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(54) Title: WATER-CONTAINING FORMULATIONS WITH PHOSPHOLIPIDS

(57) Abstract

Methods of preparing water-containing formulations of phospholipids in the presence of swelling accelerators and the use of the gels so formed for the preparation of liposomal formulations by dilution with water.

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WATER-CONTAINING FORMULATIONS WITH PHOSPHOLIPIDS

The invention refers to a method for the preparation of water-containing formulations with phospholipids using swelling accelerators and the application of these formulations for the preparation of liposomes.

Phospholipids and liposomes have been the subject of many investigations and are described in the literature in numerous publications. In these the incorporation of the phospholipids into aqueous media plays an important role on account of their economic importance. There is also great interest in the therapeutic application of liposomes as carriers of active agents of the most various types.

Thus, it is well-known that phospholipids of all types are practically insoluble in water and are only slowly swelled by it; in particular it is not possible to produce highly concentrated aqueous phospholipid-containing or liposome-containing solutions without resorting to expensive methods. The various methods of liposome preparation have been comprehensively reviewed by Szoka et al. in Ann. Rev. Biophys, Bioeng. 9 467-508 (1980).

However, for many fields of application it is desirable to be able to dissolve or at least disperse phospholipids in water.

Thus, in EP 98561 mixtures of phospholipids or mixtures containing phospholipids were brought into solution or emulsified by the addition of organic solvents and surfactants. In

DE-PS 11 41 639 choline phosphoric acid diglyceride ester compounds were solubilized with the aid of salts of the bile acids. In DE-AS 12 27 191 lecithins were emulsified in water with aliphatic polyalcohols in the presence of ethanol. In DE-OS 16 17 542 deoiled crude lecithin was made water-soluble in aqueous, sugar-containing alcohol solutions. According to US-PS 2 402 690 oil-containing lecithins can be made dispersible in water by the addition of monoglycerides. According to DE-PS 32 18 027 the addition of hydroxyethyl fatty acid amides yields liquified and water-soluble phospholipids. However, these methods have the disadvantage that they only function for specific phospholipids or particular phospholipid mixtures or lecithin mixtures and can, therefore, only be applied in a few particular cases. It is true that the method of DE-OS 36 10 873 involving the addition of specific amines can be used for the dispersion or dissolution of many phospholipids and lecithin mixtures in water, but, on account of the negative organoleptic properties (odor!) and toxic properties of the dissolution agents - the amines used -, they are unsuitable for oral, parenteral or topical applications.

Several methods, which were developed in the past for the production of liposomes from phospholipids or phospholipid mixtures and for the preparation of liposomal solutions, have since become established as standard methods. Liposomes of various compositions and sizes are obtained depending on the class of process, so that it is necessary to distinguish between numerous types.

For preparation by the method in most general use, the "film technique", the phospholipid or phospholipid mixture is dissolved in a volatile organic solvent - e.g. chloroform, ether, ethanol etc. - and the solvent is evaporated in the

rotary evaporator leaving behind a thin film of phospholipid in the round-bottomed flask. The liposomes are then produced by the addition of water or a suitable buffer solution (Bangham A.D. et al., Methods in Membrane Biology 1, 1-68 (1976)). This method yields multilammellar liposomes (MLV), which, however, suffer from the disadvantage of a very wide range of particle size and particle size distribution.

It is possible to produce unilamellar liposomes by exposure of multilamellar liposomes to ultrasound (Huang, C., Biochemistry 8, 346-352 (1969)). The possibility of contamination by abraded heavy metals is disadvantageous here.

It is also possible to prepare liposomal solutions by the injection method of Batzri S., Korn E.D., Biochim. Biophys. Acta 298, 1015-1019 (1976) in which an ethanolic solution of the lipid is injected into a buffer solution. This method cannot be carried out on an industrial scale, it is also necessary to use expensive methods to remove the solvent.

