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(54) Title: A METHOD FOR NON-THERAPEUTIC OR THERAPEUTIC PHOTODYNAMIC SKIN TREATMENT

(57) Abstract: In a method for non-therapeutic or therapeutic photodynamic treatment of a skin target area, a photosensitizer fluid incorporating a photosensitizing agent or a precursor for a photosensitizing agent is delivered to the target area, the photosensitizing agent having at least one absorption wavelength in a wavelength range higher than 400 nm. The target area is then exposed to light energy in a range of wavelengths comprising at least one absorption wavelength of the photosensitizing agent, the light energy being supplied to the target area in the form of at least one light pulse producing in the target area an accumulated energy density sufficient for photodynamic activation of the photosensitizing agent. The photosensitizing agent or precursor is incorporated in the photosensitizer fluid with a concentration in the range from 0.1 to 2.0 % preferably from 0.5 to 1.0 %, and the photosensitizer fluid is delivered to the target area in the form of a number of repeated spray doses during an overall delivery time ranging from 0.25 to 8 hours. The method can be applied to cosmetic treatments for skin rejuvenation, reduction of wrinkles, treatment of telangiectasia or removal of hair as well as a medical treatment of dermatologic diseases such as acne vulgaris, acne scars, rosacea, psoriasis, actinic keratoses, basal cell carcinoma, Bowen's disease or eczema.



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A method for non-therapeutic or therapeutic photodynamic skin treatment

5 **Technological field of the invention**

The invention relates to a method for non-therapeutic or therapeutic treatment of a skin target area of the mammal skin by a photodynamic process, by which a photosensitizer fluid incorporating a photosensitizing agent or a precursor for a photosensitizing agent, the photosensitizing agent having at least one absorption wavelength in a wavelength range higher than 400 nm, is delivered to the skin target area to produce in the skin tissue in the target area a distribution of a photosensitizing agent and the target area is exposed to light energy comprising a range of wavelengths including the at least one absorption wavelength of the photosensitizing agent distributed in the target area, the light energy being supplied to the target area in the form at least one pulse producing in the target area an accumulated energy density sufficient for photodynamic activation of the photosensitizing agent.

Non-therapeutic skin treatment, to which the invention pertains, includes in particular, but not exclusively treatment of photo damaged skin like pigmented disorders, treatment of telangiectasias and erythema as well as skin rejuvenation of dermal structures such as wrinkles, fine lines and skin pore size, and removal of unwanted hair.

Equally the invention is potentially useful to the medical treatment of dermatologic diseases and disorders including in particular, but not exclusively conditions such as actinic keratosis, basal, squamous and intraepithelial cell carcinoma, Bowen's disease, acne and seborrhoea, psoriasis and eczema.

30 **Background of the invention**

Since the late 1980's treatment of dermatologic conditions based on photodynamic activation of photosensitizing agents in affected skin regions has been proposed and reported by many scientists and disclosed in a number of patents.

Generally the proposed methods have suggested the administration of 5-aminolevulinic acid (5-ALA) or other precursors for production of the photosensitizing agent protoporphyrin IX to the affected skin region. After uptake in skin tissue 5-ALA and other precursors having similar effect metabolize in the epidermal cells to produce protoporphyrin IX, which absorbs light in the visible region of the spectrum of electromagnetic radiation and by exposure to such radiation reacts with oxygen in the cells to cause generation of free singlet oxygen radicals leading to destruction of the cell membrane and thereby cell necrosis.

10 US-A-5, 079,262 (Kennedy et al) discloses treatment of an affected skin region by topical or oral application or injection of a relatively high concentration of 17 to 33% of ALA in a Glaxal base followed by exposure to photo activating light generally three hours after exposure.

In US-B2-6, 897,238 (Anderson) the topical application of 5-ALA with a relatively low concentration of 0.1 to 1.0 % is disclosed as being effective for the treatment of acne vulgaris, acne rosacea and sebaceous gland hyperplasia by destruction of the bacteria associated with acne with the reservation, however, that the low accumulation of photosensitizer obtained hereby may require relatively frequent repetition of the treatment and proposing a relatively high concentration of 10 to 30 % 5-ALA for a suitable permanent treatment. As a main procedure for application the formulation of 5-ALA in a cream or emulsion, that can penetrate the skin, is disclosed, combined as a general practice with covering of the treated area with an occlusion material such as plastic for a period of 1 to 12 hours before activation of the photosensitizer by exposure to light energy. In a specific embodiment the use of a liposome as carrier by encapsulation of a low 5-ALA concentration combined with active driving of the carrier into follicles by massage, pressure, ultrasound or iontophoresis is disclosed.

