PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P05211/PCT	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/IB2015/053267	International filing date *(day/month/year)* 05.05.2015	Priority date *(day/month/year)* 06.05.2014

International Patent Classification (IPC) or national classification and IPC
INV. A61K9/00

Applicant
Fidia Farmaceutici S.P.A.

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 7 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 14 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and/or sheets containing rectifications authorized by this Authority, unless those sheets were superseded or cancelled, and any accompanying letters (see Rules 46.5, 66.8, 70.16, 91.2, and Section 607 of the Administrative Instructions).
      ☐ sheets containing rectifications, where the decision was made by this Authority not to take them into account because they were not authorized by or notified to this Authority at the time when this Authority began to draw up this report, and any accompanying letters (Rules 66.4bis, 70.2(e), 70.16 and 91.2).
      ☐ superseded sheets and any accompanying letters, where this Authority either considers that the superseding sheets contain an amendment that goes beyond the disclosure in the international application as filed, or the superseding sheets were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in item 4 of Box No. I and the Supplemental Box (see Rule 70.16(b)).
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing, in the form of an Annex C/ST.25 text file, as indicated in the Supplemental Box Relating to Sequence Listing (see paragraph 3*ter* of Annex C of the Administrative Instructions).

4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04.03.2016	14.04.2016

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IB2015/053267

Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a) and (b))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-22 as originally filed

   Claims, Numbers

   1-13 filed with the demand for preliminary international examination

   Drawings, Sheets

   1/1 as originally filed

   ☐ a sequence listing - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since either they are considered to go beyond the disclosure as filed, or they were not accompanied by a letter indicating the basis for the amendments in the application as filed, as indicated in the Supplemental Box (Rules 70.2(c) and (c-*bis*)):
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:

5. ☐ This report has been established:
   - ☐ taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rules 66.1(d-*bis*) and 70.2(e)).
   - ☐ without taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91(Rules 66.4*bis* and 70.2(e)).

6. ☒ With regard to top-up searches (Rules 66.1*ter* and 70.2(f)):
   - ☒ A top-up search was carried out by this Authority on 21.03.2016 (all discovered documents are listed in the Supplemental Box Relating to Top-up Search).
     - ☐ Additional relevant documents have been discovered during the top-up search.
   - ☐ No top-up search was carried out by this Authority because it would serve no useful purpose.

7. ☐ Supplementary international search report(s) from Authority(ies) has/have been received and taken into account in establishing this report (Rule 45bis.8(b) and (c)).

* *If item 4 applies, some or all of those sheets may be marked "superseded".*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-13
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item I

Basis of the report

The amendments filed with the demand for preliminary international examination meet the requirements of Article 34(2)(b) PCT in that they do not extend beyond the content of the application as filed.

Re item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1	WO 2013/171668 A1 (BROTZU GIOVANNI [IT]; BROTZU GIUSEPPE [IT]) 21 November 2013
D2	EP 0 309 086 A1 (EFAMOL HOLDINGS [GB]) 29 March 1989
D3	US 2003/064929 A1 (DURANTON ALBERT [FR] ET AL) 3 April 2003
D4	NATTAYA LOURITH ET AL: "Hair loss and herbs for treatment", JOURNAL OF COSMETIC DERMATOLOGY, vol. 12, no. 3, 1 September 2013, pages 210-222, XP055078947, ISSN: 1473-2130, DOI: 10.1111/jocd.12051
D5	US 2009/197954 A1 (MCDANIEL WILLIAM ROBERT [US]) 6 August 2009
D6	GB 2 150 588 A (KITANO AKIYOSHI) 3 July 1985
D7	WO 2011/095938 A1 (BIORICERCA DI GIOVANNI BROTZU & C SNC [IT]; BROTZU GIOVANNI [IT]) 11 August 2011
D8	US 5 962 015 A (DELRIEU PASCAL [FR] ET AL) 5 October 1999

D1-D8 are cited in the International Search Report.

D1 and D7 have been cited in the application.

Unless otherwise indicated, reference is made to the relevant passages emphasized in the International Search Report.

