:0, and oleic, C18:1, but also comprising stearic, C18:0, palmitoleic, C16:1, linolenic, C18:2, and arachidonic, C20:4(M, radicals), soybean (rich in unsaturated C18 chains, but also containing some palmitic radical, amongst a few others), coconut (rich in saturated chains), olives (rich in monounsaturated chains), saffron (safflower) and sunflowers (rich in n-6 linoleic acid), linseed (rich in n-3 linolenic acid), from whale fat (rich in monounsaturated n-3 chains), from primrose or primula (rich in n-3 chains). Preferred, natural phosphatidyl ethanolamines (used to be called cephalins) frequently originate from egg or soybeans. Preferred sphingomyelins of biological origin are typically prepared from eggs or brain tissue. Preferred phosphatidylserines also typically originate from brain material whereas phosphatidylglycerol is preferentially extracted from bacteria, such as *E. coli*, or else prepared by way of transphosphatidylation, using phospholipase D, starting with a natural phosphatidylcholine. The preferably used phosphatidylinositols are isolated from commercial soybean phospholipids or bovine liver extracts. The preferred phosphatidic acid is either extracted from any of the mentioned sources or prepared using phospholipase D from a suitable phosphatidylcholine.

Furthermore, synthetic phosphatidyl cholines ( $R^4$  in Formula II corresponds to 2-trimethylammonium ethyl), and  $R^1$  and  $R^2$  are aliphatic chains, as defined in the preceding paragraph with 12 to 30 carbon atoms, preferentially with 14 to 22 carbon atoms, and even more preferred with 16 to 20 carbon atoms, under the proviso that the chains must be chosen so as to ensure that the resulting ESAs comprise fluid lipid bilayers. This typically means use of relatively short saturated and of relatively longer unsaturated chains. Synthetic sphingomyelins ( $R^4$  in Formula IIB corresponds to 2-trimethylammonium ethyl), and  $R^1$  is an aliphatic chain, as defined in the preceding paragraph, with 10 to 20 carbon atoms, preferentially with 10 to 14 carbon atoms per fully saturated chain and with 16-20 carbon atoms per unsaturated chain.

Synthetic phosphatidyl ethanolamines ( $R^4$  is 2-aminoethyl), synthetic phosphatidic

acids ( $R^4$  is a proton) or its ester ( $R^4$  corresponds, for example, to a short-chain alkyl, such as methyl or ethyl), synthetic phosphatidyl serines ( $R^4$  is L- or D-serine), or synthetic phosphatidyl (poly)alcohols, such as phosphatidyl inositol, phosphatidyl glycerol ( $R^4$  is L- or D-glycerol) are preferred as lipids, wherein  $R^1$  and  $R^2$  are fatty residues of identical or moderately different type and length, especially such as given in the corresponding tables given before in the text. Moreover,  $R^1$  can represent alkenyl and  $R^2$  identical hydroxyalkyl groups, such as tetradecylhydroxy or hexadecylhydroxy, for example, in ditetradecyl or dihexadecylphosphatidyl choline or ethanolamine,  $R^2$  can represent alkenyl and  $R^2$  hydroxyacyl, such as a plasmalogen ( $R^4$  trimethylammonium ethyl), or  $R^1$  can be acyl, such as lauryl, myristoyl or palmitoyl and  $R^2$  can represent hydroxy as, for example, in natural or synthetic lysophosphatidyl cholines or lysophosphatidyl glycerols or lysophosphatidyl ethanolamines, such as 1-myristoyl or 1-palmitoyllysophosphatidyl choline or -phosphatidyl ethanolamine; frequently,  $R^3$  represents hydrogen.

15

A lipid of Formula IIB is also a suitable lipid within the sense of this disclosure. In Formula IIB,  $n=1$ ,  $R^1$  is an alkenyl group.  $R^2$  is an acylamido group.  $R^3$  is hydrogen and  $R^4$  represents 2-trimethylammonium ethyl (choline group). Such a lipid is known under the name of sphingomyelin.

20

Suitable lipids furthermore are a lysophosphatidyl choline analog, such as 1-lauroyl-1,3-dihydroxypropane-3-phosphoryl choline, a monoglyceride, such as monoolein or monomyristin, a cerebroside, ceramide polyhexoside, sulfatide, sphingoplasmalogen, a ganglioside or a glyceride, which does not contain a free or esterified phosphoryl or phosphono or phosphino group in the 3 position. An example of such a glyceride is diacylglyceride or 1-alkenyl-1-hydroxy-2-acyl glyceride with any acyl or alkenyl groups, wherein the 3-hydroxy group is etherified by one of the carbohydrate groups named, for example, by a galactosyl group such as a monogalactosyl glycerin.

30

Lipids with desirable head or chain group properties can also be formed by biochemical means, for example, by means of phospholipases (such as phospholipase A1, A2, B, C and, in particular, D), desaturases, elongases, acyl transferases, etc., from



natural or synthetic precursors.

Furthermore, a suitable lipid is any lipid, which is contained in biological membranes and can be extracted with the help of apolar organic solvents, such as chloroform.

5 Aside from the lipids already mentioned, such lipids also include, for example, steroids, such as estradiol, or sterols, such as cholesterol, beta-sitosterol, desmosterol, 7-keto-cholesterol or beta-cholestanol, fat-soluble vitamins, such as retinoids, vitamins, such as vitamin A1 or A2, vitamin E, vitamin K, such as vitamin K1 or K2 or vitamin D1 or D3, etc.

10

The less soluble amphiphilic components comprise or preferably comprise a synthetic lipid, such as myristoleoyl, palmitoleoyl, petroselinyl, petroselaidyl, oleoyl, elaidyl, cis- or trans-vaccenoyl, linolyl, linolenyl, linolaidyl, octadecatetraenoyl, gondoyl, eicosaenoyl, eicosadienoyl, eicosatrienoyl, arachidoyl, cis- or trans-docosaenoyl, 15 docosadienoyl, docosatrienoyl, docosatetraenoyl, lauroyl, tridccanoyl, myristoyl, pentadccanoyl, palmitoyl, heptadecanoyl, stearyl or nonadecanoyl, glycerophospholipid or corresponding derivatives with branched chains or a corresponding dialkyl or sphingosin derivative, glycolipid or other diacyl or dialkyl lipid.

20

The more soluble amphiphilic components(s) is/are frequently derived from the less soluble components listed above and, to increase the solubility, substituted and/or complexed and/or associated with a butanoyl, pentanoyl, hexanoyl, heptanoyl, octanoyl, nonanoyl, decanoyl or undecanoyl substituent or several, mutually 25 independent, selected substituents or with a different material for improving the solubility.

25

A further suitable lipid is a diacyl- or dialkyl-glycerophosphoethanolamine azopolyethoxylene derivative, a didecanoylphosphatidyl choline or a 30 diacylphosphooligomaltobionamide.

30

In certain embodiments, the amount of lipid in the formulation is from about 1% to

about 12%, about 1% to about 10%, about 1% to about 4%, about 4% to about 7% or about 7% to about 10% by weight. In a specific embodiment, the lipid is a phospholipid. In another specific embodiment, the phospholipid is a phosphatidylcholine.

5

In some embodiments, the lipid in the formulation does not comprise an alkyllysophospholipid. In some embodiments, the lipid in the formulation does not comprise a polyeneylphosphatidylcholine.

10 The term "surfactant" has its usual meaning. A list of relevant surfactants and surfactant related definitions is provided in EP 0 475 160 A1 (see, e.g., p. 6, 1. 5 to p.14. 1.17) and U.S. Pat. No. 6,165,500 (see, e.g., col. 7, 1. 60 to col. 19, 1. 64), each herein incorporated by reference in their entirety, and in appropriate surfactant or pharmaceutical Handbooks, such as Handbook of Industrial Surfactants or US  
15 Pharmacopoeia, Pharm. Eu. In some embodiments, the surfactants are those described in Tables 1-18 of U.S. Patent Application Publication No. 2002/0012680 A1 . published January 31, 2002, the disclosure of which is herein incorporated by reference in its entirety. The following list therefore only offers a selection, which is by no means complete or exclusive, of several surfactant classes that are particularly  
20 common or useful in conjunction with present patent application. Preferred surfactants to be used in accordance with the disclosure include those with an HLB greater than 12. The list includes ionized long-chain fatty acids or long chain fatty alcohols, long chain fatty ammonium salts, such as alkyl- or alkenoyl-trimethyl-, -dimethyl- and -methyl-ammonium salts, alkyl- or alkenoyl-sulphate salts, long fatty  
25 chain dimethyl- aminoxides, such as alkyl- or alkenoyl-dimethyl-aminoxides, long fatty chain, for example alkanoyl, dimethyl-aminoxides and especially dodecyl dimethyl-aminoxide, long fatty chain, for example alkyl-N-methylglucamide- s and alkanoyl-N-methylglucamides. such as MEGA-8, MEGA-9 and MEGA-IO, N-long fatty chain-N,N-dimethylglycines, for example N-alkyl- N,N-dimethylglycines, 3 -  
30 (long fatty chain-dimethylammonio)-alkane- sulphonates, for example 3-(acylidimethylammonio)-alkanesulphonates, long fatty chain derivatives of sulphosuccinate salts, such as bis(2-ethylalkyl) sulphosuccinate salts, long fatty chain-