30 In WO 03/086460 (Geronemus et al) the activation of a photosensitizer in an affected skin region by pulsed exposure to light energy with an elevated fluence rate, primarily from a pulsed dye laser has been disclosed. The main method proposed therein for 5-ALA administration is again the topical application of 20% 5-ALA formulated into a cream or

emulsion to an affected skin area followed by covering the affected area with an occlusive dressing during a period of 10 minutes to 18 hours for incubation in a low light environment prior to the exposure to irradiating light energy.

5 Although focussing mainly on the medical treatment of essential malign or severe dermatological conditions the use of the described photodynamic treatment procedure has been proposed, e.g. in WO 03/086460, also as a recommendable vehicle for the cosmetic treatment of photoaged facial skin and the removal of unwanted, in particular,
10 blonde, grey or light-coloured hair, this use involving the administration of 5-ALA as component of an alcohol solution with a typical concentration of 20 %.

 In clinical practice it has turned out, however, that the beneficial effects of the photodynamic treatment are accompanied by side effects,
15 in particular in the form of a relative long-lasting post-treatment period of heavy phototoxicity, typically for 24 to 48 hours, for the duration of which patients must be warned against skin exposure to bright daylight.

 Another disadvantage of the recommended prior art procedures has been the relatively long duration of the treatment, in particular following from the need for covering the skin area under treatment with an
20 occlusive dressing for a prolonged period of up till 18 hours for incubation of the 5-ALA into the active photosensitizing agent protoporphyrin IX.

 Whereas such inconveniences may seem as although uncomfortable,
25 able, yet acceptable conditions in the medical treatment of significant dermatologic diseases or disorders, they would be far from desirable in the cosmetic treatment of frequently occurring more daily life adverse skin conditions typically calling for treatment in the form of skin rejuvenation often performed by clinics outside a hospital environment

30

Summary of the invention

 On the background outlined above, it is the overall object of the present invention to provide a novel photodynamic treatment procedure suitable for non-therapeutic applications or mainly cosmetic derma-

tologic treatment as well as therapeutic applications for the medical treatment of dermatologic diseases and disorders, by which the described inconveniences of prior art procedures are significantly reduced.

It is a further object of the invention to provide a photodynamic treatment method which, while offering patients beneficial treatment results compared to the best state of the art procedures, can be easily practiced in non-therapeutical applications within a generally shortened overall duration of the procedure and with higher certainty against harmful and undesirable effects to patients by offering improved options for monitoring the progress of the treatment.

To accomplish these and other objects, which will become clear from the following description, the invention provides a method for non-therapeutic or therapeutic photodynamic treatment of a skin target area, comprising the steps of delivery of a photosensitizer fluid incorporating a photosensitizing agent or a precursor for a photosensitizing agent, the photosensitizing agent having at least one absorption wavelength in a wavelength range higher than 400 nm, to the target area, and exposure of the target area to light energy in a range of wavelengths comprising said at least one absorption wavelength of the photosensitizing agent, the light energy being supplied to the target area in the form of at least one light pulse producing in the target area an accumulated energy density sufficient for photodynamic activation of the photosensitizing agent.

According to the invention in its general aspect this method is characterized in that the photosensitizing agent or precursor is incorporated in the photosensitizer fluid with a concentration in the range from 0.1 to 2.0 % and that the photosensitizer fluid is delivered to the target area in the form of a number of repeated spray doses during an overall delivery time ranging from 0.25 to 8 hours.

By the term "a photosensitizer fluid" as used herein should be understood any pharmaceutically acceptable carrier fluid capable of carrying a photosensitizing agent or a precursor into a desired treatment region of the epidermis by penetration through outer layers of the skin.

