(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 November 2015 (12.11.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/170247 A1

(51) International Patent Classification:
A61K 9/00 (2006.01) *A61K 31/202* (2006.01)
A61K 9/127 (2006.01) *A61K 31/353* (2006.01)

(21) International Application Number:
PCT/IB2015/053267

(22) International Filing Date:
5 May 2015 (05.05.2015)

(25) Filing Language: Italian

(26) Publication Language: English

(30) Priority Data:
CA2014A000003 6 May 2014 (06.05.2014) IT

(71) Applicant: **FIDIA FARMACEUTICI S.P.A.** [IT/IT]; Via Ponte della Fabbrica 3/A, 1-35031 Abano Terme PD (IT).

(72) Inventors: **BROTZU, Giovanni;** Piazza Garibaldi 4, I-09127 Cagliari (CA) (IT). **BROTZU, Giuseppe;** Piazza Garibaldi 4, 1-09127 Cagliari (CA) (IT).

(74) Agents: **ASENSIO, Raffaella** et al; c/o Perani & Partners, Piazza San Babila 5, 1-20122 Milan (IT).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17 :

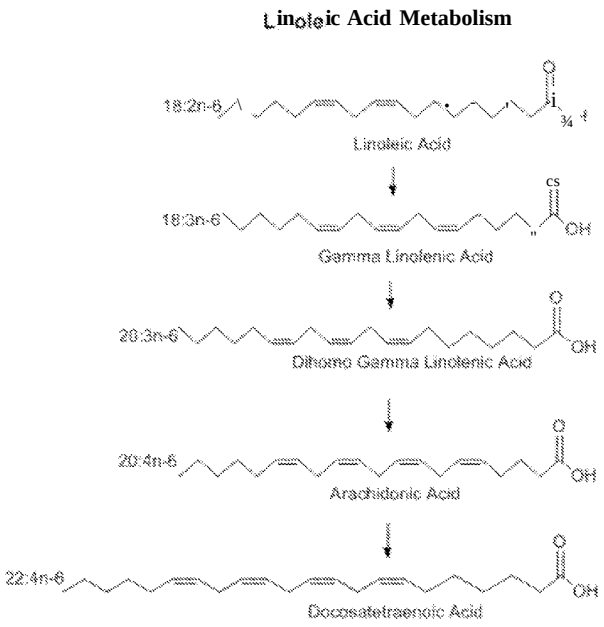
— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

(54) **Title:** LIPOSOMES CONTAINING DI-HOMO-GAMMA LINOLENIC ACID (DGLA), FORMULATIONS CONTAINING THEM AND USE THEREOF

Fig. 1



(57) **Abstract:** Phospholipid liposomes comprising at least one polyunsaturated fatty acid selected in the group consisting of dihomo-gamma-linolenic acid (DGLA), linoleic acid, gamma-linolenic acid, arachidonic acid, docosatetraenoic acid, a plant estrogen selected from equol, genistein, daidzein, glycitein; **a compound able to increase cation capacity of** the liposome selected from essential amino acids, carnitine and derivatives thereof; a stabilizer selected from cholesterol, cholesterol sulphate and stearylamine, for use in the treatment of alopecia, baldness, hair loss, and related pharmaceutical compositions containing them as active ingredients in association with suitable excipients and/or diluents.

WO 2015/170247 A1

"Liposomes containing di-homo-gamma linolenic acid (DGLA), formulations containing them and use thereof"

**** *** ****

Field of the invention

The present invention discloses and claims liposomes comprising active substances, formulated for topical and/or oral use and preferentially for topical use, to be used for the treatment of alopecia, baldness, hair loss, also in postmenopausal women, and in general for hair regrowth.

State of the art

Hair loss is an extremely common phenomenon concerning both men and women, caused by various factors such as stress, diet, seasonal changes, hereditary factors, particular diseases or pharmacological treatments; eventually, in the female population, it can be caused by the lack of sexual hormones occurring during menopause. In fact, the female menopausal population generally reports, if not hair loss, at least a decrease in the characteristics of strength, brightness, resistance to brushing .

The phenomenon often has such consequences on the patient that it is considered an actual disease, and anyway it has an unquestionable impact from the aesthetic point of view. There are many methods described for treatment

and prevention of baldness through aids acting on the hair shaft, trying to make it stronger, or on the bulb, attempting to identify the reasons why it becomes atrophied to find a solution. Some positive attempts were made by employing actual drugs, such as the well known Minoxidil and Finasteride.

