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- (71) Applicant(s)
Evangelia Protopapa; Aristotelis Xenakis; Spyridon Avramiotis; Konstantinos Sekeris
- (72) Inventor(s)
Evangelia Protopapa; Aristotelis Xenakis; Spyridon Avramiotis; Konstantinos Sekeris
- (74) Agent/Attorney
FREEHILLS CARTER SMITH BEADLE, Level 43, 101 Collins Street, MELBOURNE VIC 3000
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(21) International Application Number: PCT/GR97/00015 (22) International Filing Date: 19 May 1997 (19.05.97) (30) Priority Data: 960100168 22 May 1996 (22.05.96) GR (71)(72) Applicants and Inventors: PROTOPAPA, Evangelia [GR/GR]; 6 Agrafon Street, GR-145 65 Anixis Attikis (GR). XENAKIS, Aristotelis [GR/GR]; 48 Nikomidias Street, GR-171 24 Nea Spyrimi Attikis (GR). AVRAMIOTIS, Spyridon [GR/GR]; 137 Spartis Street, GR-176 75 Kalithea Attikis (GR). SEKERIS, Konstantinos [GR/GR]; 9 Ithakis Street, GR-112 57 Athens (GR). (74) Agent: KORIATOPOULOU, Pierrina; 16 Akademias Street, GR-106 71 Athens (GR).	(81) Designated States: AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: LECITHIN-BASED MICROEMULSIONS CONTAINING PROTEOLYTIC ENZYMES AND METHOD FOR PERMANENT ENZYMIC DEPILATION		
(57) Abstract		
<p>The invention refers to depilatory preparations containing: proteolytic enzymes solubilized in microemulsions, formed with lecithin, aliphatic hydrocarbon, aliphatic alcohol and buffer solution pH 7 to 9, value corresponding to the pH range near the optimum pH value for the catalytic activity of the proteolytic enzymes, to be applied for permanent enzymic depilation. The application of these preparations will assure, as shown by our experimental studies, more permanent depilation than the one resulting from other depilatory methods. The application of these preparations is suitable for every type of skin (fatty-resistant or dry-sensitive). The present invention introduces the use of microemulsions as a medium for the facilitated penetration of the enzymic activity in the epithelial cells of the skin, as shown by our studies.</p>		

Lecithin-based microemulsions containing proteolytic enzymes and method for permanent enzymic depilation

The present invention refers to the depilatory preparations of lecithin-based microemulsions containing proteolytic enzymes. The proposed invention introduces for the first time the use of microemulsions as media for the solubilization of proteolytic enzymic systems, that assist the penetration of active molecules through the phospholipidic membranes. In addition, the present invention refers to a depilation method, that applies the preparations of microemulsion containing the enzyme α -chymotrypsin, or the enzyme trypsin, for the depilation of any type of skin, as well as to the depilation method based on the sequential application of the preparation of microemulsion containing the enzyme α -chymotrypsin, followed by the application of the preparation of microemulsion containing the enzyme trypsin, depending on the particularities of each type of skin. The application of the depilatory preparations of microemulsions containing either the enzyme α -chymotrypsin, or the enzyme trypsin, or the sequential application first of the preparation of microemulsion containing the enzyme α -chymotrypsin, followed by the application of the preparation of microemulsion containing the enzyme trypsin, is carried out by simple spreading at appropriate intervals.

Experimental studies published by us for the first time in the international literature, have shown that the action of the enzyme α -chymotrypsin provokes destruction of the stem cells and of the hair follicle, explaining the positive results in depilation. Similar positive results in hair depilation also has trypsin, but with a milder action.

Microemulsions are systems consisting of a continuous inert organic medium, where water droplets are dispersed with the help of lecithin acting as a natural surfactant. The dispersion of the aqueous phase, where the enzyme molecules α -chymotrypsin or

trypsin are located, is achieved by simple and gentle shaking.

Our invention improves the depilatory action of the enzymes α -chymotrypsin and trypsin, because of the fact that they are applied through the microemulsion. The shape of the microemulsion is such that allows its easy spreading over the whole surface of the skin to be depilated. Furthermore, the presence of the microemulsion offers the facility to be applied on every type of skin, such as the sensitive-dry ones, or the resistant-fatty ones.