sulphobetaines, for example acyl-sulphobetaines, long fatty chain betaines, such as  
 EMPIGEN BB or ZWITTERGENT-3-16, -3-14, -3-12, -3-10, or -3-8, or polyethylen-  
 glycol- acylphenyl ethers, especially nonaethylen-glycol-octyl- phenyl ether,  
 polyethylene-long fatty chain-ethers, especially polyethylene-acyl ethers, such as  
 5 nonaethylen-decyl ether, nonaethylen-dodecyl ether or octaethylene-dodecyl ether,  
 polyethyleneglycol-isoacyl ethers, such as octaethyleneglycol-isotridecyl ether,  
 polyethyleneglycol-sorbitane-long fatty chain esters, for example polyethyleneglycol-  
 sorbitane-acyl esters and especially polyoxyethylene- monolaurate (e.g. polysorbate  
 20 or Tween 20), polyoxyethylene-sorbitan-monooleate (e.g. polysorbate 80 or Tween  
 10 80), polyoxyethylene-sorbitan-monolauroleylate, polyoxyethylene - sorbitan-  
 monopetroselinate, polyoxyethylene -sorbitan- monoelaidate, polyoxyethylene -  
 sorbitan-myristoleylate, polyoxyethylene -sorbitan-palmitoleinylate, polyoxyethylene-  
 sorbitan-p- etroselinylate, polyhydroxyethylene-long fatty chain ethers, for example  
 polyhydroxyethylene-acyl ethers, such as polyhydroxyethylene-lauryl ethers,  
 15 polyhydroxyethylene-myristoyl ethers, polyhydroxyethylene-cetylst- earyl, polyhyd  
 roxyethylene-palmityl ethers, polyhydroxyethylene-oleoyl ethers,  
 polyhydroxyethylene- palmitoleoyl ethers, polyhydroxyethylene-lino- leyl,  
 polyhydroxyethylen-4, or 6, or 8, or 10, or 12-lauryl, miristoyl, palmitoyl,  
 palmitoleyl, oleoyl or linoeyl ethers (Brij series), or in the corresponding esters,  
 20 polyhydroxyethylen-laurate, -myristate, -palmitate, -stearate or -oleate, especially  
 polyhydroxyethylen-8-stearate (Myrj 45) and polyhydroxyethylen-8-oleate,  
 polyethoxylated castor oil 40 (Cremophor EL), sorbitane-mono long fatty chain, for  
 example alkylate (Arlacel or Span series), especially as sorbitane-monolaurate  
 (Arlacel 20, Span 20), long fatty chain, for example acyl-N-methylglucamides,  
 25 alkanoyl-N-methylglucamides, especially decanoyl-N-methylglucamide, dodecanoyl-  
 N-methylglucamide, long fatty chain sulphates, for example alkyl-sulphates, alkyl  
 sulphate salts, such as lauryl-sulphate (SDS), oleoyl-sulphate: long fatty chain  
 thioglucosides, such as alkylthioglucosides and especially heptyl-, octyl- and nonyl-  
 beta-D-thioglucopyranoside; long fatty chain derivatives of various carbohydrates,  
 30 such as pentoses, hexoses and disaccharides, especially alkyl-glucosides and  
 maltosides, such as hexyl-, heptyl-, octyl-, nonyl- and decyl-beta-D-glucopyranoside  
 or D- maltopyranoside; further a salt, especially a sodium salt, of cholate,

deoxycholate, glycocholate, glycodcoxicholate, taurodeoxycholate, taurocholate, a fatty acid salt, especially oleate, elaidate, linoleate, laurate, or myristate, most often in sodium form, lysophospholipids, n-octadecylene-glycerophosphatidic acid, octadecylene- phosphorylglycerol, octadecylene-phosphorylserine, n-long fatty chain-  
5 glycerophosphatidic acids, such as n-acyl-glycerophosphatidic acids, especially lauryl glycerophosphatidic acids, oleoyl-glycerophosphatidic acid, n-long fatty chain-phosphoryl glycerol, such as n- acyl-phosphorylglycerol, especially lauryl-, myristoyl-, oleoyl- or palmitoeloyl- phosphorylglycerol, n-long fatty chain-phosphorylserine, such as n-acyl-phosphorylserine, especially lauryl-, myristoyl-,  
10 oleoyl- or palmitoeloyl-phosphorylserine, n-tetradecyl-glycerophosphatidic acid, n-tetradecyl-phosphorylglycerol, n-tetradecyl-phosphorylserine, corresponding-, elaidoyl-, vaccenyl-lysophospholipids, corresponding short-chain phospholipids, as well as all surface active and thus membrane destabilising polypeptides. Surfactant chains are typically chosen to be in a fluid state or at least to be compatible with the  
15 maintenance of fluid-chain state in carrier aggregates.

In certain embodiments, the surfactant is a nonionic surfactant. The surfactant may be present in the formulation in about 0.1% to about 5.0%, about 0.2% to 10%, about 1% to about 10%, about 1% to about 7% or about 2% to 5% by weight. In certain  
20 embodiments, the nonionic surfactant is selected from the group consisting of: polyoxyethylene sorbitans (polysorbate surfactants), polyhydroxyethylene stearates or polyhydroxyethylene laurylethers (Brij surfactants). In a specific embodiment, the surfactant is a polyoxyethylene-sorbitan- monooleate (e.g. polysorbate 80 or Tween 80) or Tween 20, 40 or 60. In certain embodiments, the polysorbate can have any  
25 chain with 12 to 20 carbon atoms. In certain embodiments, the polysorbate is fluid in the formulation, which may contain one or more double bonds, branching, or cyclo-groups.

In some embodiments, the formulations of the invention comprise only one lipid and  
30 only one surfactant. In other embodiments, the formulations of the invention comprise more than one lipid and only one surfactant, e.g., two, three, four, or more lipids and one surfactant. In other embodiments, the formulations of the invention comprise only



one lipid and more than one surfactant, e.g., two, three, four, or more surfactants and one lipid. In other embodiments, the formulations of the invention comprise more than one lipid and more than one surfactant, e.g., two, three, four, or more lipids and two, three, four, or more surfactants.

5

The formulations of the invention may have a range of lipid to surfactant ratios. The ratios may be expressed in terms of molar terms (mol lipid/mol surfactant). The molar ratio of lipid to surfactant in the formulations may be from about 1:3 to about 30:1, from about 1:2 to about 30:1, from about 1:1 to about 30:1, from about 2:1 to about 20:1, from about 5:1 to about 30:1, from about 10:1 to about 30:1, from about 15:1 to about 30:1, or from about 20:1 to about 30:1. In certain embodiments, the molar ratio of lipid to surfactant in the formulations of the invention may be from about 1:2 to about 10:1. In certain embodiments, the ratio is from about 1:1 to about 2:1, from about 2:1 to about 3:1, from about 3:1 to about 4:1, from about 4:1 to about 5:1 or from about 5:1 to about 10:1. In certain embodiments, the molar ratio is from about 10:1 to about 30:1, from about 10:1 to about 20:1, from about 10:1 to about 25:1, and from about 20:1 to about 25:1. In specific embodiments, the lipid to surfactant ratio is about 1.0:1.0, about 1.25:1.0, about 1.5/1.0, about 1.75/1.0, about 2.0/1.0, about 2.5/1.0, about 3.0/1.0 or about 4.0/1.0. The formulations of the invention may also have varying amounts of total amount of the following components: lipid and surfactant combined (TA). The TA amount may be stated in terms of weight percent of the total composition. In one embodiment, the TA is from about 1% to about 40%, about 5% to about 30%, about 7.5% to about 15%, about 6% to about 14%, about 8% to about 12%, about 5% to about 10%, about 10% to about 20% or about 20% to about 30%. In specific embodiments, the TA is 6%, 8%, 9%, 10%, 12%, 14%, 15% or 20%.

30

Selected ranges for total lipid amounts and lipid/surfactant ratios (mol/mol) for the formulations of the invention are described in the Table below:

Table 2: Total Amount and Lipid to Surfactant Ratios

TA (and surfactant) (%)	Lipid/Surfactant (mol/mol)
5 to 10	1.0 to 1.25
5 to 10	1.25 to 1.72
5 to 10	1.75 to 2.25
5 to 10	2.25 to 3.00
5 to 10	3.00 to 4.00
5 to 10	4.00 to 8.00
5 to 10	10.00 to 13.00
5 to 10	15.00 to 20.00
5 to 10	20.00 to 22.00
5 to 10	22.00 to 25.00
10 to 20	1.0 to 1.25
10 to 20	1.25 to 1.75
10 to 20	1.25 to 1.75
10 to 20	2.25 to 3.00
10 to 20	3.00 to 4.00
10 to 20	4.00 to 8.00
10 to 20	10.00 to 13.00
10 to 20	15.00 to 20.00
10 to 20	20.00 to 22.00
10 to 20	22.00 to 25.00

The formulations of the invention do not comprise a pharmaceutically active agent that has received marketing or regulatory approval in any country for the treatment of rosacea.

5

The formulations of the invention may optionally contain one or more of the following ingredients: co-solvents, chelators, buffers, antioxidants, preservatives, microbicides, emollients, humectants, lubricants and thickeners. Preferred amounts of optional components are described as follows.

10



	Molar (M) or	Rel w%*
Antioxidant:		
<i>Primary:</i>		
Butylated hydroxyanisole, BHA		0.1-8
Butylated hydroxytoluene BHT		0.1-4
Thymol		0.1-1
Metabisulphite	1-5mM	
Bisulphite	1-5mM	
Thiourea (MW=76.12)	1-10mM	
Monothioglycerol (MW=108.16)	1-20mM	
Propyl gallate (MW=212.2)		0.02-0.2
Ascorbate (MW=175.3 <sup>+</sup> ion)	1-10mM	
Palmityl-ascorbate		0.01-1
Tocopherol-PEG		0.5-5
<i>Secondary (chelator)</i>		
EDTA (MW=292)	1-10mM	
EGTA (MW=380.35)	1-10mM	
Desferal (MW=656.79)	0.1-5mM	
<i>Buffer</i>		
Acetate	30-150mM	
Phosphate	10-50mM	
Triethanolamine	30-150mM	

\* as a percentage of total lipid quantity

The formulations of the invention may include a buffer to adjust the pH of the aqueous solution to a range from pH 3.5 to pH 9, pH 4 to pH 7.5, or pH 6 to pH 7. Examples of buffers include, but are not limited to. acetate buffers, lactate buffers, phosphate buffers, and propionate buffers.

The formulations of the invention are typically formulated in aqueous media. The

formulations may be formulated with or without co-solvents, such as lower alcohols.

A "microbicide" or "antimicrobial" agent is commonly added to reduce the bacterial count in pharmaceutical formulations. Some examples of microbicides are short chain  
5 alcohols, including ethyl and isopropyl alcohol, chlorbutanol, benzyl alcohol, chlorbenzyl alcohol, dichlorbenzylalcohol, hexachlorophene; phenolic compounds, such as cresol, 4-chloro-m-cresol, p-chloro-m-xylene. dichlorophene, hexachlorophene, povidon- iodine; parabenes. especially alkyl-parabenes, such as methyl-, ethyl-, propyl-, or butyl- paraben, benzyl paraben; acids, such as sorbic acid,  
10 benzoic acid and their salts; quaternary ammonium compounds, such as alkonium salts, e.g., a bromide, benzalkonium salts, such as a chloride or a bromide, cetrimonium salts, e.g., a bromide, phenoalkecinium salts, such as phenododecinium bromide, cetylpyridinium chloride and other salts; furthermore, mercurial compounds, such as phenylmercuric acetate, borate, or nitrate, thiomersal, chlorhexidine or its  
15 gluconate, or any antibiotically active compounds of biological origin, or any suitable mixture thereof.