They act on completely different districts. Finasteride acts as an inhibitor of type 2 alpha-reductase (involved in the synthesis of steroids), while Minoxidil is a vasodilator. Both products, although quite efficient, display remarkable complications, given their nature of drugs and the inevitable appearance of side effects, and this has strongly limited their use.

Products acting on the hair shaft, and that are essentially of cosmetic type, have proven to be poorly efficient in time.

Despite the multiple remedies available on the market, the problem is far from being called satisfactorily solved. A suggestion comes from WO2013171668, wherein PGE1 is employed in association with plant sterols, and administered locally on the scalp. PGE1 is a drug acting on several levels: it inhibits type 1 and 2 alpha-reductase, is a vasodilator and improves angiogenesis .

Such compositions are delivered in the form of liposomes, which had already demonstrated to be valuable

carriers for PGE1 for systemic or local treatment of vascular diseases and their cutaneous outbreaks (ulcers). For example, WO 2011/095938 discloses unilamellar liposomes, encapsulating PGE1 and/or PGEl-a-cyclodextrin in association with L-propionyl-carnitine for the treatment of vascular diseases in diabetic subjects.

Encapsulation in liposomes is not able to increase the very poor stability of PGE1, though; the findings of WO2013171668 in fact, must be freeze-dried or, if in suspension, stored at low temperature (about -20°C) up to the time of use, if one intends to prevent the almost complete oxidation of PGE1. This obviously represents a complication not only on the industrial production level, but mainly on the product safety level. To act against the oxidative process, in fact, the trend is to resort to PGE1 doses that might be dangerous for the body, considered the repeated employment of these products, which must be administered over very long periods.

SUMMARY OF THE INVENTION

The present invention overcomes the limits of the state of the art through liposome formulations containing PGE1 precursors stable at room temperature, storable in a liquid, semi-solid or solid form, directly applicable without preventive preparation and absolutely free of any kind of side effect related to the use of an actual drug.

Clinical trials that were carried out further demonstrated a surprisingly higher efficacy of the products described herein in comparison with what is known, so as to make their use possible at concentrations markedly lower than those of already known products.

Description of figure 1

Figure 1 reports the synthetic scheme of linoleic acid metabolism.

Detailed description of the invention

Object of the present invention is liposomes comprising active substances, formulated for topical and/or oral use and preferentially for topical use, to be used for the treatment of alopecia, baldness, hair loss, also in postmenopausal women, and in general for hair regrowth. Substances enclosed in the liposomes are PGE1 precursor polyunsaturated fatty acids selected from at least one of dihomo-gamma-linolenic acid (DGLA), linoleic acid, gamma-linolenic acid, arachidonic acid, docosatetraenoic acid in association with a plant estrogen and a compound able to increase cation capacity of the liposome, then improving its adhesiveness. Liposomes further comprise a stabilizer, to improve stability of the preparations. Preferably used for the purposes of the present invention.

Gamma-linolenic acid and dihomo-gamma-linolenic acid are sometimes defined as gamma-linoleic, and dihomo-gamma-linoleic acid, respectively, due to the fact that they are intermediates in the process of prostaglandin biosynthesis starting from linoleic acid, which is progressively desaturated according to the scheme reported in figure 1.

For the purposes of the present invention, the definitions "gamma-linolenic" acid and "gamma-linoleic acid", are synonyms and identify one single compound reported in figure 1, similarly, the definitions "dihomo-gamma-linoleic acid" and "dihomo-gamma-linolenic acid", both identifying one single chemical compound reported in figure 1 too, are also synonyms, for a compound that in the present description is also identified by the acronym DGLA.

Doses of DGLA used range between 0.05% and 0.3% and preferably between 0.1% and 0.2% of the phospholipid amount used.

Liposomes according to the invention are composed of a phospholipid vesicle containing an aqueous solution core. Phospholipids composing the vesicle wall can be natural phospholipids or can be of synthetic origin, considering their high biocompatibility and absence of toxicity.

Phospholipids that can be used according to the invention are, for example: phosphatidylcholine (lecithin), phosphatidylethanolamine, phosphatidylserine, phosphatidyl glycerol, phosphatidyl inositol, dimyristoylphosphatidylcholine (DPMC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), palmitoyl-stearoylphosphatidylcholine, sphingomyelin, and other similar ones. Particularly suitable are phosphatidylcholine, dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DPMC) and preferentially phosphatidylcholine.

By plant estrogens, in general, isoflavons such as, for example, genistein, daidzein, glycitein are meant, and particularly preferred is equol, a key metabolite of daidzein, for long already in use by oral route for reducing menopausal symptoms (flushes, sweating, sudden mood changes). - Doses employed range between 0.1% and 2%, and preferably between 0.3% and 1% of phospholipid amount.