The active component for production of the desired level of photosensitivity in the treatment region could be any photosensitizing agent,

which by exposure to light is capable of causing generation of free oxygen radicals leading to therapeutic injury to skin tissue by exposure to irradiation by light energy.

As known in the art, there are many potential candidates for use as a photosensitizing agent for direct incorporation in the photosensitizer fluid of the invention. Preferably, the photosensitizing agent would comprise at least one of chlorine, a cyanine, a purpurin, a porphyrin and xanthenes.

From this group the more preferred photosensitizing agent would currently comprise a porphyrin and more preferably benzoporphyrin derivative monoacid.

As in most of the described prior art applications, the photosensitizer fluid would preferably include, however, a precursor for a photosensitizing agent capable of producing a distribution of the photosensitizing agent in the skin tissue in the target area by metabolic transformation.

In the currently most preferred implementation of the method of the invention, the precursor comprises 5-aminolevulinic acid (5-ALA), which when entering a target treatment skin region will cause production of the photosensitizing agent protoporphyrin IX.

Alternatively, the precursor may be an ALA derivative, preferably an ALA ester, which could preferably be one of ALA-methyl ester, ALA-n-pentyl ester, ALA-n-octyl ester, R, S-ALA-2-(hydroxymethyl)tetrahydro-pyranyl ester, N-acetyl-ALA or N-acetyl-ALA-ethyl ester.

Whereas the photosensitizer carrier fluid for use in the method of the invention would as already mentioned comprise any pharmaceutically acceptable carrier fluid capable of carrying a photosensitizing agent or a precursor in a desired treatment region of the epidermis by penetration through outer layers of the skin, a currently particularly preferred photosensitizer fluid would comprise a liposome preparation incorporating the photosensitizing agent or precursor by encapsulation in liposome vesicles. As known in the art typical liposome preparations are available, which offer excellent characteristics in terms of capability of penetration through outer dermal layers such as the stratum corneum.

A currently particularly preferred photosensitizer carrier fluid comprises a liposome preparation prepared from a nanokolloid liposome emulsion having a mean vesicle diameter less than 250 nm.

Liposome preparations having this characteristic have been disclosed in EP-A-1 013 265 and are available e.g. in the form of a phosphatidylcholine based concentrate available from DIANORM G. Maierhofer GmbH, München, DE. It is typically a low viscosity fluid, which is excellently suitable for application to the skin by conventional dispensing methods including spraying and can thereby be made available to biological processes in subsurface skin regions.

Whereas, with respect to prior art treatment procedures as described above it has been demonstrated that the prolonged post-treatment phototoxicity is mainly caused by a continued increase in the production of the photoactive component protoporphyrin IX for a considerable period after termination of 5-ALA administration, typically 8 to 10 hours, until a maximum level is reached, it has been demonstrated that in use of the procedure according to invention with a regular time interval of 15 minutes between the repeated spray doses the concentration of the photoactive component will reach its maximum shortly after termination of the administration of the precursor typically less than 15 minutes after delivery of the last spray dose and will return to its base level within about 8 to 10 hours.

In result, both of the inconveniences of the prior art procedures described above may be significantly reduced by performing the exposure of the target area with light energy immediately after delivery of the photosensitizer fluid by a number of repeated spray doses and the decay of the concentration of the photoactive component in the target area within a shorter time after the treatment. In particular, this would be a major benefit to the application of the treatment regime of the invention to non-therapeutic cosmetic skin treatment to meet demands for the shortest possible period of post-treatment photo-toxicity as well as the shortest possible overall duration of a treatment session.

Other investigations have indicated, however, that by shortening the interval between spray dose deliveries to the skin target area to be-

tween 3 and 10 minutes the production of protoporphyrin IX may continue to increase for at least up till 5 hours after termination of the delivery of the spray doses. Thereby, it would be possible to increase the accumulated maximum level of protoporphyrin IX, which may of significant importance to the application of the procedure according to the invention to the medical treatment of adverse dermatologic conditions such as skin cancer and pre-cancer stages.

For determination of essential parameters of the method according to the invention such as the number of spray doses and the length of intervals separating them as well as the duration and possible sequence of pulses and the totally delivered energy density by the exposure to light energy, it may be of significant advantage to monitor the progress of the treatment procedure by objective means such as repeated measuring of the skin surface fluorescence emitted from the target area in response to excitation of the photoactive component by exposure to light specifically adapted to such a fluorescence measurement.