Examples of "antioxidants" are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and di-tert-butylphenol (LY178002, LY256548, HWA-131, BF-389, CI-986, PD-127443, E-51 or 19, BI-L-239XX, etc.), tertiary  
20 butylhydroquinone (TBHQ), propyl gallate (PG), 1-O-hexyl-2,3,5-trimethylhydroquinone (HTHQ); aromatic amines (diphenylamine, p-alkylthio-o-anisidine, ethylenediamine derivatives, carbazol, tetrahydroindenoindol); phenols and phenolic acids (guaiacol, hydroquinone, vanillin, gallic acids and their esters, protocatechuic acid, quinic acid, syringic acid, ellagic acid, salicylic acid, nordihydroguaiaretic acid (NDGA), eugenol); tocopherols (including tocopherols (alpha, beta, gamma, delta) and their derivatives, such as tocopheryl-acylate (e.g. -acetate. - laurate. myristate, -palmitate, -oleate, -linoleate. etc., or any other suitable  
25 tocopheryl- lipoate). tocopheryl-POE-succinate; trolox and corresponding amide and thiocarboxamide analogues; ascorbic acid and its salts, isoascorbate, (2 or 3 or 6)-o-alkylascorbic acids, ascorbyl esters (e.g., 6-o-lauroyl, myristoyl, palmitoyl-, oleoyl, or linoleoyl-L-ascorbic acid, etc.). Also useful are the preferentially oxidised  
30



compounds, such as sodium bisulphite, sodium metabisulphite, thiourea; chellating agents, such as EDTA, GDTA, desferral: miscellaneous endogenous defence systems, such as transferrin, lactoferrin, ferritin, cearuloplasmin, haptoglobin, heamopexin, albumin, glucose, ubiquinol-10); enzymatic antioxidants, such as superoxide  
5 dismutase and metal complexes with a similar activity, including catalase, glutathione peroxidase, and less complex molecules, such as beta- carotene, bilirubin, uric acid; flavonoids (flavones, flavonols, flavonones, flavanonals, chacones, anthocyanins). N-acetylcystein, mesna. glutathione, thiohistidine derivatives, triazoles; tannines, cinnamic acid, hydroxycinnamic acids and their esters (coumaric acids and esters,  
10 caffeic acid and their esters, ferulic acid, (iso-) chlorogenic acid, sinapic acid); spice extracts (e.g., from clove, cinnamon, sage, rosemary, mace, oregano, allspice, nutmeg); carnosic acid, carnosol, carsolic acid; rosmarinic acid, rosmaridiphenol, gentisic acid, ferulic acid; oat flour extracts, such as avenanthramide 1 and 2; thioethers, dithioethers, sulphoxides, tetralkylthiuram disulphides; phytic acid, steroid  
15 derivatives (e.g., U74006F); tryptophan metabolites (e.g., 3-hydroxykynurenine, 3-hydroxyanthranilic acid), and organochalcogenides.

"Thickeners" are used to increase the viscosity of pharmaceutical formulations to and may be selected from selected from pharmaceutically acceptable hydrophilic  
20 polymers, such as partially etherified cellulose derivatives, comprising carboxymethyl-, hydroxyethyl-, hydroxypropyl-, hydroxypropylmethyl- or methyl-cellulose; completely synthetic hydrophilic polymers comprising polyacrylates, polymethacrylates, poly(hydroxyethyl)-, poly(hydroxypropyl)-, poly(hydroxypropylmethyl)methacrylate, polyacrylonitrile, methallyl- sulphonate,  
25 polyethylenes, polyoxiethylenes, polyethylene glycols, polyethylene glycol- lactide, polyethylene glycol-diacrylate, polyvinylpyrrolidone, polyvinyl alcohols, poly(propylmethacrylamide), poly(propylene fumarate-co-ethylene glycol), poloxamers, polyaspartamide. (hydrazine cross-linked) hyaluronic acid, silicone; natural gums comprising alginates, carrageenan, guar-gum, gelatine, tragacanth,  
30 (amidated) pectin, xanthan, chitosan collagen, agarose; mixtures and further derivatives or co-polymers thereof and/or other pharmaceutically, or at least biologically, acceptable polymers.

The formulations of the present invention may also comprise a polar liquid medium. The formulations of the invention may be administered in an aqueous medium. The of the present invention may be in the form of a solution, suspension, emulsion, cream, lotion, ointment, gel, spray, film forming solution or lacquer.

In some embodiments, the invention relates to the use of a vesicular formulation as described above for the preparation of a pharmaceutical composition for the treatment of disorders related to fatty acid deficiencies, fatty acid metabolism, hypertriglyceridemia and hypercholesterolemia. In some embodiments, the invention relates to a vesicular formulation or pharmaceutical composition comprising at least one phospholipid and one surfactant for the treatment of disorders related to fatty acid deficiencies, fatty acid metabolism, hypertriglyceridemia and hypercholesterolemia wherein the formulation or pharmaceutical composition is formulated for subcutaneous, topical or intravenous delivery. The surfactant may be nonionic.

While not to be limited to any mechanism of action or any theory, the formulations of the invention may form vesicles or ESAs characterized by their adaptability, deformability, or penetrability. Vesicles of this invention as described in both WO 2010/140061 and in WO 2011/022707.

### EXAMPLES

The present invention is described with reference to the following examples and figures in which:

Figure 1 shows the results of Example 2 wherein the panels are as follows

- a. Control subject, untreated. There is an absence of fluorescing components
- b. Treated subject in which the image is irradiated to display the (red) fluorescent vesicles which are observed to be localised on cartilage surfaces and absent from the surrounding tissue and synovial fluid
- c. Identical section to b) but in which the vesicles are alternatively viewed in



black and white (grey) imaging

- d. Identical section to b) + c) following treatment with DAPI stain to reveal the nuclei of cells. Bone and cartilage can be identified by the concentration of cells, synovial fluid has only limited cellular content;

5

Figure 2 shows a schematic diagram showing from where in the knee joint the section of Figure 1 was taken;

Figure 3 shows the mean percentage change from baseline in WOMAC Stiffness Subscale scores (with adjustment for analgesics) after 3 months of therapy (n=753, ITT population);

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Figure 4 shows the mean percentage change from baseline in WOMAC Function Subscale scores (with adjustment for analgesics) after 3 months of therapy (n=943, ITT population);

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Figure 5 shows the mean percentage change from baseline in WOMAC Pain, Function and Stiffness Subscale scores (with adjustment for analgesics) after 3 months of therapy with Formulation X (n=472) and celecoxib (n=233) (ITT population) in study CL-033-III-03;

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Figure 6 shows the average VAS scores for pain and stiffness over 3 weeks of treatment with Formulation X;

Figure 7 shows an Image of DIO labelled vesicles penetrating and occupying collagenous material, namely ligamentous structures taken from rat knee joints; and

25

Figure 8 shows an image of labelled vesicles occupying collagenous structures taken from a rat knee joint. Bone is shown outlined in red.

30

Example 1: Example Formulations

The following exemplary formulations for topical application may be prepared by the following procedure:

1. Organic phase production, which contains all lipophilic excipients

The organic phase is produced by weighing the lipid, the surfactant, any additional lipophilic excipients into suitable containers followed by mixing these components into an optically isotropic phase which appears as a clear solution. During mixing, the organic phase will be heated up, but temperature must not rise above 45°C.

2. Aqueous phase production

The aqueous phase is prepared by weighing the non-lipophilic components and water, which serves as solvent, into suitable containers and then mixing these components into a clear solution. During mixing, the temperature will be elevated to 40°C.

3. Production of a concentrated intermediate by combination of both phases

The isotropic organic phase and the clear aqueous phase are combined under stirring in a suitable vessel. Before and during the combination the temperature of both phases must be kept between 35°C and 45°C. The resulting intermediate is homogenised mechanically at 40°C. Before starting homogenisation, the pressure in the production vessel is lowered to - 0.08 MPa. The desired average carrier size is typically reached after 10 minutes of homogenisation.

Three process parameters must be controlled carefully during the production of the concentrated intermediate: temperature, homogeniser circulation velocity, and overall processing time.

4. Production of the final bulk product by mixing the concentrated intermediate with dilution buffer.



The concentrated intermediate is diluted with the dilution buffer to the intended final concentration. The mixture is carefully stirred in the mixing vessel at 20°C to homogeneity.

- 5 Table 8 describes the amounts of surfactant and lipids, and other excipients in the transfersomes formulations, described in terms of the percent of the total amount of formulation.

#### Example Formulation 1

10

Formulation 1 comprises sphingomyelin (brain) (47.944 mg/g) as a lipid, Tween 80 (42.05mg/g) as a surfactant, lactate buffer (pH 4). benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (.0500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

15

#### Example Formulation 2

Formulation 2 comprises sphingomyelin (brain) (53.750 mg/g) as a lipid, Tween 80 (31.250 mg/g) as a surfactant, lactate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (15.000 mg/g).

20

#### Example Formulation 3

25

Formulation 3 comprises sphingomyelin (brain) (90.561 mg/g) as a lipid, Tween 80 (79.439 mg/g) as a surfactant, lactate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

30

#### Example Formulation 4

Formulation 4 comprises sphingomyelin (brain) (47.944 mg/g) as a lipid, Tween 80 (42.056 mg/g) as a surfactant, lactate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

#### Example Formulation 5

10

Formulation 5 comprises sphingomyelin lauroyl (50.607 mg/g) as a lipid, Brij 98 (44.393 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, EDTA (3.000 mg/g) as a chelating agent, and ethanol (10.000 mg/g).

15

#### Example Formulation 6

Formulation 6 comprises sphingomyelin lauroyl (90.561 mg/g) as a lipid, Brij 98 (79.439 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

20

#### Example Formulation 7

Formulation 7 comprises sphingomyelin lauroyl (49.276 mg/g) as a lipid, Brij 98 (79.439 mg/g) as a surfactant, acetate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

30



## Example Formulation 8

Formulation 8 comprises phosphatidyl choline and phosphatidyl glycerol (53.750 mg/g) as a lipid, Brij 98 (31.250 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, HTHQ (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), and EDTA (3.000 mg/g) as a chelating agent.