To increase the adhesiveness of liposomes and to promote cell metabolism of the endothelium of micro-capillaries, compounds able to increase cation capacity of the liposome are also used; for this purpose, for example, essential amino acids are useful, for example: phenylalanine, isoleucine, methionine, lysine or

carnitine, and even more preferably one of its derivatives, L-propionylcarnitine, which, in addition, is a carrier for fatty acids and allows mitochondria to use them for ATP production. The amount of L-propionylcarnitine used ranges between 0.1% and 2%, and preferably between 0.3% and 1.3% of phospholipid amount.

Liposomes comprise stabilizers such as, for example, cholesterol, cholesterol sulphate, stearylamine or others known to the expert in the field. Within the scope of the present invention the use of stearylamine that, in addition to stabilizing the microemulsion increases adhesiveness of liposomes and further increases their cation capacity, was shown to be particularly useful. Various laboratory tests demonstrated that the percentage of stearylamine used is able to influence the size of liposomes and their charge more considerably than other substances used. Stability tests additionally highlighted that liposomes prepared with stearylamine at concentrations higher than 0.5%, preferably between 1% and 5%, and even more preferably between 2% and 4% of phospholipid, in particular phosphatidylcholine, retain their peculiar characteristics unchanged for at least 30 days.

Object of the present invention is thus phospholipid liposomes comprising:

- a PGEl precursor polyunsaturated fatty acid selected from at least one of: dihomo-gamma-linolenic acid (DGLA), linoleic acid, gamma-linolenic acid, arachidonic acid, docosatetraenoic acid,

- a plant estrogen selected from equol, preferably S-equol, genistein, daidzein, glycitein,

- a compound able to increase cation capacity of the liposome selected from essential amino acids, carnitine and derivatives thereof, a stabilizer selected from cholesterol, cholesterol sulphate and stearylamine,

for use in the treatment of alopecia, baldness, hair loss .

More preferably liposomes according to the present invention are composed of phosphatidylcholine and comprise dihomo-gamma-linoleic acid at a concentration ranging from 0.05% to 0.3% and preferably from 0.1% to 0.2% of the phospholipid amount used, equol, even more preferably S-equol, at a concentration comprised between 0.1% and 2% and preferably between 0.3% and 1% of the phospholipid amount used, L-propionylcarnitine at a concentration comprised between 0.1% and 2% and preferably between 0.3% and 1.3% of the phospholipid amount used, stearylamine at a concentration comprised between 1% and 5% and more preferably between 2% and 4% of the phospholipid amount used.

Liposomes according to the invention can be readily prepared (method A)

• admixing solutions of the various components in an organic solvent (selected from ethanol, methanol, isopropanol, acetone or mixtures thereof) and water and

• sonicating the thus obtained mixture for the time needed .

The preferably selected organic solvent is ethanol.

Alternatively, it is possible to (method B)

· sonicate the mixtures comprised of the solutions of the various components in an organic solvent only (selected from ethanol, methanol, isopropanol, acetone or mixtures thereof)

• evaporate the organic solvent

· take up in an aqueous solution.

Also in this case the preferably used solvent is ethanol .

Additionally, to increase the adherence of liposomes to the cells of the dermis avoiding their traumatic removal, the outer surface of the liposomes may be optionally coated with hydrophilic polymers, such as for example polylisine, polyornithine, fibronectin and/or mixtures thereof.

Such coating, if applied, can be very simply obtained according to known techniques, for example letting the

mixture of liposomes drop into a solution of the selected polymer .

Further object of the present invention is the administration of the liposomes of the invention in pharmaceutical compositions or formulations comprising them, suitable for topical application and/or for oral administration .

Formulations for topical use according to the invention comprising liposomes as described above are normally in classic forms for topical application, such as for example: aqueous suspensions (lotions), ointments, creams, gels, sprays, polymeric films, in which liposomes are dispersed using known methods of pharmaceutical technique for preparing said formulations. Eventually, topical administration of liposome suspensions described herein is also possible in the form of aerosols, after producing the specific pharmaceutical form according to known techniques.

Gels and polymeric films can be made of organic polymers such as for example: polyacrilates , sodium hyaluronate, hydroxypropylcellulose (HPMC) , polyethylene glycol 400 (PEG 400) and water, in suitable ratios, taken individually or in mixtures thereof, and are characterized in terms of viscoelastic properties, thickness and *in vitro* bioadhesion using a rheometer, a micrometer and a

tensiometer respectively, according to the methods known to the expert in the field.