1. Novelty (Article 33(2) PCT).

None of the cited prior art items discloses phospholipid liposomes comprising dihomo-gamma-linolenic acid (DGLA) at a concentration ranging from 0.05% to 0.3% of the phospholipid amount used, equol at a concentration comprised between 0.1% and 2% of the phospholipid amount used, a compound able to increase cation capacity of the liposome selected from carnitine and L-propionylcarnitine; a stabilizer selected from cholesterol, cholesterol sulphate and stearylamine.

In fact, D1 discloses phospholipid liposomes comprising prostaglandin E1 (PGE1), equol and carnitine for use in the treatment of alopecia, baldness and hair loss.

D2 to D6 disclose the topical or systemic use of dihomo-gamma-linolenic acid, linoleic acid, gamma-linoleic acid, arachidonic acid and docosatetraenoic acid in the treatment of alopecia, baldness and hair loss.

D7 discloses phospholipid liposomes comprising PGE1 and carnitine and suggests the use of cholesterol as liposome stabilizer.

D8 discloses phospholipid liposomes comprising stearylamine as stabilizer.

Therefore, the subject-matter of present independent claim 1, and as a consequence also of claims 2-13, is considered novel over said prior art documents in the sense of Article 33(2) PCT.

2. Inventive Step (Article 33(3) PCT).

2.1 Document D1 is regarded as being the closest prior art to the subject-matter of independent claim 1. As mentioned above, this document discloses phospholipid liposomes comprising PGE1, equol and carnitine for use in the treatment of alopecia, baldness and hair loss.

D1 does not disclose the presence in the liposomes of dihomo-gamma-linolenic acid, as well as of a stabilizer selected from cholesterol, cholesterol sulphate or stearylamine.

2.2 According to the application, the replacement of PGE1 with DGLA, which is a PGE1 precursor, aims at overcoming the disadvantages related to the use of PGE1, such as its fast deterioration and the low temperature needed as working conditions (see description, page 3, lines 7-25), while cholesterol, cholesterol sulphate and stearylamine are described as stabilizing agents for the liposomes.

In addition, the comparative studies annexed to the Applicant's letter dated 04.03.2016 show that, for lotions having comparable compositions:

1) the one comprising the DGLA and equol (lotion A') is more stable than the one comprising the association PGE1 and equol (lotion B of the application, corresponding to the compositions of D1): see stability study on page 5 of said annex;

2) the one comprising the DGLA and equol (lotion A) is more effective in treating hair loss than a comparable lotion comprising the association PGE1 and equol (lotion D'): see efficacy study on page 6 of said annex;

3) the one comprising the DGLA and equol (lotion A) is also more effective in treating hair loss than a comparable lotion comprising either only S-equol (Lotion C) or only DGLA (lotion D): see clinical test on pages 1-4 of said annex. On the basis of this result, the presence of a synergistic effect between the two component can be acknowledged.

The problem to be solved by the present invention may therefore be regarded as how to provide more stable and more effective liposome compositions for the treatment of alopecia, baldness and hair loss.

2.3 The cited prior art items neither suggests that a replacement of PGE1 with DGLA in the compositions of D1 would lead to an efficacy and stability improvement, nor they hint to a possible synergy between DGLA and equol.

In fact, neither D1 nor D4-D8 mention at all DGLA as possible component for the formulations disclosed therein.

D2 and D3 describe the use of DGLA in the treatment of alopecia, baldness and hair loss, however they neither suggest its use as replacement for PGE1 nor they relate to liposome compositions.

Therefore, the person skilled in the art would not have arrived to the solution proposed in the present application and the presence of an inventive step under Article 33(3) PCT can be acknowledged for the subject-matter of independent claim 1.

2.4 As a consequence, also the subject-matter of claims 2-13 can be regarded as meeting the requirements of Article 33(3) PCT.

3. Industrial applicability (Article 33(4) PCT).

Claims 1-13 are industrial applicable in the sense of Article 33(4) PCT.

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Milan, March 4, 2016

Our ref.: P05211/PCT – AK-RCA/sd (please always quote)

Dear Sirs,

Re: PCT: Patent Application No. **PCT/IB2015/053267**
in the name of Fidia Farmaceutici S.p.A.

With reference to our Demand for International Preliminary Examination in connection with the above-identified application, we set out herein below our arguments in support of the patentability of the claimed subject matter, taking therefore into account the objections contained in the Written Opinion enclosed to the International Search Report.