## Example Formulation 9

10

Formulation 9 comprises phosphatidyl choline and phosphatidyl glycerol (90.561 mg/g) as a lipid, Brij 98 (79.439 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, HTHQ (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDT[Lambda] (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

15

## Example Formulation 10

Formulation 10 comprises phosphatidyl choline and phosphatidyl glycerol (41.351 mg/g) as a lipid. Brij 98 (48.649 mg/g) as a surfactant, phosphate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, HTHQ (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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## 25 Example Formulation 11

Formulation 11 comprises phosphatidyl choline and phosphatidyl glycerol (47.882 mg/g) as a lipid. Brij 98 (37.118 mg/g) as a surfactant, phosphate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, HTHQ (0.200 mg/g) as an antioxidant, glycerol, EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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## Example Formulation 12

Formulation 12 comprises phosphatidyl choline and phosphatidyl glycerol (95.764 mg/g) as a lipid, Brij 98 (74.236 mg/g) as a surfactant, phosphate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, HTHQ (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

## Example Formulation 13

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Formulation 13 comprises phosphatidyl choline and phosphatidylinositol (66.676 mg/g) as a lipid, Span 20 (24.324 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g), I ITI IQ (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (25.000 mg/g).

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## Example Formulation 14

Formulation 14 comprises phosphatidyl choline and phosphatidylinositol (62.027 mg/g) as a lipid, Span 20 (22.973 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, HTHQ (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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## Example Formulation 15

Formulation 15 comprises phosphatidyl choline and phosphatidylinositol (124.054 mg/g) as a lipid, Span 20 (45.946 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, HTHQ (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), and EDTA (3.000 mg/g) as a chelating agent, and ethanol (36.510 mg/g).

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## Example Formulation 16



5 Formulation 16 comprises phosphatidyl choline and phosphatidylinositol (62.687 mg/g) as a lipid, Span 20 (32,313 mg/g) as a surfactant, acetate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, HTHQ (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent.

#### Example Formulation 17

10 Formulation 17 comprises phosphatidyl choline and phosphatidic acid (41.853 mg/g) as a lipid, Tween 80 (43.147 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDTA (3.000 mg/g), and ethanol (30.000 mg/g).

#### Example Formulation 18

15 Formulation 18 comprises phosphatidyl choline and phosphatidic acid (95.764 mg/g) as a lipid, Tween 80 (74.236 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g), and ethanol (30.000 mg/g).

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#### Example Formulation 19

25 Formulation 19 comprises phosphatidyl choline and phosphatidic acid (47.882 mg/g) as a lipid, Brij 98 and Tween 80 (37.118 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) as an antioxidant, and EDTA (3.000 mg/g).

#### Example Formulation 20

30 Formulation 20 comprises phosphatidyl choline and phosphatidic acid (45.000 mg/g) as a lipid, Span 20 and Tween 80 (45.000 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200

mg/g) as an antioxidant, and EDTA (1.000 mg/g).

#### Example Formulation 21

- 5 Formulation 21 comprises phosphatidyl choline (31.935 mg/g) as a lipid, cremophor and Span 20 (58.065 mg/g) as a surfactant, lactate (pH 5) buffer, thimerosal (5.000 mg/g) as an antimicrobial agent, BHA (0,200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (15.000 mg/g).

#### 10 Example Formulation 22

- Formulation 22 comprises phosphatidyl choline (42.500 mg/g ) as a lipid, cremophor and Tween 80 (42.500 mg/g ) as a surfactant, lactate (pH 6,5) buffer, thimerosal (5.000 mg/g) as an antimicrobial agent, BHA (0.200 mg/g) as an antioxidant, glycerol  
15 (30.000 mg/g). and EDTA (3.000 mg/g) as a chelating agent.

#### Example Formulation 23

- Formulation 23 comprises phosphatidyl choline (38.276 mg/g) as a lipid, cremophor  
20 (51.724 mg/g) as a surfactant, lactate (pH 4) buffer, thimerosal (5.000 mg/g) as an antimicrobial agent. BHA (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (36.510 mg/g).

#### Example Formulation 24

- 25 Formulation 24 comprises phosphatidyl choline (42.500 mg/g ) as a lipid, cremophor (42.500 mg/g) as a surfactant, lactate (pH 4) buffer, thimerosal (5.000 mg/g) as an antimicrobial agent, BHA (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (15.000 mg/g).

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#### Example Formulation 25



Formulation 25 comprises phosphatidyl choline (85.000 mg/g) as a lipid, cremophor (85.000 mg/g) as a surfactant, lactate (pH 4) buffer, thimerosal (5.000 mg/g) as an antimicrobial agent, BHA (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 26

Formulation 26 comprises phosphatidyl choline (38.276 mg/g) as a lipid, cremophor (51.276 mg/g) as a surfactant, lactate (pH 5) buffer, thimerosal (5.000 mg/g) as an antimicrobial agent, BHA (0.200 mg/g) as an antioxidant, and EDTA (1.000 mg/g) as a chelating agent.

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#### Example Formulation 27

Formulation 27 comprises phosphatidyl choline (36.429 mg/g) as a lipid, cremophor (48.571 mg/g) as a surfactant, lactate (pH 5) buffer, thimerosal (5.000 mg/g) as an antimicrobial agent, BHA (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 28

Formulation 28 comprises phosphatidyl choline (72.299 mg/g) as a lipid, cremophor (97,701 mg/g) as a surfactant, lactate (pH 5) buffer, thimerosal (5.000 mg/g) as an antimicrobial agent, BHA (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (15.000 mg/g).

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#### Example Formulation 29

Formulation 29 comprises phosphatidyl ethanolamine (46.250 mg/g) as a lipid, Tween 80 (46.250 mg/g) as a surfactant, phosphate (pH 6.5) buffer, thimerosal (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (20.000

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mg/g).

#### Example Formulation 30

5 Formulation 30 comprises phosphatidyl ethanolamine (38.804 mg/g) as a lipid, Tween 80 (46.196 mg/g) as a surfactant, phosphate (pH 6.5) buffer, thimerosal (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as an antioxidant, glycerol (15.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 31

Formulation 31 comprises phosphatidyl ethanolamine (36.667 mg/g) as a lipid, Brij 98 and Tween 80 (33.333 mg/g) as a surfactant, phosphate (pH 6.5) buffer, thimerosal  
15 (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

#### Example Formulation 32

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Formulation 32 comprises phosphatidyl glycerol (23.333 mg/g) as a lipid, cremophor and Brij 98 (66.667 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) as an antioxidant, and EDTA (3.000 mg/g) as a chelating agent.

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#### Example Formulation 33

Formulation 33 comprises phosphatidyl glycerol (45.833 mg/g) as a lipid, Brij 98 (41.667 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000  
30 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), and EDTA (3.000 mg/g) as a chelating agent.



Formulation 34 comprises phosphatidyl glycerol (31.957 mg/g) as a lipid, Brij 98 (38.043 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent. BHT (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 35

Formulation 35 comprises phosphatidyl glycerol (47.143 mg/g) as a lipid, Brij 98 (42.857 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDT[Lambda] (1.000 mg/g) as a chelating agent, and ethanol (25.000 mg/g).

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#### Example Formulation 36

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Formulation 36 comprises phosphatidyl glycerol (96.905 mg/g) as a lipid, Brij 98 (88.095 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (20.000 mg/g).

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#### Example Formulation 37

Formulation 37 comprises phosphatidyl glycerol (31.957 mg/g) as a lipid, Brij 98 (38.043) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 38

Formulation 38 comprises phosphatidyl ethanolamine (35.455 mg/g) as a lipid, cremophor (54.545 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium

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metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), and EDTA (3.000 mg/g) as a chelating agent.

#### Example Formulation 39

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Formulation 39 comprises phosphatidyl ethanolamine (84.457 mg/g) as a lipid, cremophor (100.543 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 40

Formulation 40 comprises phosphatidyl ethanolamine (89.048 mg/g) as a lipid, cremophor (80.952 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g), BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 41

Formulation 41 comprises phosphatidyl glycerol (41.087 mg/g) as a lipid, Tween 80 (48.913 mg/g) as a surfactant, propionate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (36.510 mg/g).

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#### Example Formulation 42

Formulation 42 comprises phosphatidyl glycerol (45.280 mg/g) as a lipid, Tween 80 (39.720 mg/g) as a surfactant, propionate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite

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(0.500 mg/g), and EDTA (3.000 mg/g) as a chelating agent.

#### Example Formulation 43

5 Formulation 43 comprises phosphatidyl glycerol (107.500 mg/g) as a lipid, Tween 80 (62.500 mg/g) as a surfactant, propionate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 44

Formulation 44 comprises phosphatidyl glycerol (77.243 mg/g) as a lipid, Tween 80 (67.757 mg/g) as a surfactant, propionate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent. BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants. EDTA (3,000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 45

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Formulation 45 comprises phosphatidyl glycerol (45.280 mg/g) as a lipid, Tween 80 (39.720 mg/g) as a surfactant, propionate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 46

Formulation 46 comprises phosphatidyl glycerol (90.561 mg/g) as a lipid, Tween 80 (79.439 mg/g) as a surfactant, propionate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, EDTA (3.000 mg/g) as a chelating agent, and ethanol

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(30.000 mg/g).

#### Example Formulation 47

- 5 Formulation 47 comprises phosphatidyl glycerol (47.944 mg/g) as a lipid, Tween 80 (42.056 mg/g) as a surfactant, propionate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, EDTA (3.000 mg/g) as a chelating agent, and ethanol (10.000 mg/g).

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#### Example Formulation 48

- Formulation 48 comprises phosphatidyl serine (50.607 mg/g) as a lipid, Brij 98 (44.393 mg/g) as a surfactant, phosphate (pH 5.5) buffer, thimerasol (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), and EDTA (1.000 mg/g) as a chelating agent.

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#### Example Formulation 49

- 20 Formulation 49 comprises phosphatidyl serine (107.500 mg/g) as a lipid, Brij 98 (62.500 mg/g) as a surfactant, phosphate (pH 5.5) buffer, thimerasol (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), and EDTA (3.000 mg/g) as a chelating agent.

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#### 25 Example Formulation 50

- Formulation 50 comprises phosphatidyl serine (47.944 mg/g) as a lipid, Brij 98 (42.056 mg/g) as a surfactant, phosphate (pH 5.5) buffer, thimerasol (5.000 mg/g) as an antimicrobial agent, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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## Example Formulation 51

Formulation 51 comprises phosphatidyl glycerol (46.364 mg/g) as a lipid, Brij 98 (38.636 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (25.000 mg/g).