Unilamellar liposomes can be produced using the "French press" at low pressures by passing multilamellar liposomes produced by conventional means through a narrow orifice (Hamilton R.L. et al., J. Lipid. Res. 21, 981-992 (1980)).

According to another method phospholipids and a surface-active substance plus a solvent are used to produce solvent-lipid-detergent micelles, liposomes are produced on removal of the detergent. However, it is only possible to achieve complete removal of the detergent by very expensive means and the liposome preparation often contains traces of detergent.

However, all these methods have the disadvantage that they require the use of organic solvents such as chloroform,

ether, ethanol or other organic solvents. Such solvents are, at the very least, irritating to the human skin and some of them have toxic properties and, therefore, have to be removed entirely from the phospholipid solutions and aqueous lipid preparations by the use of expensive processes.

The aim of the present invention is to create a process which allows the easy preparation of aqueous formulations of phospholipids of widely different composition and concentration for the manufacture of liposomes.

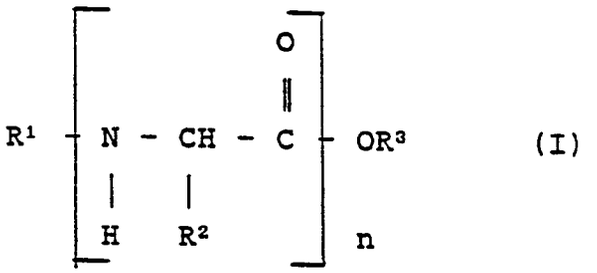
It was found, completely unexpectedly, that phospholipids of very different origin and phospholipid mixtures of widely different composition and concentration could be worked directly into water with spontaneous swelling in the presence of certain quantities of swelling accelerator or mixtures of swelling accelerators.

The aim was fulfilled according to the invention by a method of preparation of water-containing formulations with phospholipids by admixture of phospholipids in water with stirring in the presence of swelling accelerators, whereby a mixture of saturated or unsaturated organic carboxylic acids and their salts with a strong base yielding a pH of 5 to 7 is used as swelling accelerator in proportions from 1 to 30% by weight, the concentration of phospholipid is 20 to 50% by weight and the remainder water, based on the total weight of the formulation.

Proportions of 1 to 10% by weight of swelling accelerator with respect to the total weight of the formulation are especially preferred. Preferred saturated or unsaturated carboxylic acids are those with 10 to 20 carbon atoms of natural or synthetic origin. These include, for example,

capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, margaric acid, arachic acid, behenic acid, undecanoic acid, 10-undecanoic acid, tridecanoic acid, pentadecanoic acid, nonadecanoic acid, heneicosanoic acid, lauroleic acid, myristoleic acid, palmitoleic acid, petroselaidic acid, oleic acid, elaidic acid, linoleic acid, linolaidic acid, linolenic acid, eleostearic acid, gadoleic acid, arachidonic acid, erucic acid, brassidic acid, clupanodonic acid, hydroxyundecanoic acid, petroselinic acid, parinaric acid, 10-methyloctadecanoic acid, isotridecanoic acid (a mixture of isomeric C_{13} acids), 10-methylstearic acid and mixtures of these.

Collagen hydrolysates or corresponding acylated hydrolysates of collagen of general formula I



(I)

can also be used as swelling accelerators, where

R^1 is hydrogen or a saturated or unsaturated acyl group with 1 to 22 carbon atoms,

R^2 is the side chains of the amino acids of collagen and

R^3 is hydrogen or an alkali metal ion and

n is an integer between 1 and 10.

Collagen mainly consists of the amino acids glycine, proline and hydroxyproline together with small amounts of glutamic acid, arginine, alanine, aspartic acid, lysine, leucine, serine and isoleucine.