1. Amendments

Before analysing in detail the objections raised in the Written Opinion, the Applicant submits herewith an amended set of claims (clean copy and copy with annotations).
All these amendments find support in the originally filed description and claims

Claim 1
The selection made by the Applicant in this claim of the specific polyunsaturated fatty acid at specific concentrations finds support in the as filed description at page 5 lines 15-17, and in the originally filed claim 3.

The selection of the specific phytoestrogen at specific concentrations range finds support in the as filed description at page 6 lines 13-19 and in the originally filed claim 4.

The selection of the specific compound, able to increase the stability of the cation capacity of the liposome, besides finding support in the originally filed claim 1, it also finds support in the originally filed description at page 6 line 20-page 7 line 6.

The selection of the specific stabilizer finds support in the as filed description at page 7 line 9 -14.

The amended claims 3 and 4 find respectively support in the as filed claim 3, and 4 deprived of the first range of concentration of DGLA and S-equol, now in claim 1.

As far as the other claims are concerned, they are identical with the corresponding originally filed claims.

2. ***Comparative studies***

The Applicant encloses in the demand also comparative studies, wherein most of the studies reported in the originally filed application and further experimental tests clearly demonstrate the efficacy of the invention as presently claimed.

In fact the Applicant has realized that some errors were present in the experimental part of the description of the present application.

First of all as also correctly pointed out by the same ISA the prior art lotion B was prepared as described in Example 1 of WO2013171668 (D1 mentioned in the International Search report) and not WO2011095938 (i.e.D7 mentioned in the Search report).
In addition the lotion C contains S- equol and **not** PGE1 as erroneously reported in the originally filed description.

In fact the comparative studies, carried out with lotion C and D have the aim to demonstrate that both DGLA and S-equol, when used alone in the same amounts as those present in Lotion A (using them in association), notwithstanding they are incorporated in liposomes and they are in the presence of the other components of lotion A, they are **completely ineffective** for hair regrowth.

Therefore the Applicant has demonstrated the synergy of the association of DGLA with S-equol in the treatment of hair loss and hair regrowth.

Moreover the clinical studies carried out with the formulation C clearly evidence that S-equol, when used as the sole active ingredient, is **completely ineffective,** even when used in high amounts.

As a consequence, the results obtained with formulation C clearly evidence that only the association of DGLA and S-equol is effective.

Moreover the Applicant in the enclosed comparative study has submitted further experimental data demonstrating what above stated i.e. that the association of DLGA and S-equol **is far more effective** than PGE1 and S-equol

Comparative study (carried out with weight ratio of DGLA/s-equol and PGE1/S-equol =1:7.25 i.e. the same weight ratio utilized in lotion A

The Applicant in the enclosed comparative study has carried out a comparative study, wherein the effectiveness of lotion D' differing from the lotion A, as it it contains PGE1 in the same amount as DGLA of lotion A, was compared with the results obtained with lotion A.

The conclusion of this study is the following.

Starting from the following premises:

1. being the components of the lotion A and D' identical with the exclusion of one component i.e. PGE1 for lotion D' and DGLA for lotion A,
2. being the amounts of all the components present in lotion A identical with those of D',

from this comparative study it appears clearly evident that:

a) the association of DGLA with S-equol is far more **efficient** than that of PGE1 with S-equol, and the association of DGLA exerts its effect **far earlier** than the association of PGE1 with S-equol, used also at the same weight range **1,25:7 used for the lotion (A);**
b) the better efficacy of DGLA and S.equol with respect to the association of PGEI and S-equol is confirmed in the same type of liposomial formulation as that of DGLA and S-equol of lotion A.

It follows that the comparative study has demonstrated that the association of PGE1 and S-equol is **less** effective than that of the invention also in liposomial systems like those presently claimed.

Finally the Applicant has further demonstrated, in the enclosed comparative study, that the composition as claimed in claim 1 is decidedly more stable than the composition of WO2013171668 (lotion B) as the Pζ of lotion A' at t= 0 and after 30 days is > 30 mVolt, whereas that of lotion B both at t=0 and after 30 days have Pζ >-30mV.

1. Inventive Step

According to the Opinion drawn up by the International Searching Authority, document D1 is considered the closest prior art to the subject matter of claim 1, since it discloses a composition comprising prostaglandin E1, equol and carnitine for use in the treatment of alopecia and hair loss.