## Example Formulation 52

Formulation 52 comprises phosphatidyl glycerol (46.364 mg/g) as a lipid, Brij 98 (38.636 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (20.000 mg/g).

## Example Formulation 53

Formulation 53 comprises phosphatidyl glycerol (46.098 mg/g) as a lipid, Brij 98 (43.902 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, glycerol (15.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

## Example Formulation 54

Formulation 54 comprises phosphatidyl glycerol (43.537 mg/g) as a lipid, Brij 98 (41.463 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), and EDTA (3.000 mg/g) as a chelating agent.

## Example Formulation 55

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Formulation 55 comprises phosphatidyl glycerol (45.000 mg/g) as a lipid, Brij 98 (45.000 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000

mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

#### Example Formulation 56

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Formulation 56 comprises phosphatidyl glycerol (59.492 mg/g) as a lipid, Brij 98 (30.508 mg/g) as a surfactant, acetate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 57

Formulation 57 comprises phosphatidyl glycerol (39.054 mg/g) as a lipid, Brij 98 (45.946 mg/g) as a surfactant, acetate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, and EDTA (3.000 mg/g) as a chelating agent.

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#### Example Formulation 58

Formulation 58 comprises phosphatidyl glycerol (35.854 mg/g) as a lipid, Brij 98 (34.146 mg/g) as a surfactant, acetate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), and EDTA (3.000 mg/g) as a chelating agent.

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#### Example Formulation 59

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Formulation 59 comprises phosphatidyl choline (50.000 mg/g) as a lipid, Tween 80 (40.000 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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## Example Formulation 60

Formulation 60 comprises phosphatidyl choline (38.571 mg/g) as a lipid, Tween 80 (51.429 mg/g) as a surfactant phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g), and ethanol (30.000 mg/g).

## Example Formulation 61

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Formulation 61 comprises phosphatidyl choline (41.954 mg/g) as phospholipid, Tween 80 (50.546 mg/g) as surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g), and ethanol (30.000 mg/g).

## Example Formulation 62

Formulation 62 comprises phosphatidyl choline (42.632 mg/g) as a lipid, Tween 80 (47.368 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

## 25 Example Formulation 63

Formulation 63 comprises phosphatidyl choline (46.098 mg/g) as a lipid, Tween 80 (43.902 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

## Example Formulation 64

Formulation 64 comprises phosphatidyl choline (39.721 mg/g) as a lipid, Tween 80 (50.279 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BH T (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), ED TA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

## Example Formulation 65

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Formulation 65 comprises phosphatidyl choline (44.198 mg/g) as a lipid, Tween 80 (50.802 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0,200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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## Example Formulation 66

Formulation 66 comprises phosphatidyl choline (46.453 mg/g) as a lipid, Tween 80 (51.047 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial. BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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Formulation 67 comprises phosphatidyl choline (51.221 mg/g) as a lipid, Tvveen 80 (43.779 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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## Example Formulation 68



Formulation 68 comprises phosphatidyl choline (54.167 mg/g) as a lipid, Tween 80 (43.333 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

#### Example Formulation 69

Formulation 69 comprises phosphatidyl choline (66.440 mg/g) as a lipid, Brij 98 (23.560 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g). Example formulation 69 is an emulsion.

#### Example Formulation 70

Formulation 70 comprises phosphatidyl choline (66.440 mg/g) as a lipid, Brij 98 (23.560 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g). Example formulation 70 is a suspension.

#### Example Formulation 71

Formulation 71 comprises phosphatidyl choline (66.440 mg/g) as a lipid, Brij 98 (23.560 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

Formulation 72 comprises phosphatidyl choline (40.000 mg/g) as a lipid, Tween 80 (50.000 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben

(5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g). Example formulation 72 is an emulsion.

5      Example Formulation 73

Formulation 73 comprises phosphatidyl choline (40.000 mg/g) as a lipid, Tween 80 (50.000 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500  
10 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g). Example formulation 73 is a suspension.

Example Formulation 74

15      Formulation 74 comprises phosphatidyl choline (40.000 mg/g) as a lipid, Tween 80 (50.000 mg/g) as a surfactant, acetate (pH 5.5) buffer, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

20      Example Formulation 75

Formulation 75 comprises phosphatidyl choline (40.000 mg/g) as a lipid, Tween 80 (50.000 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500  
25 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

Example Formulation 76

30      Formulation 76 comprises phosphatidyl choline (40.000 mg/g) as a lipid, Brij 98 (50.000 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzalkonium chloride (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500



mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

#### Example Formulation 77

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Formulation 77 comprises phosphatidyl choline (40.000 mg/g) as a lipid, Tween 80 (50.000 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5,000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 78

Formulation 78 comprises phosphatidyl choline (66.440 mg/g) as a lipid, Brij 98 (23.560 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzalkonium chloride (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 79

Formulation 79 comprises phosphatidyl choline (66.440 mg/g) as a lipid, Brij 98 (23.560 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 80

Formulation 80 comprises phosphatidyl choline (40.000 mg/g) as a lipid, Tween 80 (50.000 mg/g) as a surfactant, acetate (pH 5.5) buffer, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000

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mg/g) as a chelating agent, and ethanol (30.000 mg/g).

#### Example Formulation 81

- 5 Formulation 81 comprises phosphatidyl choline (40.000 mg/g) as a lipid, Tween 80 (50.000 mg/g) as a surfactant, acetate (pH 5.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial. BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 82

- Formulation 82 comprises phosphatidyl choline (44.444 mg/g) as a lipid, Tween 80 (55.556 mg/g) as a surfactant, acetate (pH 5.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 83

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- Formulation 83 comprises phosphatidyl choline (66.440 mg/g) as a lipid, Tween 80 (23.560 mg/g) as a surfactant, acetate (pH 5.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 84

- Formulation 84 comprises phosphatidyl choline (54.000 mg/g) as a lipid, Tween 80 (36.000 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHA (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and

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ethanol (30.000 mg/g).

#### Example Formulation 85

5 Formulation 85 comprises phosphatidyl choline (50.000 mg/g) as a lipid, Tween 80 (40.000 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHA (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g)

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#### Example Formulation 86

Formulation K6 comprises phosphatidyl choline (48.61 1 mg/g) as a lipid. Tween 80 (38.889 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BH[Lambda] (0.200 mg/g) and sodium metabisulfite (0,500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 87

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Formulation 87 comprises phosphatidyl choline (46.575 mg/g) as a lipid, Tween 80 (38,425 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHA (0.200 mg/g) and sodium metabisulf[iota]te (0.500 mg/g) as antioxidants, glycerol (30,000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g). Example formulation 87 is an emulsion.

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#### Example Formulation 88

Formulation 88 comprises phosphatidyl choline (46.575 mg/g) as a lipid, Tween 80 (38.425 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHA (0.200 mg/g) and sodium metabisulf[iota]te (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating

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agent, and ethanol (30.000 mg/g). Example formulation 88 is suspension.

#### Example Formulation 89

5 Formulation 89 comprises phosphatidyl choline (46.575 mg/g) as a lipid, Tween 80 (38.425 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BUT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 90

Formulation 90 comprises phosphatidyl choline (50.000 mg/g) as a lipid, Tween 80 (40.000 mg/g) as a surfactant, acetate (pH 4.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 91

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Formulation 91 comprises phosphatidyl choline (94.444 mg/g) as a lipid, Tween 80 (75.556 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 92

Formulation 92 comprises phosphatidyl choline (46.712 mg/g) as a lipid, Tween 80 (38.288 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial. BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g). EDTA (3.000 mg/g) as a chelating agent, and

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ethanol (30.000 mg/g).

#### Example Formulation 93

5 Formulation 93 comprises phosphatidyl choline (48.889 mg/g) as a lipid, Tween 80 (39.111 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 94

Formulation 94 comprises phosphatidyl choline (39.721 mg/g) as a lipid, Tween 80 (50.279 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.25 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 95

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Formulation 95 comprises phosphatidyl choline (90.000 mg/g) as a lipid, phosphate buffer (pH 6.5), benzyl alcohol or paraben as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 96

Formulation 96 comprises phosphatidyl choline (68.700 mg/g) as a lipid, Tween 80 (8.500 mg/g) as a surfactant, phosphate (pH 7.5) buffer, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, glycerol (30.000 mg/g), EDTA (1.000 mg/g) as a chelating agent, and ethanol (36.51 mg/g).

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### Example Formulation 97

5 Formulation 97 comprises phosphatidyl choline (71.460 mg/g) as a lipid, Tween 80 (4.720 mg/g) as a surfactant, phosphate (pH 7.5) buffer. BHA (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, glycerol (50.000 mg/g), EDTA (3.000 mg/g) as a chelating agent and ethanol (35.000 mg/g).

### 10 Example Formulation 98

15 Formulation 98 comprises phosphatidyl choline (71.460 mg/g) as a lipid, Tween 80 (4.720 mg/g) as a surfactant, phosphate (pH 7.8) buffer. BHA (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, glycerol (15.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (35.000 mg/g).

### Example Formulation 99

20 Formulation 99 comprises phosphatidyl choline (71.460 mg/g) as a lipid, Tween 80 (4.720 mg/g) as a surfactant, phosphate (pH 7.8) buffer, BHA (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (50.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (15.000 mg/g).

### 25 Example Formulation 100

30 Formulation 100 comprises phosphatidyl choline (71.4600 mg/g) as a lipid, Tween 80 (4.720 mg/g) as a surfactant, phosphate (pH 7.5) buffer, BHA (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (50.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (35.000 mg/g).

### Example Formulation 101



Formulation 101 comprises phosphatidyl choline (46.575 mg/g) as a lipid, Tween 80 (38.425 mg/g) as a surfactant, phosphate (pH 4) buffer, BHT (0.500 mg/g) and sodium metabisulfite (0.200mg/g) as antioxidants, and EDTA (3.000 mg/g) as a chelating agent. Example formulation 101 is an emulsion.

#### Example Formulation 102

Formulation 102 comprises phosphatidyl choline (46.575 mg/g) as a lipid, Tween 80 (38.425 mg/g) as a surfactant, phosphate (pH 4) buffer, BHT (0.500 mg/g) and sodium metabisulfite (0.200mg/g) as antioxidants, and EDTA (3.000 mg/g). Example formulation 102 is a suspension.