A novel method and apparatus suitable for performance of fluorescence measurement during a dermatological treatment session has been disclosed in co-pending international patent application entitled "Method and apparatus for in vivo determination of skin surface fluorescence" in the name of Dia-Medico ApS, Denmark et al.

In the non-therapeutic applications of the method according to the invention, including procedures for skin rejuvenation, reduction of wrinkles, treatment of telangiectasia or removal of hair, the essential parameter for delivery of the photosensitizer fluid to the treatment area would preferably comprise incorporation of the photosensitizing agent in the photosensitizer fluid in a concentration from 0.1 to 2.0 %, preferably from 0.5 to 1.0 % and delivery to the target area in from 2 to 36 successive spray doses separated by intervals between 3 and 15 minutes.

Whereas application of the method according to the invention to medical treatment of dermatological conditions including acne vulgaris, acne scars, rosacea, psoriasis, actinic keratosis, basal cell carcinoma Bowen's disease or eczema has so far been conducted to a limited scale only, the indications currently available are that the same ranges of

treatment parameters would be very useful also for therapeutic treatment.

In a second aspect, the invention further relates to a liposome based photosensitizer fluid for use in any of the described treatment procedures. Conforming to the currently preferred implementation of the method this photosensitizer fluid is characterized by comprising a nano-kolloid liposomic emulsion having a mean vesicle diameter not exceeding 250 nm, in which a photosensitizing agent or a precursor for a photosensitizing agent is encapsulated in liposome particles with a concentration in the range from 0.1 to 2.0 %.

In a still further aspect, the invention relates to the use of a photosensitizing agent or a precursor for a photosensitizing agent in the manufacture of a photosensitizer fluid for application in photodynamic treatment of a dermatologic disorder, wherein the photosensitizing agent or precursor is incorporated in the photosensitizer fluid with a concentration in the range from 0.1 to 2.0 % and the photosensitizer fluid is delivered to the target area in the form of a number of repeated spray doses during an overall delivery time ranging from 0.25 to 8 hours.

20 Brief description of the drawings

In the following, the invention will be explained more in detail with reference to the accompanying drawings and by way of a clinical example.

In the appended drawings

25 figures 1 to 4 are graphic representations of some of the results obtained by a preliminary investigation of prospects of treatment procedures according to invention compared to characteristics of established prior clinical practice for photodynamic therapy;

figure 5 is a graphic representation illustrating the potential benefit of the photodynamic procedure of the invention to medical therapy of actinic keratosis; and

figures 6 and 7 are graphic representations of results obtained in a clinical investigation of a photodynamic cosmetic treatment for reduction of wrinkles.

Detailed description

As will appear from the foregoing description the mechanism of the use of 5-ALA in photodynamic therapy is based on intracellular transformation of 5-ALA into protoporphyrin IX followed by exposure to light energy, by which photo-toxicity is induced. The efficacy of the treatment is determined by a number of factors including a sufficient metabolic transformation rate for 5-ALA to obtain the intended phototoxicity upon subsequent light exposure, inclusion of the major absorption bands of protoporphyrin IX in the optical spectrum used for the light exposure and selection of the light dose in terms of energy density to be sufficient to obtain oxygen concentration in the treatment area.

Since in contrast to 5-ALA the photoactive component protoporphyrin IX produces a pronounced fluorescence at a wavelength of 634 nm by excitation with a wavelength of 407 nm, a preliminary investigation of the metabolic transformation of 5-ALA into protoporphyrin IX has been performed by detection of skin surface fluorescence, which provides a fair representation of the distribution and concentration of protoporphyrin IX in a skin region.

The investigation was conducted to obtain empirical verification of the assumption that use of liposome encapsulated 5-ALA in a low concentration would provide the benefits of the invention as compared to the conventional clinical practice of incorporation of a concentration of 20 % 5-ALA in a standard moisturizing cream and also to optimize treatment parameters like concentration and incubation time for 5-ALA to obtain a standardized fluorescence response from the protoporphyrin IX produced in the treatment region. The results of the investigation were obtained by skin fluorescence measurements implying visual assessment of fluorescence photographs obtained by a digital camera as well as objective fluorescence quantification by a novel fluorescence detection device as disclosed in the international patent application mentioned above, the disclosure of which is incorporated herein by reference.