The main essential difference between the liposomial compositions disclosed in the present invention and the liposomial composition disclosed in D1 is the replacement of PGE1 with DGLA.

The other difference between the claimed invention and D1 is the presence of stearyl amine as stabilizer.

The technical effect obtained with the first main difference is that the topical composition of the invention results decidedly **more effective than liposomial composition containing the association of PGE1 and S-equol** as demonstrated not only by the clinical tests reported in the originally filed description, but also by the enclosed comparative studies.

In fact the better efficacy of the association of DGLA with S-equol, if compared to PGE1 and S-equol is evidenced

- not only by the comparative study disclosed at page 17 line 15-page 22 of the description (see in particular the results obtained with lotion according to the present invention in comparison with the lotion B prepared as described in example 1 of D1),
- but also by the comparative study herewith enclosed, wherein the clinical results obtained with lotion A of the invention are compared with those obtained with lotion D', wherein the liposomial system is the same as that of lotion A of the invention.

The second difference has as the technical effect that the compositions of the invention results decidedly more stable than those disclosed in D1 not only at t=0 but also after 30 days from the preparation. .

Therefore starting from D1, the objective technical problem solved with the present invention is the provision of decidedly much more efficacious liposomial composition and more stable with the time.

The skilled person, faced with the above technical problem would not have been addressed to the solution thereof from the teaching contained in the prior art although these prior art disclose that DLGA is used in the treatment of baldness.

As above pointed out in the enclosed comparative studies but also in the experimental part of the originally filed description, the Applicant has demonstrated that DGLA administered as the sole component, although in the liposomial formulation claimed is completely ineffective in the treatment of baldness at daily dosages **of 0.03 mg.**[1]

By contrast the Applicant has found that DLGA when administered at the same daily dosages of 0,03mg in association with S-equol (see the results obtained with lotion A) is **very effective** in the treatment of hair loss.

In view of the foregoing, the skilled person in the art faced with the problem to find more efficacious and more stable composition than those disclosed in D1, and in the same time being already aware that:

i) DGLA notwithstanding being incorporated in liposomes at the daily dosages of 0.03 mg is ineffective,

ii) S-equol although administered at daily dosages of 0.167mg [2]is ineffective:

(s)he, in no way would have been addressed towards the invention as presently claimed from the other prior art references mentioned in the international search report and in particular:

- from D2 (EP309086) teaching that DGLA is active in the treatment and prevention of baldness both in men and women comprising topically administering an amount of DGLAin concentrations at least **33.3** times higher than those administered with the liposomial formulation as presently claimed in claim 1 (see the abstract of D2);
- from D3 US2003/0064929 teaching that DGLA is topically administered as a lipoxygenase inhibitor for the treatment of baldness alone or in association with other lipoxygenase inhibitors (claim 1,3, 33 and 34) in any case in amount not lower than 100 mg[3] (see example 1 i.e. dosages being about **3333** times the administered dosages of DLGA in accordance with the invention.
- from D4 (Nattaya Lourith et al "Hair loss and herbs for treatment Joutrnal of Cosmetic Deramtology vo.12 no. 3 pages 201-222) i.e. a generic review reporting all the known treatments of alopecia including among them the treatment with essential fatty acids and/or phytoestrogens,
- D5(US2009/197954) disclosing that linoleic acid and **not** DGLA is efficacious in the treatment of a specific alopecia i.e.androgenetic alopecia associated with acne provoked by Propionibacterium acnes (see the abstract) .
- D6 (GB2150588) disclosing shampoo containing extracts of safflower oils that are effective only when contains linoleic acid and **not** DGLA, at daily dosages of **935**mg [4] therefore at dosages of about **31166** times the administered dosages of DGLA in association with S-equol.

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[1] This dosage was found as follows: the total amount of DGLA present in each vial: 1.25/5= 0.25mg the total volume in each vial before dilution is 6: 5 = 1.2 ml; the concentration in each vial of the DGLA before dilution is = 0.21 mg/ml; the concentration after dilution of DGLA to the final volume of 7 ml in each vial is 0.03 mg/ml. The daily dosage administered to each patient is 0.03mg/ml X 1ml /day=0.03 mg/day.