#### Example Formulation 103

Formulation 103 comprises phosphatidyl choline (54.643 mg/g) as a lipid, Tween 80 (30.357 mg/g) as a surfactant, phosphate (pH 4) buffer, BHA (0.500 mg/g) and sodium metabisulfite (0.200mg/g) as antioxidants, and EDTA (3.000 mg/g) as a chelating agent.

#### Example Formulation 104

Formulation 104 comprises phosphatidyl choline (39.72 mg/g) as a lipid, Tween 80 (50.279 mg/g) as surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.00 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g) as emollient, EDTA (3.000 mg/g) as the chelating agent, and ethanol (30.000 mg/g).

#### Example Formulation 105

Formulation 105 comprises phosphatidyl choline (90.00 mg/g) as a lipid, phosphate (pH 6.5) buffer, benzyl alcohol or paraben as antimicrobial (5.000 mg/s), BHT (0.200

mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g) as emollient, EDTA (3.000 mg/g) as the chelating agent, and ethanol (30.000 mg/g).

#### Example Formulation 106

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Formulation 106 comprises phosphatidyl choline (46.57 mg/g) as a lipid, Tween 80 (38.425 mg/g) as a surfactant, phosphate (pH 4) buffer, BHT (0.500 mg/g) and sodium metabisulfite (0.200mg/g) as antioxidants, and EDTA (3.000 mg/g) as the chelating agent. Formulation 106 is formulated as an emulsion.

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#### Example Formulation 107

Formulation 107 comprises phosphatidyl choline (46.57 mg/g) as a lipid, Tween 80 (38.425 mg/g) as a surfactant, phosphate (pH 4) buffer, BHT (0.500 mg/g) and sodium metabisulfite (0.200mg/g) as antioxidants, and EDTA (3.000 mg/g) as the chelating agent. Formulation 107 as a suspension.

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#### Example Formulation 108

Formulation 108 comprises phosphatidyl choline (54.64 mg/g) as a lipid, Tween 80 (30.357 mg/g) as a surfactant, phosphate (pH 4) buffer, BHA (0.500 mg/g) and sodium metabisulfite (0.200mg/g) as antioxidants, EDTA (3.000 mg/g) as the chelating agent.

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#### Example Formulation 109

Formulation 109 comprises phosphatidyl glycerol and lysophospholipid (46.364 mg/g) as a lipid, Brij 98 (38.636 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (25.000 mg/g).

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## Example Formulation 110

Formulation 110 comprises phosphatidyl glycerol and lysophospholipid (46.364 mg/g) as a lipid, Brij 98 (38.636 mg/g) as a surfactant, acetate (pH 4) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (20.000 mg/g).

## Example Formulation 111

Formulation 111 comprises phosphatidyl glycerol and lysophospholipid (46.098 mg/g) as a lipid, Brij 98 (43.902 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, glycerol (15.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

## Example Formulation 112

Formulation 112 comprises phosphatidyl glycerol and lysophospholipid (43.537 mg/g) as a lipid, Brij 98 (41.463 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), and EDTA (3.000 mg/g) as a chelating agent.

## Example Formulation 113

Formulation 113 comprises phosphatidyl glycerol and lysophospholipid (45.000 mg/g) as a lipid, Brij 98 (45.000 mg/g) as a surfactant, acetate (pH 5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, EDTA (3.000 mg/g) as a chelating agent, and ethanol (30,000 mg/g).

## Example Formulation 114

Formulation 114 comprises phosphatidyl glycerol and lysophospholipid (59.492

mg/g) as a lipid, Brij 98 (30.508 mg/g) as a surfactant, acetate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 115

Formulation 115 comprises phosphatidyl glycerol and lysophospholipid (39.054 mg/g) as a lipid, Brij 98 (45,946 mg/g) as a surfactant, acetate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, and EDTA (3.000 mg/g) as a chelating agent.

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#### Example Formulation 116

Formulation 116 comprises phosphatidyl glycerol and lysophospholipid (35.854 mg/g) as a lipid, Brij 98 (34.146 mg/g) as a surfactant, acetate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) as an antioxidant, glycerol (30.000 mg/g), and EDTA (3.000 mg/g) as a chelating agent.

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#### Example Formulation 117

Formulation 117 comprises phosphatidyl choline and lysophospholipid (50.000 mg/g) as a lipid, Tween 80 (40.000 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 118

Formulation 118 comprises phosphatidyl choline and lysophospholipid (38.571 mg/g) as a lipid, Tween 80 (51.429 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium

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metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g), and ethanol (30.000 mg/g).

#### Example Formulation 119

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Formulation 119 comprises phosphatidyl choline and lysophospholipid (41.954 mg/g) as phospholipid, Tween 80 (50.546 mg/g) as surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA

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(3.000 mg/g), and ethanol (30.000 mg/g).

#### Example Formulation 120

Formulation 120 comprises phosphatidyl choline and lysophospholipid (42.632 mg/g) as a lipid, Tween 80 (47.368 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 121

Formulation 121 comprises phosphatidyl choline and lysophospholipid (46.098 mg/g) as a lipid, Tween 80 (43.902 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 122

Formulation 122 comprises phosphatidyl choline and lysophospholipid (39.721 mg/g) as a lipid, Tween 80 (50.279 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium

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metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

#### Example Formulation 123

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Formulation 123 comprises phosphatidyl choline and lysophospholipid (44.198 mg/g) as a lipid, Tween 80 (50.802 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 124

Formulation 124 comprises phosphatidyl choline and lysophospholipid (46.453 mg/g) as a lipid, Tween 80 (51.047 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 125

Formulation 125 comprises phosphatidyl choline and lysophospholipid (51.221 mg/g) as a lipid. Tween 80 (43.779 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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#### Example Formulation 126

Formulation 126 comprises phosphatidyl choline (54.167 mg/g) as a lipid, Tween 80 (43.333 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500

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mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

#### Example Formulation 127

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Formulation 127 comprises phosphatidyl choline and lysophospholipid (66.440 mg/g) as a lipid, Brij 98 (23.560 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g) Example formulation 69 is an emulsion.

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#### Example Formulation 128

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Formulation 128 comprises phosphatidyl choline and lysophospholipid (66.440 mg/g) as a lipid, Brij 98 (23.560 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial, BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g). Example formulation 70 is a suspension.

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#### Example Formulation 129

Formulation 129 comprises phosphatidyl choline and lysophospholipid (66.440 mg/g) as a lipid. Brij 98 (23.560 mg/g) as a surfactant, phosphate (pH 6.5) buffer, benzyl alcohol or paraben (5.000 mg/g) as an antimicrobial. BHT (0.200 mg/g) and sodium metabisulfite (0.500 mg/g) as antioxidants, glycerol (30.000 mg/g), EDTA (3.000 mg/g) as a chelating agent, and ethanol (30.000 mg/g).

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It will be understood that the exact amounts of the components of the formula may be adjusted slightly without departing from the scope of the invention. For example, in each of the above formulations, the amount antimicrobial be anywhere from about 1

mg/g to about 15 mg/g, or about 5 mg/g to about 12 mg/g, or 5.25 mg/g, 6, mg/g, 7 mg/g, 8 mg/g, 9 mg/g, 10 mg/g, or 10.25 mg/g. Furthermore, the antimicrobial can be a combination of ingredients, for example benzyl alcohol and parabenes (e.g., ethyl and/or propyl).

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Example Formulations 1 through 129 may also optionally include thickeners such as pectin, xanthan gum, HPMC gel, methylcellulose or carbopol.

### Example 2

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This experiment was conducted on 6 week old CD® Hairless female rats (Charles River labs).

The DiO-labeled deformable vesicles were applied to the knee joints of four hairless rats at a dose of 10mg per joint. The test article (10mg per knee) was applied 2-5 times per day at 4 hour intervals for 3 days. The formulation, consisting of Soy Phosphatidylcholine (68.7 mg/g,) Tween 80 (8.5 mg/g) to form the vesicular component which contained the fluorescent species DIO + carbopol (12.5 mg/g) +preservatives, antioxidants and stabilisers in water (816 mg/g) was applied on to the skin of the joint and allowed to dry. Extra care was taken to avoid any mechanical damage to the skin. The animals were sacrificed following the last application of the test article. One knee of each animal was dissected and flash frozen in Acrytol Mounting Media (Leica Inc.) which contains 10% PolyVinyl Alcohol (PVA). The frozen 10µm thick sections was imaged and analyzed for DIO staining and number of synovial vesicle density as described below.

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All collected joint tissues were cryo-sectioned and fixed (and stained with DAPI for cell location purposes). The images were recorded and show the localisation of the vesicles of the formulation of the invention in Figures 1 and 2.

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### Examples 3 to 5



Six clinical trials were conducted with Formulation X on patients with osteoarthritis of the knee. One efficacy measure was the change from baseline to the end of the study in patients' perception of joint stiffness and physical function. In studies CL-033-III-03 and CL-033-III-06, joint stiffness was assessed using two single items  
5 describing the severity of stiffness, both of which were measured using an 11-point numerical rating (NRS) on the stiffness subscale of the Western Ontario and McMaster Universities (WOMAC) Osteoarthritis Index (version 3.1) instrument. A measure of physical function was performed in all six studies using the WOMAC Osteoarthritis Index (version 3.1), which consisted of 17 single items describing the  
10 difficulty in performing daily activities. Each item was rated on a VAS or an 11-point numerical scale.

Studies CL-033-III-02, CL-033-III-03 and CL-033-III-06 examined the efficacy and safety of epicutaneously applied Formulation X (4.4 g or 2.2 g twice daily) or  
15 ketoprofen in Transfersome<sup>®</sup> gel administered for 12 weeks. CL-033-III-03 was an active-controlled study comparing 12 weeks of Formulation X (4.4 g or 2.2 g twice daily) with celecoxib (100 mg twice daily), oral placebo or ketoprofen in Transfersome<sup>®</sup> gel.

20 Studies CL-033-II-03, CL-033-III-04 and CL-033-III-05 provided additional supportive efficacy and safety data for Formulation X combination regimens. Study CL-033-II-03 evaluated 6 weeks' treatment with Formulation X (4.8 g twice daily) in combination with celecoxib (100 mg twice daily) or oral placebo versus ketoprofen in Transfersome<sup>®</sup> gel. Study CL-033-III-04 compared 12 weeks' treatment with  
25 Formulation X (4.95 g, 2.65 g and 1.45 g twice daily) in combination with oral naproxen (500 mg twice daily) or oral placebo versus ketoprofen in Transfersome<sup>®</sup> gel. CL-033-III-05 was a double-blind extension of study CL-033-III-04 over 52 weeks in which patients received Formulation X 4.95 g in combination with oral naproxen (500 mg twice daily) versus ketoprofen in Transfersome<sup>®</sup> gel plus oral  
30 placebo.