R^2 has for the individual amino acids the following meaning:

$R^2 = -H$	(for glycine)
$R^2 = -CH_2-CH_2-CH_2-$	(for proline)
$R^2 = -CH-CHOH-CH_2-$	(for hydroxyproline)
$R^2 = -CH_2-CH_2-COOH$	(for glutamic acid)
$R^2 = -(CH_2)_3-NH-C(=\overset{\oplus}{N}H_2)(NH_2)$	(for arginine)
$R^2 = -CH_3$	(for alanine)
$R^2 = -CH_2-COOH$	(for aspartic acid)
$R^2 = -(CH_2)_4-\overset{\oplus}{N}H_3$	(for lysine)
$R^2 = -CH_2-CH(CH_3)-CH_3$	(for leucine)
$R^2 = -CH_2OH$	(for serine)
$R^2 = -CH(CH_3)-CH_2-CH_3$	(for isoleucine)

Hydrolysates or acylated hydrolysates of casein, keratin or O-acyl derivatives of hydroxyproline can also be used as the carboxylic acid.

Acylated amino acids, acylated peptides or choline and their salts can also serve as the carboxylic acid.

Suitable strong bases for the formation of the salts of the organic carboxylic acids are, in particular, sodium hydroxide, potassium hydroxide, ammonium hydroxide and amines, such as ethanolamine and triethanolamine.

All natural and synthetic phospholipids in both the hydrogenated and nonhydrogenated states can be used as phospholipids for aqueous formulations. For example:

Soya lecithin: ca. 30% phosphatidylcholine, 1-2% lysophosphatidylcholine, 22% phosphatidylethanolamine, 1-2% lysophosphatidylethanolamine, 3-4% phosphatidylserine, 18% phosphatidylinositol, 13% phytoglycolipids, 2% phosphatidic acid, 8% accompanying lipids.

Rape lecithin: 30-32% phosphatidylcholine, 3% lysophosphatidylcholine, 30-32% phosphatidylethanolamine, 2-3% lysophosphatidylethanolamine, 14-18% phosphatidylinositol, 1% lysophosphatidylinositol, 10% phytoglycolipids, 1% phosphatidic acid, 2-3% accompanying lipids.

Safflower lecithin: 32-39% phosphatidylcholine, 1-2% lysophosphatidylcholine, 14-17% phosphatidylethanolamine, 2% lysophosphatidylethanolamine, 21-27% phosphatidylinositol, 1% lysophosphatidylinositol, 15-28% accompanying lipids.

Egg lecithin: 73% phosphatidylcholine, 5-6% lysophosphatidylcholine, 15% phosphatidylethanolamine, 2-3% lysophosphatidylethanolamine, 1% phosphatidylinositol, 2-3% sphingomyeline, 1% plasmologen.

The individual lecithins can be purified by known methods and the phospholipids separated into their individual components such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylglycerol, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylserine, lysophosphatidylglycerol, *n*-acylphosphatidylethanolamine, phosphatidic acid, cardiolipin, sphingomyeline, plasmologens and other substances or into olefinic mixtures.

Thus, for instance, pure phospholipid products are available commercially which can have the following compositions:

Phospholipon^{R} 100 (96% phosphatidylcholine), Phospholipon^{R} 100 H (98% phosphatidylcholine, completely hydrogenated), Phospholipon^{R} 80 (80% phosphatidylcholine, 10% phosphatidylethanolamine), Phospholipon^{R} 55 (55% phosphatidylcholine, 25% phosphatidylethanolamine, 2% phosphatidylinositol),

Phospholipon^{R} 38 (38% phosphatidylcholine, 16% N-acetylphosphatidylethanolamine), Phospholipon^{R} 25 (25% phosphatidylcholine, 25% phosphatidylethanolamine, 20% phosphatidylinositol). Such phospholipids can be manufactured according to the methods of EP 68 295.

The synthetic phospholipids that are suitable include, for example:

Dihexadecanoylphosphatidylcholine, ditetradecanoylphosphatidylcholine, dioleoylphosphatidylcholine, dilinoloylphosphatidylcholine, dibutyroylphosphatidylcholine, dihexanoylphosphatidylcholine, dimyristoylphosphatidylcholine, distearoylphosphatidylcholine, but, in particular, dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylglycerol.