[2] In the same way the total amount of S-equol present in each vial is 7/5=1.4mg the total volume in each vial before dilution is 6/5=1.2 ml, therefore S-equol concentration before dilution is 1.17 and after dilution is 1.17/7=0.167mg/ml therefore the daily dosage is 0.167mg/ml x 1 ml/day= 0.167mg/day

[3] This dosage is reported for the linoleic acid

[4] The daily dosage of D6 is calculated from the sample 3 of example 1 containing 75% of linoleic acid, as is it is more effective than sample 1 and 2. The total formulation weight is =249+100+52.3= 401,3g (see page 2 lines 21-26) the amount of linoleic acid in % by weight is: 18.6%; the daily amount of linoleic acid is 0.186x 5 g = 935 mg.

Also D7 (WO2011095938) is unable to fill the gap between the invention as now claimed and D1 not only because it is unable to suggest that the association of DGLA to S-equol in liposomial formulations, but also because it is far from suggesting the advantageous use of stearyl amine as a stabilizer in place of cholesterol or cholesterol sulfate.

In fact the applicant has found that in the formulations of the invention stearyl amine besides being a stabilizer has also adhesive properties.(see page 7 lines 9-13 of the description as originally filed).

Finally D8(US5962015) taught away from using stearyl amine as stabilizer for liposomes, since table 1 of example 1 it reports that liposomes stabilized with stearylamine are unstable.

It follows from the above that the addition of D7 and D8 in D1 would in no way have addressed the skilled person in the art towards the invention ad presently claimed, that are decidedly more stable than the liposomial composition of D1.

In view of the foregoing, we deem that the invention involves an inventive step in view of the prior art mentioned by the Examiner.

Clarity objection

The objection concerning the lack of clarity of carnitine derivative should now be overcome in view of the new claim 1, clearly specifying the type of carnitine derivative.

* * *

For the above reasons, Applicant respectfully submits that the invention as claimed in the originally filed application besides being novel, also involves an inventive step over the prior art mentioned in the ISR and respectfully request that a favourable IPER be issued.

Anyway should the IPEA be of a different opinion, Applicant would be pleased to argue further the matter in writing replying to an IPEA'S a written opinion or orally in a telephone interview with the IPEA, before Chapter II is closed.

Yours faithfully,
Perani & Partners
Raffaella Consuelo Asensio

Encl.: - Comparative Studies
- Amended claims (copy with annotations)
- Amended claims (clean copy)

Comparative studies supporting the stability and the efficacy of DGLA/Equol-containing liposomes over PGE1/Equol-containing liposomes.

1. AMENDED EXPERIMENTAL STUDY CONTAINED IN THE ORIGINALLY FILED APPLICATION

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 whose composition is disclosed in example 4 was evaluated,.i.e. that on previous tests displayed the best characteristics of homogeneity and stability.

Treatments:

Lotion A, prepared according to the present example 4 in other words, by using the amounts of the compounds reported in the following table 1.

Table 1

Phosphatidylcholine (Lipid S75 Humangrade)	1 g
DGLA	1.25 mg
S-Equol	7 mg
Ethanol	1 ml
L-propionylcarnitine	7 mg
Stearylamine	30 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. This topical formulation is administered at daily doses of 1 ml.

Lotion C

The information concerning the composition C reported in the as filed description contains an error.

The formulation C is prepared with the same components of the lotion (A) with the only difference that it contains only S-equol and not PGE1 as erroneously reported in the description.

Therefore the composition used for preparing lotion C is reported in the following table 2.

Table 2

Phosphatidylcholine (Lipid S75 Humangrade)	1 g
S-Equol	7 mg
Ethanol	1 ml
L-propionylcarnitine	7 mg
Stearylamine	30 mg
Sterile water	5 ml

DGLA 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. This topical formulation is administered at daily doses of 1 ml.

Lotion D

The formulation D is prepared with the same components of the lotion (A) with the only difference that it contains only DLGA.

Therefore the composition used for preparing lotion D is reported in the following table 3.

Table 3

Phosphatidylcholine (Lipid S75 Humangrade)	1 g
DGLA	1.25 mg
Ethanol	1 ml
L-propionylcarnitine	7 mg
Stearylamine	30 mg
Sterile water	5 ml

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. This topical formulation is administered at daily doses of 1 ml.
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/cm2). 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.

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 and S-equol, even at the concentrations described in WO2013/171668, experience a very modest variation of the situation. Still different and most important is the observation of groups 4 and 5, treated respectively with the formulation C and D containing respectively S-equol and 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.