Films are used for preparing dressings for topical application and are comprised, for example, of strips of various sizes for application to the skin. Such films are particularly useful when the area to be treated is very wide, and thus administering the product as a lotion, cream or gel becomes less comfortable.

Lotions, eventually, can also be dispensed from "spray no-gas", then propellant free, bottles.

Additionally, it is possible to administer liposomes by oral route after having them properly delivered in capsules of gelatine or other substances suitable for the purpose and known to the expert in the field.

Regardless of the selected pharmaceutical form, it is clear that all excipients and all tactics known to the technical formulator in order to ensure stability, storability, absence of contamination, etc. of the preparations will be employed.

The present invention relates to pharmaceutical compositions for the treatment of alopecia, baldness, hair loss, also in postmenopausal women, and in general to promote hair regrowth, such compositions comprise phospholipid liposomes comprising:

- a PGE1 synthesis precursor polyunsaturated fatty acid, selected at least from one of the following: dihomo-gamma-linolenic acid (DGLA), linoleic acid, gamma-linolenic acid, arachidonic acid, docosatetraenoic acid,

- a plant estrogen selected from equol, preferably S-equol, genistein, daidzein, glycitein,

- a compound able to increase the cation capacity of the liposome selected from essential amino acids, carnitine and derivatives thereof,

- a stabilizer selected from cholesterol, cholesterol sulphate and stearylamine and containing pharmaceutically acceptable excipients.

Preferably the pharmaceutical compositions described herein comprise phospholipid liposomes composed of phosphatidylcholine, dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DPMC) and preferably phosphatidylcholine, and

- DGLA, at a concentration ranging from 0.05% to 0.3% and preferably from 0.1% to 0.2% of the phospholipid amount used;

- equol, at a concentration ranging from 0.1% to 2% and preferably from 0.3% to 1% of the phospholipid amount used;

- L-propionylcarnitine at a concentration comprised between 0.1% and 2% and preferably between 0.3% and 1.3% of the phospholipid amount used;

- stearylamine at a concentration comprised between 1% and 5% and more preferably between 2% and 4% of the phospholipid amount used

and suitable pharmaceutically acceptable excipients.

The invention is now better decsribed by the following examples, which have merely demonstrative purposes.

Example 1: preparation of a liposome mixture in the form of a lotion comprising 1% stearylamine (method A)

Phosphatidylcholine (Lipid S75 Humangrade)	1 g
DGLA	1.25 mg
S-Equol	7 mg
Ethanol	1 ml
L-propionylcarnitine	7 mg
Stearylamine	10 mg
Sterile water	5 ml

DGLA, S-equol and stearylamine are dissolved in the dose of ethanol and phosphatidylcholine is added to the solution. Propionylcarnitine is dissolved in 5 ml of water and the thus obtained solution is added to the previous one. The resulting mixture is placed in a sonicator (Sonipress 150 kw) and submitted to 25 sonication cycles. Each cycle is composed of 5 seconds of full power

sonication alternating with 2 seconds of rest. The lotion thus obtained is divided into 5 vials and each one is brought to the volume of 7 ml with additional sterile water. The lotion thus obtained is ready for use.

Example 2: preparation of a liposome mixture in the form of a lotion comprising 1% stearylamine (method B)

Phosphatidylcholine (Lipid S75 Humangrade)	1 g
DGLA	1.25 mg
S-Equol	7 mg
Ethanol	1 ml
L-propionylcarnitine	7 mg
Stearylamine	10 mg
Sterile water	5 ml

DGLA, S-equol, stearylamine and L-propionylcarnitine are dissolved in the dose of ethanol and the solution obtained, after addition of phosphatidylcholine, is placed in a sonicator (Sonipress 150 kw) and submitted to 25 sonication cycles. Each cycle is composed of 5 seconds of full power sonication alternating with 2 seconds of rest. Ethanol is then completely evaporated and the liposomes obtained are brought into contact with 5 ml of sterile water. The lotion thus obtained is divided into 5 vials and each one is brought to the volume of 7 ml with additional sterile water. The lotion is ready for use.

Example 3: preparation of a liposome mixture in the form of a lotion comprising 2% stearylamine (method A)

See example 1, with the only variation of the dose of stearylamine employed, equal to 20 mg.

Example 4: preparation of a liposome mixture in the form of a lotion comprising 3% stearylamine (method A)

See example 1, with the only variation of the dose of stearylamine employed, equal to 30 mg.