Efficacy outcomes reported here focus on the 943 patients who received Formulation X alone.

“Formulation X” is as set out below, and is in accordance with example formulation 5 96 as described above:

Trade name	Concentration [mg/g]
Soy Phosphatidylcholine	68.70
Polysorbate 80	8.50
Butylated hydroxytoluene (BHT)	0.20
Disodium edetate	1.00
Methyl paraben	2.50
Ethyl paraben	2.50
Glycerol	30.00
Sodium hydroxide	6.30
Disodium hydrogenphosphate dodecahydrate	7.55
Sodium dihydrogenphosphate dihydrate	0.61
Carbopol	12.50
Linalool	1.00
Ethanol (96%)	36.51
Benzyl alcohol	5.25
Purified water	816.38
Sodium metabisulphate	0.50

### Example 3



In studies CL-033-III-03 and CL-033-III-06, the difference in joint stiffness from baseline to the end of the 3-month treatment period with Formulation X alone was measured. Joint stiffness ratings improved by up to 43.8% in 753 patients (Figure 3) (CSR CL-033-III-03; CSR CL-033-III-06).

At the end of the 3-month treatment period in the clinical trials assessing Formulation X alone, enhanced physical function of up to 42.3% was noted in 943 patients (Figure 4) (CSR CL-033-III-02; Conaghan et al. 2012; Rother et al. 2012a). Quality of life, assessed using the EURO QoL, improved by almost 40% in patients who received Formulation X in study CL-033-III-02.

Improved physical function was reported with Formulation X alone and in combination with celecoxib at the end of the 6-week treatment period in study CL-033-II-03 (CSR CL-033-II-03): 10.2% improvement for Formulation X alone versus 16.6% for Formulation X/celecoxib ( $p=0.01$ ) and 14.6% for topical ketoprofen in Transfersome<sup>®</sup> gel ( $p=0.077$ ).

In study CL-033-III-04, both the Formulation X/naproxen arm and the Formulation X/placebo arm showed improvements in physical function after 3 months (41.8% and 29.9%, respectively) (CSR CL-033-III-04), and this was maintained after 52 weeks of treatment in study CL-033-III-05 (42.3% vs 8.6% for topical ketoprofen in Transfersome<sup>®</sup> gel [ $p=0.05$ ]) (CSR CL-033-III-05). An improvement of 36.5% in mental health scores (SF-36 health survey) was also observed for both Formulation X-containing study arms in study CL-033-III-04.

#### Example 4

Pain, joint mobility and physical function outcomes were compared in study CL-033-III-03. Celecoxib (Celebrex<sup>®</sup>) is known to reduce pain in OA and was considered to be an appropriate active comparator in this study, controlling for bias and providing a reference for comparing the treatment effect of Formulation X. In study CL-033-III-

03 patients were assigned to treatment with celecoxib (100 mg twice daily), placebo or active comparator (diclofenac) for 6 weeks (McKenna et al. 2001).

At the end of 3 month treatment period in study CL-033-III-03, Formulation X was associated with reductions in joint pain (up to 39.8%) and stiffness (up to 35.9%), and an improvement in physical function (up to 37.0%) that were comparable with the effects of oral celecoxib (40.4%, 37.9% and 38.2%, respectively) (Figure 5) (CSR CL-033-III-03; Conaghan et al. 2012). Reductions in pain achieved with Formulation X were statistically significantly non-inferior to oral celcoxib, and superior to oral placebo at a magnitude similar to that of celecoxib (Table 1). These response rates for Formulation X compare favourably with those reported for patients who received therapy with oral celecoxib for 12 weeks (percentage reduction in WOMAC pain, function and stiffness approximately 30%, 26% and 26%, respectively [estimated data]) in a large double-blind, placebo-controlled study by Bensen et al. (1999). This demonstrates consistency between the efficacy data for Formulation X in Study CL-033-III-03 and published celecoxib data.

**Table 3.** Confirmatory analysis of effect size (Mann—Whitney estimator) for WOMAC pain and function after 3 months of therapy with Formulation X in Study CL-033-III-03. The pre-specified benchmark for superiority was  $MW > 0.5$ . Non-inferiority versus celecoxib was tested for pain and function in exploratory and post hoc analyses using a pre-specified lower equivalence margin of  $MW = 0.4$ .

	2.2 g Formulation X (n=238)	4.4 g Formulation X (n=234)	Oral celecoxib 100 mg b.i.d. (n=233)	Oral placebo matching celecoxib (n=227)
<b>WOMAC pain subscale score</b>				
Effect size versus oral placebo	MW=0.6006 LB: 0.5404 *p=0.0001	MW=0.5779 LB: 0.5176 *p=0.0019	MW=0.5873 LB: 0.5268 *p=0.0006	—



Effect size versus oral celecoxib	MW=0.5101 LB: 0.4506 **p=0.3526	MW=0.4897 LB: 0.4299 **p=0.6504	—	—
<b>WOMAC function subscale score</b>				
Effect size versus oral placebo	MW=0.6054 LB: 0.5452 *p<0.0001	MW=0.5788 LB: 0.5184 *p=0.0017	MW=0.5949 LB: 0.5344 *p=0.0002	—
Effect size versus oral celecoxib	MW=0.5062 LB: 0.4467 **p=0.4076	MW=0.4829 LB: 0.4232 **p=0.7388	—	—

LB, lower boundary of confidence interval; MW, Mann–Whitney estimator; SD, standard deviation; WOMAC, Western Ontario and McMaster Universities.

\*P-values calculated using Wilcoxon–Mann–Whitney U test based on one-sided

5 98.75% confidence intervals.

\*\*P-values represent test for superiority.

#### Example 5

10 The results of a meta-analysis of the change from baseline in WOMAC Osteoarthritis Index pain and function subscales from five of the clinical studies (CL-033-II-03; CL-033-III-02; CL-033-III-03; CL-033-III-04; CL-033-III-06) suggest that the treatment effects seen with Formulation X in the clinical trials represent genuine improvements in outcomes for patients with OA, rather than being only due to a placebo response  
15 which has been reported in interventional trials in OA (Zhang et al. 2010).

The change from baseline in WOMAC Osteoarthritis Index pain and function from each study were standardised to a 0–100 scale. The resulting pre-post effect size (ES) was calculated as the standardised difference of the change from baseline of the  
20 WOMAC pain subscale score at various times. The results were compared with data from the study by Zhang et al. (2010) who examined determinants of the placebo response in a meta-analysis of 198 randomised OA trials.

**Table 4.** Effect size for individual studies and their meta-analysis evaluated by the standardised difference and 95 % CIs applied for WOMAC pain and function after 6 weeks of treatment with Formulation X (ITT analysis, LOCF)

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	<b>Standard difference</b>	<b>95% CI</b>	<b>N1/N2</b>
<b>Pain</b>			
Combined Formulation X groups			
CL-033-II-03	0.6	0.42-0.77	126/126
CL-033-III-02	1.28	1.13-1.42	190/190
CL-033-III-03	1.03	0.94-1.13	472/472
CL-033-III-04	1.08	0.92-1.23	162/162
CL-033-III-06	1.06	0.94-1.18	281/281
Combined Formulation X studies (Hedges- Olkin)	1.04	0.98-1.09	1231/1231
Meta-analysis (Zhang et al.)			
All placebo	0.54	0.49-0.6	
Topical placebo	0.63	0.47-0.8	
<b>Function</b>			
Combined Formulation X groups			
CL-033-II-03	0.64	0.46-0.82	127/127
CL-033-III-02	0.88	0.73-1.02	185/185
CL-033-III-03	0.97	0.87-1.05	472/472
CL-033-III-04	0.95	0.80-1.11	162/162
CL-033-III-06	1.01	0.89--1.13	281/281
Combined Formulation	0.93	0.87-0.93	1061/1061



X studies (Hedges-Olkin)			
Meta-analysis (Zhang et al.)			
All placebo	0.49	0.44-0.54	

Higher ESs for pain relief for Formulation X studies versus the data of Zhang et al. (2010) were reported in studies using a flare design (ES: 1.00 [95% CI: 0.93–1.07]), and in patients with high (ES: 1.08 [95% CI: 1.00–1.17]) or low baseline pain severity (ES: 1.03 [95% CI: 0.95–1.11]). These data, and the magnitude of the ES with Formulation X, demonstrate that its effect is unlikely to be solely as a result of a placebo response.

#### Example 6

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A survey was conducted among a community for older people to assess the effect of Formulation X when used by arthritis sufferers in a daily routine setting, and to understand their views on the benefits and attributes of the formulation.

15 In total, 390 subjects with OA were recruited to test Formulation X on an index joint. The baseline characteristics indicated that this was a fairly standard population of individuals with OA. The average age of respondents was 65 years, 85% had comorbid conditions and 83% reported having OA in more than one joint. From the enrolled population of 390 subjects, a population of 177 subjects have completed 3 weeks of treatment with Formulation X. Among this group, the average baseline pain was 6.85 and the average baseline stiffness was 5.29, both measured using a 10-point Visual Analogue Scale (VAS).

25 From the enrolled population of 390 subjects, 334 used Formulation X at least once, and after 1, 2 and 3 weeks, 333, 248 and 177 subjects were still using Formulation X, respectively. A variety of reasons were given for stopping treatment, including side effects (discussed later) and finishing the two tubes of Formulation X gel.

Although subjects were requested to use the gel on one index joint only, 172 (51.5%) reported using the gel on one or more joints. The joints that were treated are listed in Table 5.

5

Table 5: Joints treated with Formulation X

Knee, n (%)	Elbow, n (%)	Wrist, n (%)	Shoulder, n (%)	Ankle, n (%)	Finger, n (%)	Other, n (%)
222 (66.5)	21 (6.3)	57 (17.1)	59 (17.7)	35 (10.5)	91 (27.2)	87 (26.0)

10

Among the total population, the average baseline pain was 6.92 and the average baseline stiffness was 5.15. These values were similar to the baseline values of the subjects who completed 3 weeks' treatment (6.85 for pain and 5.29 for stiffness), which indicated that the 3-week completers were representative of the entire dataset and provided the opportunity to evaluate the effect of Formulation X on pain and stiffness in a subset of subjects who had received 3 weeks' treatment.