This investigation was attended by ten healthy volunteers with no previous skin diseases and with a mean age of 25.7 years. In the ad-

ministration of 5-ALA to skin target areas the photosensitizer carrier was a nanokolloid liposome preparation as described above and the investigation comprised preparations containing 1.0 and 0.5 % 5-ALA, respectively, which were applied to the skin in the form of repeated spray
5 doses with regular intervals of 15 minutes. For comparison, the investigation included the administration of 5-ALA by way of a conventional standard moisturizing cream preparation containing 20 % 5-ALA, which was applied to the skin and followed by the standard procedure of covering the test area with an impermeable plastic occlusion film during the
10 incubation period.

As schematically illustrated in figure 1, 16 test areas of equal size and in a 4 x 4 configuration were selected on the back of each of the participating volunteers. For each of the three defined 5-ALA preparations four test were used to investigate the effect of using incubation
15 times of 30 minutes, 1 hour, 2 hours and 3 hours, respectively. The test areas in the remaining column in the 4 x 4 matrix was used for negative control and comprised two non-treated areas positioned adjacent the test area supplied with 20 % 5-ALA cream-based preparation for the shortest and longest incubation times of 30 minutes and 3 hours, respectively,
20 whereas the two intermediate test areas were supplied with the moisturizing cream base and the liposome carrier emulsion both without any content of 5-ALA.

During the investigation, skin surface fluorescence was measured for all test areas before application of the 5-ALA preparations and the
25 empty control preparations. For each test area subsequent fluorescence measurements were performed immediately after termination of the incubation time and every 30 minutes during the following 3.5 hours and ultimately with extended time intervals of 1 hour for the subsequent 7 hours.

30 The main results of the investigation are summarized in the graphic representations in figures 2, 3 and 4.

Figure 2 is a representation of the average increase for the ten volunteers of the fluorescence level measured at the end of the incubation time for each test area for each of the three 5-ALA preparations defined

by the curve 1 for the cream-based 20 % 5-ALA concentration, the curve 2 for the 0.5 % 5-ALA liposome preparation and the curve 3 for 1.0 % 5-ALA liposome preparation. Whereas the curve 1 shows a continued linear increase in fluorescence level for the cream-based 20 % 5-ALA concentration with incubation time, the curves 2 and 3 show an approximate saturation in fluorescence level from an incubation time of 2 hours and a higher maximum fluorescence level for the lower 0.5 % 5-ALA concentration than for the 1 % concentration.

Figure 3 is a representation of the average increase for the ten volunteers of the fluorescence level measured during a period of 10 hours after the incubation period for the cream-based 20 % 5-ALA concentration administered to the skin with incubation times of 3 hours for curve 1, 2 hours for curve 2, 1 hours for curve 3 and 30 minutes for curve 4, respectively. The representation demonstrates that independent of incubation time the fluorescence level by administration of the cream-based 20 % 5-ALA concentration continues to increase to a saturation level, which is not reached until about 8 hours after termination of the incubation time. This provides a clear indication of the prolonged post-treatment period of phototoxicity, which is a main inconvenience in the use of this preparation for treatment purposes. Further the representation in figure 3 shows a statistically ignorable difference between the maximum fluorescence level obtained for the incubation times of 3 hours, 2 hours and 1 hour.

Figure 4 is a representation corresponding to figure 3, but showing an average decrease of the fluorescence level during the 10 hours period following termination of the incubation period for the 0.5 % 5-ALA liposome preparation administered to the skin with incubation times of 3 hours for curve 1, 2 hours for curve 2, 1 hour for curve 3 and 30 minutes for curve 4, respectively. The representation provides a clear indication that, by application of the method according to the invention with intervals of 15 minutes between deliveries of the repeated spray doses, the fluorescence level will independently of the duration of the incubation period start decaying within 15 minutes from termination of the incubation period, and the decay progresses at a rate, by which the fluo-

rescence level is back to the base line after 8 to 10 hours. Like the representation in figure 3 also the representation in figure 4 illustrates a statistically ignorable difference between the maximum fluorescence levels obtained for the incubation times of 3 hours, 2 hours and 1 hour.