It is of intrinsic importance that the type, amount and, if necessary, the ratio of the carboxylic acid to its salt is adjusted to the particular phospholipid or mixture of phospholipids.

The formulation should exhibit a pH of 5 to 7 in order to avoid degradation of the phospholipids or their hydrolysis to lysophospholipids.

The admixture of the phospholipids and any other additives and components to be incorporated can be carried out in a conventional stirring apparatus.

Vigorous stirring is necessary to cause intensive mixing. Anchor stirrers, blade stirrers, propeller stirrers and turbine mixers are all employed, whereby it is advantageous to fit them with scrapers. Before the phospholipid is added it should be ensured that the apparatus is disinfected and

sterilized when pyrogen-free formulations are being prepared. The mixing is carried out at room temperature or at elevated temperatures up to 50°C. The aqueous phospholipid formulations prepared according to the invention can be diluted with water when they form liposomal phases or liposomes. If electrolytes are included when the liposomes are produced it is possible to produce vesicle sizes from 10 nm to 1500 nm in simple vessels equipped with stirrers. Depending on the intensity of stirring and intensity of mixing the phospholipids require 10 to 60 minutes before they are swollen. The formulations according to the invention are gel-like mixtures which can be diluted with water. The particular advantage of the method according to the invention is that it is possible to produce the liposomes in a single piece of apparatus, namely a mixer.

In order to obtain better wetting and more rapid swelling of the phospholipids in water, additives, such as lower alcohols (methanol, ethanol, propanol), chloroform, dichloromethane and other volatile organic solvents can be included for the mixing process. The use of organic solvents is particularly appropriate if they are to be included in the lipid components that are to be produced later. However, it is preferable to use no solvents or only very little if they must later be removed from the formulation. Other additives during admixture of the phospholipids with water can be electrolytes such as NaCl, $CaCl_2$, Na_2HPO_4, $NaHCO_3$, choline chloride, choline phosphate, sodium acetate or mixtures of these. The additives are preferably included to the extent of 0 to 20% by weight of the total formulation. The phospholipids can be introduced into the water in the presence of the additives but it is possible to add them in the form of an aqueous solution after the phospholipid has been introduced into the water.

Carbohydrates and/or starch hydrolysates, mono- and disaccharides and mixtures of these substances can also be added to the formulations.

However, these additional adjuvant substances should not make up more than 30% by weight with respect to the total formulation.

Various ingredients can be enclosed in the liposomes. For example:

Antiasthmatics	aminophylline, adrenaline, ephedrine, isoprotenol, metaproterenol, norepinephrine, theophylline, terbutaline
Cardiac glycosides	digitalis, digitoxin, digoxin, lanatoside C
Antihypertensives	apresoline, atenolol, captopril
Antiparasitics	praziquantel, pentamidine, metronidazole
Antiarrhythmics	atenolol, isosorbide, propranolol, verapamil
Hormones	corticosteroids, testosterone, antidiuretics, oestrogen, thyroid growth hormone, progesterone, gonadotropin, mineral corticoids, calcitonin, ACTH
Antidiabetics	insulin, diabenese
Cancer drugs	adriamycin, daunorubicin, bleomycin, azathioprine, cyclophosphamide, vincristine, methothrexate, vinblastin, cisplatin
Tranquillizers	benzodiazepines, chlorpromazine, butyrophenone, hydroxyzine, meprobamate, phenothiazine, reserpine, thioxanthene
Steroids	betamethasone, dexamethasone, hydroxycortisone