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Three weeks of treatment saw a decrease of the average pain score by 2.13 (31.1%) and an improvement in the average stiffness score of 1.52 (28.7%) (Figure 6).

### Responder analysis

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After 3 weeks of treatment with Formulation X, 73.4% and 57.1% of subjects reported a  $\geq 1$  point or  $\geq 2$  point improvement in pain in their index joint, respectively, while 64.8% and 46.1% of subjects reported a  $\geq 1$  point or  $\geq 2$  point improvement in stiffness in their index joint, respectively.

25

Although very few patients reported when they initially started to see a beneficial effect, over half (52.8%) reported an improvement of  $\geq 1$  point after one week of treatment.

Analysis of the response according to the level of starting pain indicated that the most pronounced improvements in pain were observed in the subjects with moderate or higher pain at baseline (Table 6).



**Table 6:** Analysis of effect according to baseline pain

<b>Starting pain</b>	<b>Average starting pain score</b>	<b>No subjects</b>	<b>Av delta at 3 weeks</b>	<b>Av delta as % of starting score</b>
<b>1–4</b>	3.64	28	0.39	10.7
<b>5–7</b>	5.89	65	1.89	32.1
<b>8–10</b>	8.59	84	2.89	33.6

In an analysis of feedback on the formulation, 97% of respondents reported that

5 Formulation X was equal or better than other products with respect to its effects on pain, while 74% reported that it was slightly or much better (Table 7). Similarly, with respect to stiffness, 99% reported that Formulation X was equal or better than other OA products, while 67% reported that it was slightly or much better.

10 Overall, 94% of respondents reported that Formulation X was equally or better tolerated than other products while over half of respondents reported that it was slightly or much better. A similar pattern was observed when respondents were questioned about ease of use with Formulation X compared with other products, with only 8% reporting that the formulation was worse than other products.

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**Table 7:** Respondent views on Formulation X compared with other OA products

	<b>Pain (131/177), n (%)</b>	<b>Stiffness (124/177), n (%)</b>	<b>Side effects (108/177), n (%)</b>	<b>Ease of use (125/177), n (%)</b>
<b>Worse</b>	4 (3)	1 (1)	6 (6)	10 (8)
<b>Equal</b>	31 (24)	40 (32)	44 (41)	53 (42)
<b>Slightly better</b>	52 (40)	45 (36)	17 (16)	26 (21)
<b>Much better</b>	44 (34)	38 (31)	41 (38)	36 (29)

The positive findings in this observational study reinforce those of the clinical trials with Formulation X (Examples 3 to 5) that showed that this innovative, drug-free treatment is efficacious and well tolerated for the treatment of pain and stiffness associated with OA. The benefits of Formulation X are further reflected in the high patient satisfaction reported by those using the product. As Formulation X improves symptoms of OA without active pharmaceutical ingredients, it represents a real breakthrough for the management of this condition, allowing it to be used with confidence in a population where comorbid conditions and concomitant medication use is widespread.



Claims

1. A vesicular formulation comprising a phospholipid and a surfactant for use in the treatment of pain or reduced mobility associated with a loss of lubrication and/or structural integrity and/or swelling of a collagen structure in an animal by topically applying the vesicular formulation to the skin surrounding the collagen structure.
2. A vesicular formulation comprising a phospholipid and a surfactant for use according to claim 1, wherein the collagen structure is cartilage within an articulated joint.
3. A vesicular formulation comprising a phospholipid and a surfactant for use according to claim 1, wherein the collagen structure is a tendon or a ligament.
4. A vesicular formulation comprising a phospholipid and a surfactant for use according to claim 2, wherein the loss of lubrication and/or structural integrity and/or swelling is due to degradation of phospholipids upon the surface of cartilage within an articulated joint.
5. A vesicular formulation comprising a phospholipid and a surfactant for use according to claim 2, wherein the loss of lubrication and/or structural integrity and/or swelling is due to the depletion of phospholipids within the synovial fluid in an articulated joint.
6. A vesicular formulation comprising a phospholipid and a surfactant for use according to claim 3, wherein the loss of lubrication and/or structural integrity and/or swelling is due to depletion of phospholipids between the tendon or ligament and a surface over which it moves.

7. A vesicular formulation comprising a phospholipid and a surfactant for use according to any one of claims 1 to 6, wherein the pain or reduced mobility is treated by visco-supplementation.
- 5 8. A vesicular formulation comprising a phospholipid and a surfactant for use according to claim 2, in the treatment of joint pain or reduced mobility.
9. A vesicular formulation comprising a phospholipid and a surfactant for use according to claim 2, wherein the joint pain or reduced mobility is associated  
10 with a reduction in phospholipids in the synovial fluid.
10. A vesicular formulation comprising a phospholipid and a surfactant for use according to claim 2, wherein the joint pain is associated with osteoarthritis.
- 15 11. A vesicular formulation comprising a phospholipid and a surfactant for use according to claim 3, for the treatment of tendonitis.
12. A vesicular formulation comprising a phospholipid and a surfactant for use according to claim 3, for the treatment of carpal tunnel syndrome.
- 20 13. A vesicular formulation comprising a phospholipid and a surfactant for use according to any one of claims 1 to 12, wherein the animal is a human, a companion animal or an agricultural animal.
- 25 14. A vesicular formulation comprising a phospholipid and a surfactant for use according to any one of claims 1 to 13, wherein the animal is a human and is from about 45 to about 85 years old.
- 30 15. A vesicular formulation comprising a phospholipid and a surfactant for use according to any one of claims 1 to 14, wherein the formulation does not contain a pharmaceutically active agent.



16. A method of treating pain or reduced mobility associated with a loss of lubrication and/or structural integrity and/or swelling of a collagen structure comprising topically applying a vesicular formulation comprising a phospholipid and a surfactant to the skin surrounding the collagen structure.

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17. A method of treating osteoarthritis, carpal tunnel syndrome or tendonitis comprising topically applying a vesicular formulation comprising a phospholipid and a surfactant to the skin surrounding a collagen structure associated with the osteoarthritis, carpal tunnel syndrome or tendonitis.

10

Application number / Numéro de demande: 2,870,111

Figures: \_\_\_\_\_

Pages: 1,2,3,4,5,7,8

Unscannable items received with this application  
(Request original documents in File Prep. Section on the 10<sup>th</sup> floor)

Documents reçu avec cette demande ne pouvant être balayés '  
(Commander les documents originaux dans la section de la préparation  
des dossiers au 10ième étage)



6/8

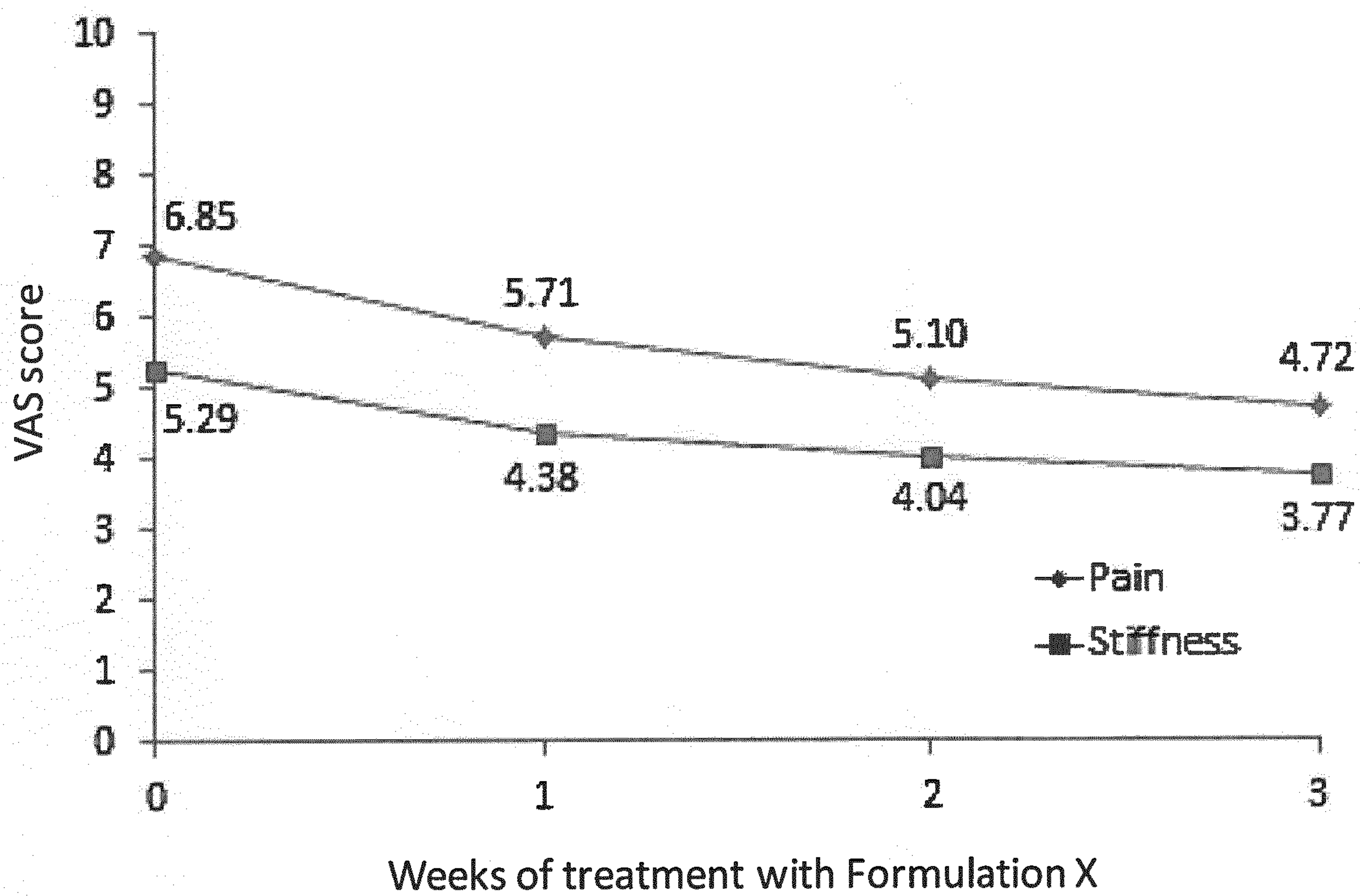


Fig. 6