5 An additional visual observation made during the investigation was that, for eight out of the ten volunteers, the fluorescence level measured for application of the 0.5 % 5-ALA liposome preparation with incubation times of 1 hour and 2 hours, which are close to each other, as also confirmed by figure 2, were equivalent to the fluorescence level measured
10 for application of the cream-based 20 % 5-ALA concentration with an incubation time of 30 minutes. As this relatively short incubation time for use of the conventional cream-based 5-ALA preparations have become clinical practice for cosmetic treatment in an attempt to reduce the prolonged period of post-treatment photo-toxicity, this observation provides
15 a clear indication of the efficiency of the procedure according to the invention for use in non-therapeutic cosmetic skin treatment compared to the use of cream-based 5-ALA preparations.

 An additional significant observation made during the investigation followed from the presence of localized acne inflammations in some test
20 areas. Fluorescence measurement made in connection with administration of the 0.5 % 5-ALA liposome preparation to such test areas demonstrated a highly selective fluorescence concentrated in the inflamed acne lesions, whereas in skin areas treated with the cream-based 20 % 5-ALA preparation fluorescence was practically uniformly distributed over the
25 detection area. This observation provides an indication of rapid transformation of 5-ALA into protoporphyrin IX in cells with a high metabolism and thereby a high potential utility for the application of the procedure according to the invention in the treatment of dermatologic diseases like acne vulgaris, acne scars, rosacea, psoriasis, actinic keratosis,
30 basal cell carcinoma, Bowen's disease or eczema.

 This observation is in line with other investigations made for administration of 5-ALA to the treatment of actinic keratosis, which as illustrated in the graphic representation in figure 5 also demonstrates a high selective fluorescence in cancer cells with high metabolism as com-

pared to normal cells.

The usefulness of the method according to the invention to non-therapeutic skin rejuvenation procedures is confirmed by the following clinical examples:

5

Example 1

A clinical multi-centre study of the effect of the method according to the invention to wrinkle reduction was attended to and finalized by 20 volunteers with ages ranging from 37 to 70 years and Fitzpatrick skin type varying from I to IV. Suntan in the treated areas was between none to heavy. The volunteers had visible periorbital and/or perioral wrinkles, On Fitzpatrick's wrinkle scale, periorbital wrinkles around the eyes averaged 5.88 and perioral wrinkles (around the mouth averaged 5.48. All patients were instructed to avoid sun exposure and use efficient sun protection every morning and prior to out door activities in sunny weather.

15

Wrinkle severity according to Fitzpatrick's wrinkle scale was evaluated before the start of the treatment and at 1 and 3 months post treatment follow-up sessions with the results for reduction in periorbital wrinkles and perioral wrinkles shown in the diagrams in figures 6 and 7, respectively.

20

The reduction in Fitzpatrick's wrinkle severity for periorbital wrinkles was in average 0.96 at the 1month follow-up session and increased to an average of 1.49 at the 3 months follow-up session. Corresponding figures for the reduction of perioral wrinkles were 1.2 at the 1 month follow-up and 1.57 at the 3 months follow-up sessions. The distribution with respect to perioral and periorbital wrinkles, respectively are shown in the representations in figures 6 and 7.

25

Wrinkle reduction and improvements in skin texture was evaluate at the 1 month follow-up session to be fair, good or excellent by 57.8 % and 43.8 %, respectively, of the patients. At the 3 months follow-up session these scores had increased to 64.5 % for wrinkle reduction as well as for skin texture.

30

The overall satisfaction of the patients with the treatment procedure was evaluated by the range: unsatisfied, slightly satisfied, satisfied,

very satisfied and extremely satisfied. At the 1 and 3 months follow-up sessions 71.0 and 70.8, respectively, of the patients rated the treatment with a score satisfied or higher.

5 Example 2

A clinical multi-centre study was finalized by a total number of 27 volunteers in the age range from 25 to 70 years and belonging to Fitzpatrick skin types I to III with a degree of suntan in the treated skin areas varying from none to heavy. The volunteers had visible periorbital and perioral wrinkles and/or fine lines and suffered from sun damages skin with benign vascular and pigmented lesions.