Antihistamines	pyribenzamine, chlorpheniramine, diphenhydramine
Sedatives + analgesics	morphium, Dilaudid, codeine, codeine-like synthetics, Demerol, oxymorphone, phenobarbital, barbiturates
Antibiotics	amoxicillin, ampicillin, carbenicillin, cefadroxil, cefazolin, cefoxitin, cephalothin, erythromycin, gentamycin, moxalactam, imipenem, penicillin, piperacillin, tetracycline, tobramycin, vancomycin and other aminoglycosides
Proteins + glycoproteins	lymphokines, interleukins 1, 2, 3, 4, 5, 6, cytokines: GM-CFS, M-CSF, G-CFS, inhibin, nerve growth factors, tumour growth factors, tumor tissue killing factors, Muller's inhibiting substance, insulin, collagen, fibronectin, laminin, other proteins accessible by DNA recombination
Immunotherapeutics	interferon, interleukin-2, γ-globulin, monoclonal antibodies
Antimycotics	amphotericin B, myconazole, muramyl dipeptide, chlortrimazole
Hypertonics	dopamine, dextroamphetamine
Vaccines	influenza vaccine
Antivirals	acylovir and derivatives, Winthrop 511711, ribavirin, rimantadine, amantadine, azidothymidine and derivatives, adenine arabinoside, protease inhibitors of the amidine type
Nucleic acids and analogues	DNA, RNA, methylphosphonates and analogues

Other outer cell surface receptor blockers

Preferred examples of pharmaceutically, cosmetically and dietetically active ingredients include, for example, the following substances for incorporation in the liposome formulation:

Actinomycin D, acylglutamate, AD 32, adenosine triphosphate, adrenaline, adriamycin, alanine, albumin, allopurinol, aminobenzoic acid, amphetamine sulphate, amgloglucosidase, angiotensin, anthracyclines, ascorbic acid, L-asparginase, azathioprine, bacteria, benaxoprofen, betamethasone, 2,3-biphosphoglycerate, bitolterol mesylate, bromazepam, bromocriptine, butaconazol nitrate, calcitonin, carbazochrome, carotene, casein, castor oil, chloroquine, chymotrypsin, clonazepam, coagulation factors, coenzymes, colchincine, collagen, corticosteroids, cosmetics, cyanocobalamin, cyclosporin, cytosine arabinoside, daunomycin, decaglycerol monolaurate, dexamethasone, dextran, diagnostics, diazepam, diacetyl phosphate, dihydroxyergotoxin, dihydroxyacetone, diltiazem, dipyridamole, DNA, doxorubicin, EDTA, elastin, ephedrine, epinephrine, ergot alkaloids, erythromycin, ethyl mercuriothiosalicylate, extract of aloes, ferritin, fibroin, flunitrazepam, fluocinolone acetonide, 5-fluoracil, frentizol, α-globulin, glycogen, **glucose, glutathione, glycerol, glycine, glycoproteins, gold salts**, griseofulvin, guanine, haemoglobin, heparin, herpes antigen, hyaluronic acid, hydroquinone, hydrocortisone, hydroxyproline, hysothiamine, ibuprofen, imidocarb, imipramine, immunoglobulins, indomethacin, inositol hexaphosphate, inositol pentaphosphate, inositol tetraphosphate, insulin, interferon, inulin, isopropyl myristate, kallikrein inhibitor, ketoprofen, lactalbumin, lanosterol, LH-RH, linolenic

acid, linoleic acid, mazidol, medazepam, meflopquin, meglumine antimonate, methasone valerate, methionine, methotrexate, muramyl peptide, naproxen, nitrazepam, sodium cromoglycate, sodium sulfite, oestradiol, oil of sesame, oil of sweet almonds, orylzanol, oxytoxin, PEG, penicillamine, penicillin, pentamidin, perhydrosqualane, phenylbutazone, poly A, E, polyvinyl carbonate, prednisolon, primaquine, progesterone, propranolol, proteins, protein hydrolysates, pyrenzepin, pyroglutamic acid, pyrrolidine, pyrrolidine carboxylic acid, radio isotopes, retinoids, RNA, salbutamol, salicylates, scopolamine, secretin, serum albumin, sitosterol, stanozolol, steroylglutamate, stearylamine, stigmasterol, streptomycin, strophanthin, sucrose distearate, superoxide dismutase, tartaraldehydetheophylline, timepidium bromide, tocopherol, tretinoids, tretoquinol, triethanolamine, triethanol salicylate, trimebutin maleate, trypsin, ubiquinone, urokinase, vaccines, vanillin, vasopressin, vindesine, vitamins.