The Applicant has furthermore carried out experimental tests that demonstrate the excellent results obtained with the compositions encompassed in the present invention.

Lotion A'

A lotion A') was prepared according to the following modality by using as starting components those reported in the following Table 4, then containing DGLA and S-Equol in weight ratio 1:1.
Table 4

Phosphatidylcholine (Lipid S75 Humangrade)	1 g
DGLA	**1.0** mg
S-Equol	**1.0** mg
Ethanol	1 ml
L-propionylcarnitine	7 mg
Stearylamine	30 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.

Lotion D'

A liposome composition prepared as prescribed for the above-mentioned composition Lotion A was prepared with the same components as those of lotion A with the sole difference that it contains 1.25 mg of PGE1 in place of DGLA.
The composition thereof is therefore reported in the following table 5:

Table 5

Phosphatidylcholine	1 g
PGE-1	**1.25 mg**
Equol	**7 mg**
Ethanol	1 mL
L-propionylcarnitine	7 mg
Stearylamine	30 mg
Sterile Water	5 mL

1. STABILITY

In order to assess the stability of the liposomes composition of the present invention over the formulations of the prior art, Lotion A' and lotion B prepared as described in example 1 of D1 of the prior art were compared in terms of Zeta Potential (Pζ), a stability marker for colloidal systems, measuring the electrophoretic mobility of particles (in this case, liposomes) within a thermostatic cell.
For the purposes of stability assessment, the ratio between ingredients is far more important than the absolute concentrations. However in order to have absolutely comparative results lotion A' was so formulated to have also identical weight amounts of DGLA and S-equol with those disclosed in example 1 of D1 .

Zeta Potentials have been measured at the time of preparation (day "0", t = 0) and after 30 days (days "30", t = 30) by means of M3-PALS (Phase Analysis Light Scattering) with the instrument Zetasizer nano (Malvern Instrument, UK).
As defined by International Standards (ASTM Standard D 4187-82, American Society for Testing and Materials), a colloidal system is stable at values of Pζ > 30 mVolt and < -30V.
Results collected by the measurements at t = 0 and at t = 30 are displayed in the following table:

Samples	Pζ (mVolt) t = 0	Pζ (mVolt) t = 30
Lotion A'	+40,2	+32,5
Lotion B	-23,7	-11,8

From the results displayed in Table 3, it is apparent that the liposomes prepared according to the present invention give rise to a suspension that is extraordinary more stable than the suspensions available in the prior art.

The higher stability registered performing the aforementioned comparative studies, reveals a lesser extent of degradation through time, which helps not only the preservation of the active principles over time, but also a maintenance of the efficacy in the exertion of the biological/pharmacological effect.

2. EFFICACY

Comparative study carried out with weight ratio of DGLA/s-equol and PGE1/S-equol =1:7.25 i.e. the same weight ratio utilized in lotion (A)

Patients of female and male gender, characterised by massive hair reduction due to various causes, were selected in order to compare the clinical efficacy of Composition A against Composition D'.

Group of Patients:

- Group 1: 10 (6 males, 4 females) 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 males, 5 females) patients treated for 120 days with 1 mL/day of Composition D' to be applied on a well defined area of the scalp.

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

Results:

On day 7: both Group 1 (treated with composition A) and Group 2 (treated with composition D') reported a reduction of hair loss, defined "consistent" by patients of Group 1 and "moderate" by patients of Group 2.

On day 20: all the patients of Group 1 have stopped losing hair and reported the presence of a substantial fuzz. In Group 2 hair loss has stopped in 4 patients and fuzz, where present, is more modest (in terms of number/ cm2).

On day 45: fuzz has grown markedly stronger and takes the colour and consistency of natural hair in Group 1. In Group 2 all the patients have stopped losing hair, but only some of them reported a certain degree of regrowth, fuzz was very poor and not completely coloured yet.

On day 90: all the patients of Group 1 reported total disappearance of hair reduction areas that have been replaced by robust and shiny hair. In Group 2 hair reduction was still visible, and the areas were just covered with fuzz.

The observation of data suggests that, even being the concentration of active principles and the other components the same in both Composition A and Composition D', Composition A containing DGLA is far more efficient than the other and exerts its effect far earlier than Composition D'.