The volunteers finalizing the study were divided into three groups comprising 10, 8 and 9 volunteers, respectively, treated at individual clinical sites in Denmark, Sweden and The Netherlands.

The volunteers in group 1 received a full face spraying with a 0.5 % 5-ALA liposome spray delivered in 8 spray doses with intervals of 15 minutes for an overall duration of 2 hours immediately followed to exposure to light energy using the standard IPL (Intensified Pulse Light) equipment ELLIPSE® from Danish Dermatologic Development A/S. One side of the face was subjected to exposure with a standard applicator emitting a wavelength range from 530 to 750 nm and supplying an energy density of 7 J/cm² in a pulse train consisting of two pulses of a duration of 2.5 ms spaced by an interval of 10 ms. The opposite side was treated with the same IPL equipment using a special applicator emitting the wavelength range from 400 to 720 nm in three subsequent passes each with a single pulse of a duration of 30 ms and an energy density of 3.5 J/cm² adding up to a total delivered energy density of 10.5 J/cm².

The volunteers in group 2 had a randomly selected face side pre-sprayed in 12 doses with intervals of 15 minutes for an overall duration of 3 hours and the other face side pre-sprayed in 4 doses with intervals of 15 minutes for an overall duration of 1 hour, for both sides with the same liposome-based 5-ALA preparation as the group 1 patients. The exposure was conducted as a full face treatment using the same special applicator as for group 1 in three subsequent passes each with a single

pulse of a duration of 30 ms and an energy density of 3.5 J/cm^2 adding up to a total delivered energy density of 10.5 J/cm^2 .

The volunteers in group 3 had randomly selected face sides pre-sprayed for 3 hours and 1 hour, respectively, using the same spray delivery procedure as for group 2 and exposure was performed immediately after termination of the pre-spraying by use of the same equipment as for group 1 using the standard applicator emitting a wavelength range from 530 to 750 nm and supplying an energy density of 7 J/cm^2 in a pulse train consisting of two pulses of a duration of 2.5 ms spaced by an interval of 10 ms.

The conclusions of this study were

- Pre-spraying with the 0.5 % liposome encapsulated 5-ALA resulted in an improved reduction in wrinkles and fine lines compared to a standard photo rejuvenation procedure using light exposure without pre-spraying with the same standard applicator emitting a wavelength range from 530 to 750 nm and supplying an energy density of 7 J/cm^2 in a pulse train consisting of two pulses of a duration of 2.5 ms spaced by an interval of 10 ms: This improvement was observed without any statistically significant difference for the standard light applicator and the special applicator;
- For reduction of perioral wrinkles a statistically significant trend for higher efficacy for 3- hours pre-spraying over 1 hour pre-spraying was observed;
- For improvement in pigmentation, diffuse redness and telangiectasis a statistically significant superiority was observed for use of the standard light applicator emitting a wavelength range from 530 to 750 nm and supplying an energy density of 7 J/cm^2 in a pulse train consisting of two pulses of a duration of 2.5 ms spaced by an interval of 10 ms;
- Superiority of the special light applicator emitting the wavelength range from 400 to 720 nm three subsequent passes each with a single pulse of a duration of 30 ms and an energy density of 3.5 J/cm^2 adding up to a total delivered energy density of 10.5 J/cm^2

was observed in the sense that it can be used for treatment of all skin types even medium to heavy pigmented ones up to skin type V on the Fitzpatrick scale.