The separation of non-enclosed substances can be carried out by dialysis, gel chromatography, flotation, centrifugation or ultracentrifugation. The choice of method depends on the method by which the liposomes have been prepared. Such methods are familiar to the specialist.

However, the separation of non-enclosed substances is of **minor importance and is usually unnecessary when the liposomes** are to be used for cosmetic purposes.

The separation of non-enclosed substances is of relevance when the liposome preparations are intended for pharmaceutical or medical purposes.

The following examples serve to explain the invention in more detail:

Example 1

A mixture of 3 g phospholipid (containing 80% phosphatidylcholine), 0.1 g sodium stearate and 10 g demineralized water are vigorously stirred for 30 minutes at 50°C in a commercial laboratory mixer. The phospholipid swells after a very short time and produces a uniform, highly viscous swollen phase with a pH of ca. 6. A liposomal formulation can be produced from the gel by dilution with water.

Example 2

A mixture of 0.1 g potassium oleate, 0.8 g oleic acid and 10 g Phospholipon 100 (highly concentrated phosphatidylcholine) in 100 g demineralized water are vigorously stirred for 30 minutes at 50°C in a laboratory mixer. The phospholipid swells after a very short time and produces a uniform, viscous swollen phase with a pH of ca. 7.

Example 3

A mixture of 10 g enriched phospholipid (containing 80% phosphatidylcholine), 0.1 g potassium oleate and 0.8 g oleic acid in 100 g demineralized water are vigorously stirred for 20 minutes at 50°C in a laboratory mixer. The phospholipid swells after a very short time and produces a uniform, viscous swollen phase. The pH of the swollen phase is 6.5.

Example 4

A mixture of 0.1 g sodium stearate, 0.4 g stearic acid and 3 g Phospholipon 100 H (fully hydrogenated, highly concentrated phosphatidylcholine) in 100 g demineralized water are vigorously stirred at 80°C in a laboratory mixer. The phospholipid swells after a very short time and produces a uniform, highly viscous swollen phase with a pH of ca. 8.5.

Example 5

A mixture of 0.2 g potassium palmitate and 15 g Phospholipon 100 H (highly concentrated phosphatidylcholine) in 100 g demineralized water are vigorously stirred for 10 minutes at 50°C in a laboratory mixer. The homogeneous, viscous swollen phase, produced from the swollen phospholipid after a few minutes, possesses a pH of 6.5.

Example 6

A mixture of 0.2 g palmitic acid, 0.1 g triethanolamine and 15 g Phospholipon 100 H (highly concentrated phosphatidylcholine) in 100 g demineralized water is vigorously stirred for 10 minutes at 50°C in a laboratory mixer. The phospholipid swells after a short time and forms a homogeneous, viscous swollen phase with pH = 7.5.

Example 7

A mixture of 0.1 g potassium oleate and 10 g phospholipid containing 80% phosphatidylcholine in 100 g demineralized

water is vigorously stirred at 50°C in a laboratory mixer. The phospholipid swells after a short time and forms a homogeneous, viscous swollen phase with a pH of 7.5.

Example 8

A mixture of 0.1 g potassium oleate and 10 g phospholipid containing 50% by weight phosphatidylcholine in 100 g demineralized water is vigorously stirred for 30 minutes at 50°C in a laboratory mixer. The phospholipid swells extensively and forms a homogeneous, viscous swollen phase with a pH of 5.7.