We have also observed that the sequential application first of the preparation of microemulsion containing the enzyme α -chymotrypsin, followed by the application of the preparation of microemulsion containing the enzyme trypsin, provokes important degradation of the hair follicle, and that the sequential application first of the preparation of microemulsion containing the enzyme α -chymotrypsin and then of the preparation of microemulsion containing the enzyme trypsin has better depilatory results than the sequential application first of the preparation of microemulsion containing the enzyme trypsin followed by the application of the one containing the enzyme α -chymotrypsin. We have also observed that the sequential application first of the preparation of microemulsion containing α -chymotrypsin followed by the application of the preparation of microemulsion containing trypsin, has much better depilatory results than the application of only one of each depilatory preparation of microemulsion containing either enzyme. In addition, we have observed that the sequential application of the preparation of microemulsion containing α -chymotrypsin followed by the application of the preparation of microemulsion containing trypsin, even at low concentrations of 0.05 mg/ml, had a degrading effect on the hair follicle, rendering thus, the preparations with low enzyme concentrations appropriate to be applied on dry, sensitive areas of the skin. On the other

hand we have observed that the use of high enzyme concentrations on dry-sensitive skins, leads to a significant depilation but with an intense irritation. Therefore it is recommended to apply on such type of skins preparations with low enzyme concentrations. Moreover, the use of microemulsions as a carrier for the transdermal transfer of active
5 macromolecules, such as the proposed enzymes, offers a considerable improvement of the enzyme depilation methodology, since the lecithin of the microemulsion interferes with the phospholipidic membrane of the epithelial cells, allowing the penetration of the enzymes in the cells.

The proposed proteolytic enzymes α -chymotrypsin and trypsin will be used in
10 absolutely pure state. The enzymes will be stored lyophilized in a freezer (-18 °C) so that they are kept constant, avoiding denaturation. For the production of the preparations the enzymes will be dissolved in a solution of hydrochloric acid 0.001 M in doubly distilled water (pH 3) obtaining concentrations of 1 to 5 mg/ml, and will be kept in a sterile environment in a freezer (-18 °C). By this procedure the enzyme activity is kept unvaried
15 for at least 8 months.

In parallel, lecithin-based microemulsions will be prepared according to the following: For every ml of final solution, 0.75 to 1 ml of aliphatic hydrocarbon are mixed with 20 to 75 mg of lecithin, 0 to 0.25 ml of aliphatic alcohol and 0.005 to 0.05 ml of buffer solution, pH 7 to 9. By mixing the above components, after gentle shaking, a clear
20 solution (microemulsion) is obtained. This solution is thermodynamically stable and has, thus, unlimited time life. To avoid perturbing the stability by a possible partial evaporation of the organic constituents and consequently altering the total microemulsion composition, the solution is stored in cupped vessels.

The choice of the buffer solutions used for the preparations of microemulsions

containing the proteolytic enzymes α -chymotrypsin or trypsin, is based on the optimum pH value for the activities of the above enzymes, which are 7.8 and 8.8 respectively. One possible buffer solution to use for the preparation of the lecithin-based microemulsions, is the solution of 0.1 M tris-hydroxymethylamino-methane- hydrochloric acid, for the pH range 7.5-9, although many other buffers, covering the desired range of pH values, can be used.

Just before applying the preparations the enzyme solution will be mixed with the lecithin-based microemulsion for the preparation of the final product, which, if stored at a temperature of about 2-6 °C, retains its depilative action for at least three days.

According to a suggested materialization of the present invention, in sterilized vials of 2.5 ml will be placed 2 ml of lecithin-based microemulsion in isooctane, prepared by mixing 1.8 ml isooctane, 64 mg soy bean lecithin, 0.2 ml propanol-1 and, either 0.03 ml buffer solution 0.1 M tris-hydroxymethylamino-methane-hydrochloric acid, pH 7.5-8 for the preparations of α -chymotrypsin, or 0.03 ml buffer solution 0.1 M tris-hydroxymethylaminomethane-hydrochloric acid, pH 8.5-9 for the preparations of trypsin. In small sterilized capsules located in the vials and incorporated in a special cap, will be placed 0.03 ml of the solution containing, either the enzyme α -chymotrypsin, or the enzyme trypsin at a concentration of 3.4 mg/ml. The microemulsion containing vials, and the small capsules will be stored at a temperature of about 2-6 °C. Before using the preparations and before opening the vial, by slightly pressing the cap, the capsule will be broken and the contained enzymic solution will be mixed with the microemulsion contained in the vial. By repeatedly shaking the vial, the final microemulsion will be prepared containing the enzyme α -chymotrypsin or the enzyme trypsin at a final concentration of 0.05 mg/ml, ready to be used. Alternatively to the small capsule, the enzymic solution may be kept in

a separate vial and mixed in a similar way with the microemulsion solution just before use.

The application of the final preparations on the skin to be depilated will be carried out immediately after and for three consecutive days. During this interval the preparations will be stored in a freezer (-18°C).