To increase their shelf-life, the lotions thus prepared can also be freeze-dried according to methods known by the expert in the field, and reconstituted with water at the time of application.

Characterization of liposomes

Liposomes comprising various percentages of stearylamine are characterized in terms of size, polydispersity index (PI) and zeta potential (Pζ); PI is measured via Photon Correlation Spectroscopy (PCS) and expresses the size uniformity of the particles constituting the dispersed system. The lower the PI value, the higher the size uniformity of the particles considered and then, the higher the homogeneity of the dispersion.

As a convention, a system has high uniformity for PI values of <1.1.

The zeta potential (Pζ) expresses the electrophoretic mobility of particles (in this case, liposomes) in a

thermostated cell and it is representative of the stability of the dispersed systems; it was measured through M3-PALS (Phase Analysis Light Scattering) with the Zetasizer nano instrument (Malvern Instrument, UK). It should be remembered that Pζ values higher than -30 mV are an index of stability.

Morphological-structural characteristics were studied through transmission electron microscopy (TEM) and polarized light optical microscopy.

The diameter of liposomes results to be, regardless of the amount of stearylamine employed, always lower than 100 nm and the polydispersity index lower than 0.2. Pζ values demonstrate that the preparations are stable. Samples A, B and C were prepared according to examples 1, 3, and 4 respectively, and after detection of the characteristics, were stored for 30 days at room temperature. At the end, a further detection of Pζ values was performed and data are summarized in the following table 1.

Table 1

Samples	Diameter (nm)	PI	Pζ (mVolt) (starting)	Pζ (mVolt) (30 d)
A (1% stearylamine)	74.84±0.88	0.187	-24.7	-30.05
B (2% stearylamine)	93.52±1.17	0.150	+24.9	+8.52
C (3% stearylamine)	96.02±1.12	0.178	+42.9	+23.5

It is clear that the samples prepared are all homogeneous, and this ensures uniformity of distribution of the active substances, and stability, both just prepared and, mainly and surprisingly, even after 30 days after preparation. Such observations are true for all the concentrations of stearylamine used, and particularly for sample c, which contains it at a concentration of 3%.

Encapsulation efficiency (E%) of DGLA and plant estrogen in liposomes was determined by HPLC, after carrying out a rupture of the liposomes with a suitable membrane lysating agent, such as for example Triton X-100. The amount of active substances present after purification is in the order of 85% of the starting concentration and remains the same also after 30-day storage.

Experimental study

Patients of female and male gender characterized by massive hair reduction due to various causes, and also a small number of patients of female gender of postmenopausal age, reporting hair thinning, hair reduction and loss of hair strength were selected. The clinical efficacy of preparation 4 that on previous tests displayed the best characteristics of homogeneity and stability was evaluated .

Treatments :

Lotion A, prepared according to the present example 4;

Lotion B, comparison, prepared as described in WO 2011/095938 (example 1) and then containing PGE1 and S-equol in 1:1 ratio

Lotion C, containing 7 mg of PGE1 enclosed in liposomes as the only active substance, prepared according to method A;

Lotion D, containing 1,25 mg of DGLA enclosed in liposomes as the only active substance, prepared according to method A.

Groups of patients:

- Group 1: 10 (6 male, 4 female) patients treated for 120 days with 1 ml/day of Lotion A to be applied on a well defined area of the scalp;
- Group 2: 10 (5 male, 5 female) patients treated for 120 days with 1 ml/day of Lotion B to be applied on a well defined area of the scalp;
- Group 3: 6 female menopausal patients for about 3 years, without specific diseases related to hair reduction, treated for 120 days with 1 ml/day of Lotion A to be applied on a well defined area of the scalp.
- Group 4: 4 (2 male, 2 female) patients treated for 120 days with 1 ml/day of Lotion C to be applied on a well defined area of the scalp;

• Group 5: 4 (2 male, 2 female) patients treated for 120 days with 1 ml/day of Lotion D to be applied on a well defined area of the scalp;

Evaluation was carried out at pre-determined time points by dermoscopy and direct observation with photographic detections.