Example 9

A mixture of 0.2 g potassium palmitate, 15 g Phospholipon 100 H (concentrated phosphatidylcholine) and 2 g thistle oil in 100 g demineralized water is vigorously stirred for 10 minutes at 50°C in a laboratory mixer. The phospholipid swells after a short time and forms a homogeneous, viscous swollen phase with a pH of 6.5.

Example 10

A mixture of 3.5 g Lipacide (acylated collagen hydrolysate), 0.2 g potassium hydroxide and 1 g phospholipid containing 80% by weight phosphatidylcholine in 5.5 g demineralized water is vigorously stirred for 5 minutes at 60°C in a laboratory mixer. After swelling the swollen phase has a pH of 6. It can be used directly as a lotion for cosmetic purposes.

Example 11

A mixture of 3.5 g Lipacide PCO (acylated collagen hydrolysate), 0.3 g potassium hydroxide and 6 g phospholipid containing 80% by weight phosphatidylcholine in 10 g demineralized water is vigorously stirred for 10 minutes at 60°C in a laboratory mixer. The phospholipid swells after a short time and forms a swollen phase with a pH of 7. It can, for example, be used as a creme base.

Example 12

A mixture of 10.5 g Lipacide PCO (acylated collagen hydrolysate), 0.4 g sodium hydroxide, 3 g phospholipid containing 80% by weight phosphatidylcholine and 283 g demineralized water is vigorously stirred for 10 minutes at 60°C in a laboratory mixer. The swollen phase formed in a short time has a pH of 6 and can, for example, be used as a lotion for cosmetic purposes.

The water-containing formulations according to the invention described in examples 2 to 12 can be converted into liposomal formulations by dilution with water. The direct preparation of liposomal formulations via the swollen phase will be described in the examples that follow:

Example 13

A mixture of 0.5 g citric acid, 0.3 g sodium hydroxide, 10 g anhydrous glucose and 100 g demineralized water is vigorously stirred in a suitable mixer and 30 g Phospholipon 100

(highly concentrated phophatidylcholine) is worked homogeneously into the solution at room temperature. The pH is 6.5. A liposomal formulation is produced with a mean particle size of 100 nm.

Example 14

A mixture of 143 g 10% sodium hydroxide solution, 100 g Lipacide PCO (acylated collagen hydrolysate), 375 g phospholipid with 80% by weight phosphatidylcholine and 4018 g demineralized water is homogenized intensively in a mixer for 30 minutes and then mixing is continued while 300 g 15% sodium chloride solution is worked in. A liposomal formulation is produced with a pH of 6.4 and a particle size of ca. 129 nm. The liposomal formulation is very suitable for cosmetic purposes, for example as a base for skin care or similar preparations. The storage life can be increased by the addition of a preservative, for instance 1 g Kathon CG.

Example 15

A mixture of 4.4 g 10% sodium hydroxide solution, 6 g phospholipid, 22.5 g Phosal 80 and 264 g demineralized water are homogenized intensively in a mixer for 30 minutes and then mixing is continued while 2.7 g sodium chloride is worked in. A liposomal formulation is produced which is suitable for cosmetic purposes, e.g. skin care preparations and hair rinses. The pH of the formulation is 6.1 and, hence, it is very gentle to the skin. The mean particle size is 120 nm. The formulation can also contain 0.06 g Kathon CG as preservative to prolong its storage life.

Example 16

A mixture of 1.3 g 10% sodium hydroxide solution, 1.5 g phospholipid, 22 g Phosal 80 and 272 g demineralized water is homogenized intensively in a suitable mixer for 60 minutes and then mixing is continued while 2.7 sodium chloride is worked in. The liposomal formulation which is produced has a pH of 6.3 and a mean particle size of 150 nm. It is suitable for cosmetic purposes. The formulation can also contain 0.06 g Kathon CG as preservative.