5 By this way we finally offer for use:

a) Preparations of lecithin-based microemulsions containing the enzyme α -chymotrypsin at a concentration of 0.05 mg/ml and pH 7.5-8, and according to a proposed application of the invention at pH 7.8.

b) Preparations of lecithin-based microemulsions containing the enzyme trypsin
10 at a concentration of 0.05 mg/ml and pH 8.5-9, and according to a proposed application of the invention at pH 8.8.

For areas of fatty resistant skins it is proposed to apply sequentially first the preparation of microemulsion containing the enzyme α -chymotrypsin and then the preparation of microemulsion containing the enzyme trypsin, whereas at areas of dry
15 sensitive skins it is proposed to apply the preparations containing trypsin. It is up to the judgement of the specialist to propose the use of the preparations of microemulsion containing either α -chymotrypsin or trypsin for sequential applications, to increase the total quantity of either enzyme, depending on the type of skin.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A two part depilatory composition comprising first and second components which, when combined together, can effectively be spread on the skin to initiate depilatory activity, the first component comprising the following ratio of ingredients:

40 to 150 mg of a lecithin;

1.5 to 2 ml of an aliphatic hydrocarbon;

0 to 0.5 ml of an aliphatic alcohol; and

an amount of a buffer solution sufficient to provide a pH of 7 to 9, and

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the second component comprising the following ration of ingredients:

water; and

a proteolytic enzyme in an amount sufficient to provide a proteolytic

enzyme concentration of the second component of from 1 to 5 mg/ml,

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wherein the proteolytic enzyme concentration of the combination of the first and second components is from 0.01 to 0.1 mg/ml.

2. The two part depilatory composition of Claim 1 wherein the aliphatic hydrocarbon is isooctane.

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3. The two part depilatory composition of Claim 1 or Claim 2 wherein the amount of the buffer solution is 0.01 to 0.1 ml.

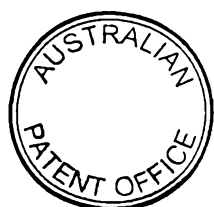
4. The two part depilatory composition of any one of Claims 1 to 3 wherein the aliphatic alcohol is propanol-1.

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5. The two part depilatory composition of any one of the preceding claims wherein the proteolytic enzyme is selected from the group consisting of a α -chymotrypsin, trypsin and combinations thereof.

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6. The two part depilatory composition of any one of the preceding claims wherein the second component further comprises an effective amount 0.001M



hydrochloric acid.

7. The two part depilatory composition of any one of the preceding claims wherein the amount of the proteolytic enzyme in the second component is sufficient to
5 provide a concentration of the proteolytic enzyme in the second component of about 3.4 mg/ml and a concentration of the proteolytic enzyme in the composition of about 0.05 mg/ml.

8. The two part depilatory composition of any one the preceding claims
10 wherein the proteolytic enzyme is α -chymotrypsin and the pH of the combination of the first and second components is 7.5 to 8.0.

9. The two part depilatory composition of any one of the preceding claims wherein the proteolytic enzyme is trypsin and the pH of the combination of the first and
15 second components is 8.5 to 9.0.

10. The two part depilatory composition of Claim 1 wherein the first component comprises:

20 64 mg of a lecithin;
1.8 ml of isooctane;
0.2 ml of propanol-1; and
0.03 ml of a buffer providing a pH for said first component of 7.5 to 8.0; and

25 the second component comprises:

0.03 ml of water containing α -chymotrypsin at a concentration of 3.4 mg/ml.

11. The two part depilatory composition of Claim 1 wherein the first
30 component comprises:

64 mg of a lecithin;
1.8 ml of isooctane;
0.2 ml of propanol-1;



20. The depilatory composition of any one of Claims 12 to 18 wherein the proteolytic enzyme is trypsin and the pH of the combination of the first and second components is 8.5 to 9.0.

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21. A method of removing hair from the skin of a warm blooded animal comprising treating the skin of the warm blooded animal with an effective amount of the depilatory composition of any one of Claims 12 to 20.

10 22. The method of Claim 21 comprising:

a) treating the skin with a first composition containing one of α -chymotrypsin and trypsin; and

b) treating the skin with a second composition containing the other of
15 α -chymotrypsin and trypsin.

23. The method of Claim 22 wherein the first composition contains α -chymotrypsin and has a pH of 7.5 to 8.0.

20 24. The method of Claim 22 or Claim 23 wherein the second composition contains trypsin and has a pH of 8.5 to 9.0.

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Freehills Carter Smith Beadle

Patent Attorneys for the Applicants:

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**Evangelia PROTOPAPA, Aristotelis XENAKIS,
Spyridon AVRAMIOTIS and Konstantinos SEKERIS**