Results

On day 7: both group 1 and group 2 experience a reduction of hair loss, defined "substantial" by patients of group 1 and "modest" by those of group 2. Group 3 feels hair as more robust. Groups 4 and 5 do not report any variation;

On day 20: in group 1 all the patients have stopped losing hair and report the presence of a substantial fuzz. In group 2 hair loss has stopped in 6 patients and fuzz, where present, is more modest (in terms of number/cm ^{2}). Group 3 reports an aesthetic improvement related to hair brightness. Group 4 reports a minimal reduction in hair loss. Group 5 does not report any variation;

On day 45: in group 1, fuzz has grown markedly stronger and takes the colour and consistency of natural hair. In group 2, as expected, all the patients have stopped losing hair, but only some of them report a certain degree of regrowth, fuzz is very weak and not completely coloured yet. Group 3 reports disappearance of split ends. Group 4

reports arrested hair loss in all the subjects and, in some of them, presence of weak fuzz. Group 5 does not report any variation;

On day 90: in group 1 all the patients report total disappearance of hair reduction areas that have been replaced by robust and shiny hair. In group 2 hair reduction is still visible, even if areas are all covered with thick fuzz. Group 3 confirms a general hair improvement. Groups 4 and 5 do not record variations from the previous evaluation;

On day 120: group 1 confirms results already seen at day 90. Group 2 continues improvement, even if in some patients of male gender the fuzz has not got the characteristics of strength and colour typical of hair yet. Group 3 confirms results previously obtained. Groups 4 and 5 do not record variations from the previous evaluation .

During the whole phase of the study the patients' skin was regularly assayed by dermoscopy, highlighting an improvement of circulation. Additionally, treated skin did not display allergic or inflammatory reactions.

From the observations reported here, first of all, it can be inferred that the formulations object of the present invention are clinically efficient in:

· stopping hair loss;

• promoting hair regrowth in areas with alopecia;

• strengthening and fortifying hair, bringing it back to its original state

both in male and female subjects with forms of hair reduction or actual alopecia due to different causes, and in menopausal women.

Formulations set up here additionally:

• do not contain potentially toxic substances or that may interfere with other active ingredients;

· are not irritating for the skin: even after long term application no evidence of irritation or inflammation was reported;

• are stable in time: analysis carried out on the content of the vials 30 days after preparation detects

· significant Pζ values in terms of stability, particularly for formulations containing 2% or, better, 3% stearylamine

• an unchanged content of active substances enclosed in liposomes;

· can be stored at room temperature and in a ready-to-use form;

and, surprisingly

• act more quickly and more effectively: comparison tests clearly demonstrate that group 1 has a more rapid, more substantial improvement and, mainly, it involves all

the patients as compared with group 2, treated with Lotion B.

Again, it should be recalled that Lotion B contains PGE1 and equol in 1:1 ratio, while Lotion A contains 30% less of active substance (DGLA). This means that the findings of the present invention exert an unexpectedly superior effect than what known, at markedly lower concentrations of active substances.

These results should be attributed only to the formulation set up herein. In fact, it is clear from data analysis that patients treated with PGE1 alone, even at the concentrations described in WO2011/095938, experience a very modest variation of the situation. Still different and most important is the observation of group 5, treated with DGLA alone at the same concentration employed in Lotion A: there is no reduction in hair loss and even less any regrowth, not even of fuzz.

The extraordinary effects that are displayed by the compositions of the present invention are then due to synergies between single components, promoted by the characteristics of the liposome suspensions used.

CLAIMS

1. Phospholipid liposomes comprising at least one polyunsaturated fatty acid selected in the group consisting of dihomo-gamma-linolenic acid (DGLA), linoleic acid, gamma-linolenic acid, arachidonic acid, docosatetraenoic acid, a plant estrogen selected from equol, genistein, daidzein, glycitein; a compound able to increase cation capacity of the liposome selected from essential amino acids, carnitine and derivatives thereof; a stabilizer selected from cholesterol, cholesterol sulphate and stearylamine.

2. The phospholipid liposomes according to claim 1, wherein the phospholipids are selected from phosphatidylcholine, phosphatidylethanol amine, phosphatidylserine, phosphatidylglycerol, phosphatidyl inositol, dimyristoylphosphatidylcholine (DPMC), dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), palmitoyl-stearoylphosphatidylcholine, sphingomyelin and, preferably, are selected from phosphatidylcholine, dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DPMC) and even more preferably from phosphatidylcholine.

3. The phospholipid liposomes according to claim 1 or 2, comprising DGLA at a concentration ranging from 0.05%

to 0.3% and preferably from 0.1% to 0.2% of the phospholipid amount used.

4. The phospholipid liposomes according to any one of claims 1-3, wherein said plant estrogen is equol, preferably S-equol, at a concentration comprised between 0.1% and 2% and preferably between 0.3% and 1% of the phospholipid amount used.

5. The phospholipid liposomes according to any one of claims 1-4, wherein said compound able to increase the cation capacity of the liposome is L-propionylcarnitine at a concentration comprised between 0.1% and 2%, and preferably between 0.3% and 1.3% of the phospholipid amount used.