Example 17

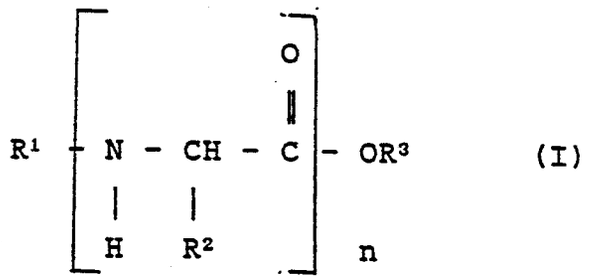
A mixture of 2 g 10% sodium hydroxide solution, 6 g phospholipid, 22.5 g Phosal 80 and 267 g demineralized water is homogenized intensively in a mixer for 60 minutes and 2.7 g sodium chloride is added. After the sodium chloride has been worked in a liposomal formulation is produced which has a pH of 5.6 and a mean particle size of 134 nm. The formulation is very gentle to the skin and can be used for cosmetic purposes. The formulation can also contain 0.06 g Kathon CG as preservative.

Patent claims

1. Methods for the preparation of water-containing formulations with phospholipids by admixture of phospholipids with water under stirring in the presence of swelling accelerators, whereby a mixture of saturated or unsaturated organic carboxylic acids and their salts with a strong base yielding a pH of 5 to 7 is used as swelling accelerator in proportions from 1 to 30% by weight, the concentration of phospholipid is 20 to 50% by weight and the remainder water, based on the total weight of the formulation.

2. A method according to claim 1,
whereby saturated or unsaturated carboxylic acids with 10 to 22 carbon atoms and their alkali metal salts, ammonium salts or amine salts are used.

3. A method according to claim 1,
whereby the carboxylic acids are a hydrolysate of collagen and their alkali metal salts according to formula I:



(I)

are used, where

R^1 is hydrogen or a saturated or unsaturated acyl group with 1 to 22 carbon atoms,

R^2 is the side chains of the amino acids of collagen and

R^3 is hydrogen or an alkali metal ion and

n is an integer between 1 and 10.

4. A method according to claim 1,
whereby the carboxylic acids used are a hydrolysate or acylated hydrolysate of casein, keratin or an O-acylated derivative of hydroxyproline.

5. A method according to claims 1 to 4,
whereby the proportion of swelling accelerator used is 1 to 10% by weight of the total formulation.

6. A method according to claims 1 to 5,
whereby choline phosphate, sodium acetate, sodium chloride, calcium chloride, Na_2HPO_4, NaH_2PO_4, $NaHCO_3$, choline chloride or mixtures of the same in proportions of 0 to 20% by weight with respect to the total weight of the formulation are present during admixture of the phospholipid to the water or are added to the formulation afterwards as aqueous solution.

7. The use of any of the formulations prepared according to claims 1 to 6 for the formation of liposomes by dilution with water to a liposome concentration of 0.1 to 20% by weight with respect to the weight of the total formulation.

8. Application according to claim 7,
whereby the liposomes enclose hydrophilic substances or lipophilic substances, whereby the enclosed substances are added to the formulation during admixture of the phospholipids with water or with stirring before dilution of the formulation with water.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 90/00621

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: A 61 K 9/127

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	FR, A, 2597345 (L'OREAL) 23 October 1987 see page 4, lines 20-27; page 6, lines 16-24; page 11, line 23 - page 12, line 4; pages 16,17; example 3	1-8
X	FR, A, 2609393 (LABORATOIRES SEROBIOLOGIQUES) 15 July 1988 see page 2, line 1 - page 6, line 6; claims	1-8
X	FR, A, 2540381 (PARFUMS CHRISTIAN DIOR) 10 August 1984 see claims	1-8
X	EP, A, 0088046 (CIBA-GEIGY) 7 September 1983 see page 10, paragraphs 3-7; claim 1	1,2,5-8

* Special categories of cited documents: [10]

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

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

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

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

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

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

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28th June 1990	31 JUIL. 1990
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9000621 SA 36022

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 26/07/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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