

FORM 25**(12) PATENT granted by****(19)****AP**
**AFRICAN REGIONAL INDUSTRIAL PROPERTY
ORGANISATION (ARIPO)**
1077**(11)****(A)**

(21)	Application Number:	AP/P/1998/001405	(73)	Applicant(s): PROTOPAPA Evangelia 6 Agrafo Street GR-145 65 Anixis Attikis Greece (See Overleaf)
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(24)	Date of Grant &	20020701		Same As Above
(45)	Publication		(74)	Representative HONEY & BLANCKENBERG P O BOX 85 HARARE ZIMBABWE
(30)	Priority Data			
(33)	Country:	GR		
(31)	Number:	960100168		
(32)	Date:	19960522		
(84)	Designated States:			
	KE			

 (51) **International Patent Classification (Int.Cl.7):** A61K 7/155

 (54) **Title:** Lecithin-Based Microemulsions Containing Proteolytic Enzymes And Method For Permanent Enzymic Depilation

 (57) **Abstract:**

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.

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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 soubilization 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

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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 5 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.

10 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 isoctane, prepared by mixing 1.8 ml isoctane, 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-hydroxymethyl-15 aminomethane-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 20 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

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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.

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CLAIMS

1. Depilatory preparation in liquid form, based on the action of proteolytic enzymes solubilized in microemulsions and applied by simple spreading. The concentration 5 of the enzyme ranges from 0.01 to 0.1 mg/ml and the microemulsion consists of 40 to 150 mg lecithin, 1.5 to 2 ml aliphatic hydrocarbon, 0 to 0.5 ml aliphatic alcohol and 0.01 to 0.1 ml buffer solution, pH 7-9, covering the pH range that is optimum for the enzyme activity.
2. Depilatory preparation in liquid form, as mentioned in claim 1, characterized by 10 the fact that the proteolytic enzyme is α -chymotrypsin, solubilized in microemulsions and applied by simple spreading. The concentration of the enzyme is 0.05 mg/ml and the microemulsion consists of 64 mg lecithin, 1.8 ml isooctane, 0.2 ml propanol-1 and 0.06 ml buffer solution of tris-hydroxymethylamino-methane-hydrochloric acid, pH 7.5-8, covering the pH range that is optimum for the activity of the enzyme α -chymotrypsin.
3. Depilatory preparation in liquid form, as mentioned in claim 1, characterized by 15 the fact that the proteolytic enzyme is trypsin, solubilized in microemulsions and applied by simple spreading. The concentration of the enzyme is 0.05 mg/ml and the microemulsion consists of 64 mg lecithin, 1.8 ml isooctane, 0.2 ml propanol-1 and 0.06 ml buffer solution of tris-hydroxymethylamino-methane-hydrochloric acid, pH 7.5-8, 20 covering the pH range that is optimum for the activity of the enzyme α -chymotrypsin.
4. Method of preparation of depilatory preparations characterized by the following: 2 ml of a microemulsion containing 1.5 to 2 ml of aliphatic hydrocarbon, 40 to 150 mg of lecithin, 0 to 0.5 ml of aliphatic alcohol and 0.01 to 0.05 ml of buffer solution, pH 7 to 9, are placed in sterilized vials of 2.5 ml. In small sterilized capsules located in the

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vials and incorporated in the cap, 0.01 to 0.05 ml of a solution of proteolytic enzymes at a concentration of about 1 to 5 mg/ml are placed. Before using the preparations and before opening the vial, by slightly pressing the cap the capsule is broken and the contained enzymic solution is mixed with the microemulsion contained in the vial. By 5 repeatedly shaking the vial, the final depilatory preparation is obtained ready to be used. Alternatively to the small capsule, the enzymic solution can be kept in a separate vial and mixed in a similar way with the microemulsion solution just before use.

5. Method of depilation for intermediate particularities of skin types, characterized by the application of the depilatory preparation according to claim 2.

10 6. Method of depilation for dry and sensitive types of skin, characterized by the application of the depilatory preparation according to claim 3.

7. Method of depilation for fatty and resistant types of skin, characterized by the sequential application first of the depilatory preparation according to claim 2, followed by consecutive applications of the depilatory preparation according to claim 3.