6. The phospholipid liposomes according to any one of claims 1-5 wherein said stabilizer is stearylamine at a concentration comprised between 1% and 5% and more preferably between 2% and 4% of the phospholipid amount used.

7. The phospholipid liposomes according to any one of claims 1-6, on the external surface of which hydrophilic polymers selected from polylisine, polyornithine, fibronectin and/or mixtures thereof are present.

8. The phospholipid liposomes according to any one of claims 1-7 comprising: phosphatidylcholine DGLA at a concentration ranging from 0.05% to 0.3% and preferably

from 0.1% to 0.2% of the phospholipid amount used, equol at a concentration comprised between 0.1% and 2% and preferably between 0.3% and 1% of the phospholipid amount used, L-propionylcarnitine at a concentration comprised between 0.1% and 2% and preferably between 0.3% and 1.3% of the phospholipid amount used, stearylamine at a concentration comprised between 1% and 5% and more preferably between 2% and 4% of the phospholipid amount used, and further containing pharmaceutically acceptable excipients.

9. Phospholipid liposomes according to any one of claims 1-8 for use in the treatment of alopecia, baldness, hair loss.

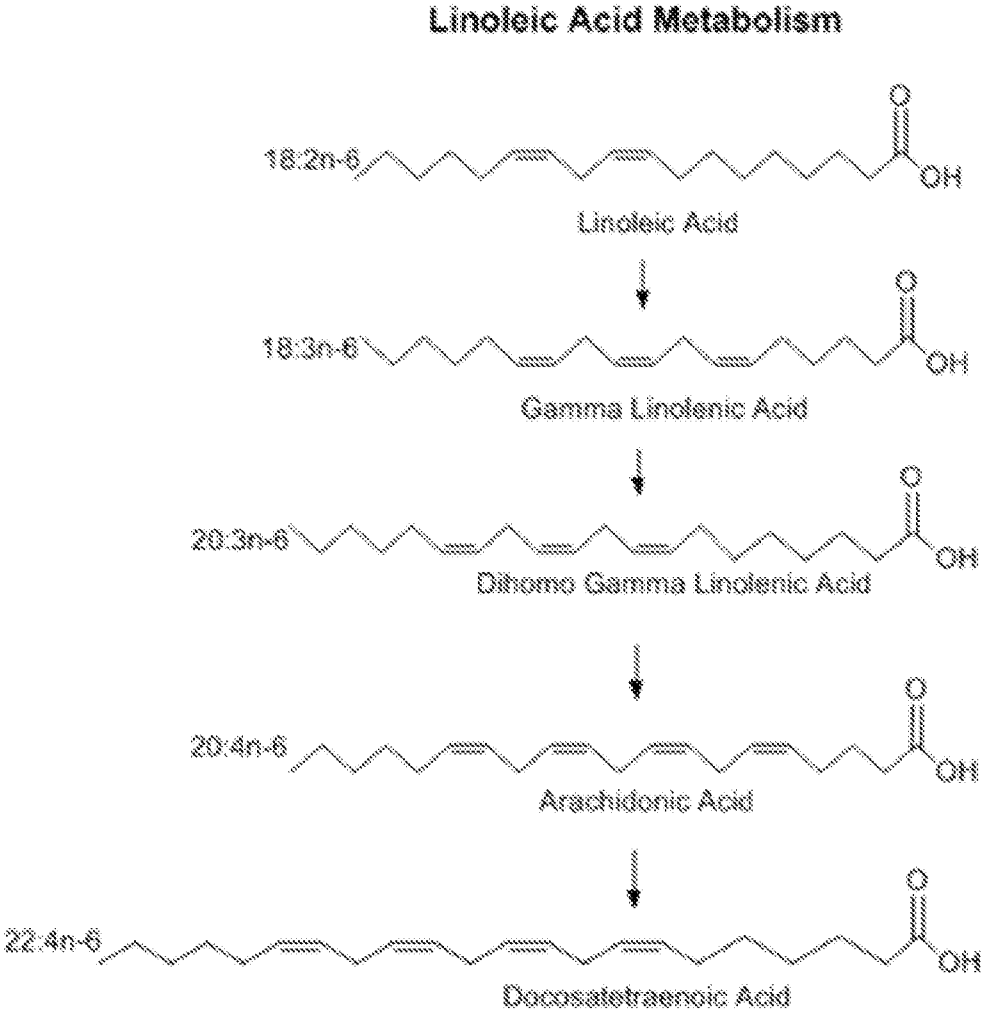
10. Pharmaceutical compositions comprising liposomes according to any one of claims 1 to 8, in association with suitable pharmaceutically acceptable excipients and/or diluents .

11. The pharmaceutical compositions according to claim 10 suitable for oral and/or topical administration.

12. The pharmaceutical compositions according to claims 10-11 for topical application in the form of aqueous suspensions (lotions), ointments, creams, gels, sprays, polymeric films.

13. The pharmaceutical compositions according to claims 10-11 for oral administration.

Fig. 1


Linoleic Acid Metabolism
18:2n-6
OH
Linoleic Acid
18:3n-6
OH
Gamma Linolenic Acid
20:3n-6
OH
Dihomo Gamma Linolenic Acid
20:4n-6
OH
Arachidonic Acid
22:4n-6
OH
Docosatetraenoic Acid

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2015/053267

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/00 A61K9/127 A61K31/202 A61K31/353
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2013/171668 Al (BROTZU GIOVANNI [IT] ; BROTZU GIUSEPPE [IT]) 21 November 2013 (2013-11-21) the whol e document	1-13
Y	EP 0 309 086 Al (EFAM0L HOLDINGS [GB]) 29 March 1989 (1989-03-29) page 2, line 52 - page 5, line 27 exampl es cl aims	1-13

-/- ·

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent but published on or after the international filing date

"L" documentwhich may throw doubts on priority claim(s) orwhich is cited to establish the publication date of another citation or other special reason (as specified)

"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 when the document is taken alone

"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

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 2015	04/08/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Gi rő, Annal i sa

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2015/053267

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2003/064929 Al (DURANTON ALBERT [FR] ET AL) 3 Apri l 2003 (2003-04-03) paragraph [0002] - paragraph [0005] paragraph [0008] - paragraph [0009] paragraph [0045] - paragraph [0046] paragraph [0051] - paragraph [0054] paragraph [0069] cl aims	1-13
Y	NATTAYA L0URITH ET AL: "Hai r l oss and herbs for treatment" , JOURNAL OF COSMETIC DERMATOLOGY, vol . 12, no. 3, 1 September 2013 (2013-09-01) , pages 210-222 , XP055078947 , ISSN: 1473-2130, D0I: 10. 1111/jocd. 12051 page 213, col umn 1, l i ne 11 - l i ne 16 tabl e 3	1-13
Y	US 2009/197954 Al (MCDANI EL WI LLIAM ROBERT [US]) 6 August 2009 (2009-08-06) paragraph [0001] paragraph [0010] - paragraph [0011] exampl es	1-13
Y	GB 2 150 588 A (KITAN0 AKIY0SHI) 3 July 1985 (1985-07-03) the whol e document	1-13
Y	W0 2011/095938 Al (BIORICERCA DI GIOVANNI BR0TZU & C SNC [IT] ; BR0TZU GIOVANNI [IT]) 11 August 2011 (2011-08-11) ci ted i n the appl i cati on the whol e document	1-13
Y	US 5 962 015 A (DELRI EU PASCAL [FR] ET AL) 5 October 1999 (1999-10-05) exampl e 1	1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2015/053267

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013171668 A1	21-11-2013	EP 2849714 A1	25-03-2015
		WO 2013171668 A1	21-11-2013
EP 0309086 A1	29-03-1989	AU 2099988 A	09-03-1989
		EP 0309086 A1	29-03-1989
		JP H01131110 A	24-05-1989
US 2003064929 A1	03-04-2003	CA 2132507 A1	14-04--1995
		DE 69426351 D1	04-01--2001
		DE 69426351 T2	05-04--2001
		EP 0648488 A1	19-04--1995
		ES 2154287 T3	01-04--2001
		FR 2711060 A1	21-04--1995
		JP 2883821 B2	19-04--1999
		JP H07238037 A	12-09--1995
		US 6465421 B1	15-10--2002
		US 2003064929 A1	03-04--2003
US 2009197954 A1	06-08-2009	NONE	
GB 2150588 A	03-07-1985	DE 3430166 A1	05-06-1985
		FR 2555442 A1	31-05-1985
		GB 2150588 A	03-07-1985
		JP S60120809 A	28-06-1985
WO 2011095938 A1	11-08-2011	CA 2787725 A1	11-08-2011
		CN 102811745 A	05-12-2012
		DK 2531222 T3	23-06-2014
		EP 2531222 A1	12-12-2012
		ES 2477233 T3	16-07-2014
		US 2013039975 A1	14-02-2013
		WO 2011095938 A1	11-08-2011
US 5962015 A	05-10-1999	NONE	