P A T E N T C L A I M S

1. A method for non-therapeutic or therapeutic photodynamic treatment of a skin target area, comprising the steps of
delivery of a photosensitizer fluid incorporating a photosensitizing
5 agent or a precursor for a photosensitizing agent to the target area, the photosensitizing agent having at least one absorption wavelength in a wavelength range higher than 400 nm, and
exposure of the target area to light energy in a range of wave-
lengths comprising said at least one absorption wavelength of the photo-
10 sensitizing agent, the light energy being supplied to the target area in the form of at least one light pulse producing in the target area an accumulated energy density sufficient for photodynamic activation of the photosensitizing agent,
c h a r a c t e r i z e d i n
15 that the photosensitizing agent or precursor is incorporated in the photosensitizer fluid with a concentration in the range from 0.1 to 2.0 % and that the photosensitizer fluid is delivered to the target area in the form of a number of repeated spray doses during an overall delivery time ranging from 0.25 to 8 hours.
- 20 2. A method as claimed in claim 1, c h a r a c t e r i z e d i n that the photosensitizer fluid incorporates a photosensitizing agent.
3. A method as claimed in claim 2, c h a r a c t e r i z e d i n that the photosensitizing agent comprises at least one of a chlorin, a cyanine, a purpurin, a porphyrin and a xanthenes.
- 25 4. A method as claimed in claim 3 , c h a r a c t e r i z e d i n that the photosensitizing agent comprises a porphyrin.
5. A method as claimed in claim 4, c h a r a c t e r i z e d i n that the porphyrin is benzoporphyrin derivative monoacid.
6. A method as claimed in claim 1, c h a r a c t e r i z e d i n that
30 the photosensitizer fluid incorporates a precursor for a photosensitizing agent capable of producing a distribution of the photosensitizing agent in the skin tissue in the target area by metabolic transformation.
7. A method as claimed in claim 6, c h a r a c t e r i z e d i n that the precursor comprises ALA.

8. A method as claimed in claim 6, characterized in that the precursor comprises an ALA derivative.
9. A method as claimed in claim 8, characterized in that the ALA derivative is an ALA ester.
- 5 10. A method as claimed in claim 9, characterized in that the ALA ester is one of ALA-methyl ester, ALA-n-pentyl ester, ALA-n-octyl ester, R,S-ALA-2-(hydroxymethyl)tetrahydropyranyl ester, N-acetyl-ALA or N-acetyl-ALA-ethyl ester.
- 10 11. A method as claimed in any of claims 1 to 10, characterized in that the photosensitizer fluid comprises a liposome preparation incorporating the photosensitizing agent or precursor by encapsulation in liposome vesicles.
12. A method as claimed in claim 11, characterized in that the liposome preparation is prepared from a nanokolloid liposome emulsion having a mean vesicle diameter less than 250 nm.
- 15 13. A method as claimed in any of claims 1 to 12, characterized in that the photosensitizing agent or precursor is incorporated in the photosensitizer fluid with a concentration in the range from 0.5 to 1.0 %.
- 20 14. A method as claimed in any of claims 1 to 13, characterized in that the number of repeated spray doses is between 2 and 12, the spray doses being delivered to the target area with intervals of 10 to 15 minutes.
- 25 15. A method as claimed in any of claims 1 to 13, characterized in that the number of repeated spray doses is between 3 and 36, the spray doses being delivered to the target area with intervals of 3 to 10 minutes.
- 30 16. A method as claimed in claim 14, characterized in that the exposure of the target area to light energy is initiated with a time delay not exceeding 5 minutes from termination of said overall delivery time for the photosensitizer fluid to the target area.
17. A method as claimed in claim 15, characterized in that the exposure of the target area to light energy is initiated with a time delay from 1 to 8 hours from termination of said overall delivery

time for the photosensitizer fluid to the target area.

18. A method as claimed in any of claims 1 to 17, c h a r a c t e r -
i z e d in that the distribution of the photosensitizing agent in the tar-
get area is monitored during the overall delivery time for the photosensi-
5 tizer fluid.

19. A method as claimed in claim 18, c h a r a c t e r i z e d in
that the distribution of the photosensitizing agent in the target area is
monitored by measuring skin fluorescence from the target area.

20. A method as claimed in claim 18 and 19, c h a r a c t e r i z e d
10 in that the number of repeated spray doses and/or said regular interval
and/or said overall delivery time is determined by assessment of skin
fluorescence from the target area.

21. A non-therapeutic method as claimed in any of claims 1 to 20
for skin rejuvenation, reduction of wrinkles, treatment of telangiectasia
15 or removal of hair, c h a r a c t e r i z e d in that the photosensitizer
fluid incorporates the photosensitizing agent or precursor in a concentra-
tion from 0.1 to 2.0 % and is delivered to the target area in from 2 to
36 successive spray doses with a duration of said regular intervals be-
tween 3 and 15 minutes.

20 22. A non-therapeutic method as claimed in claim 21, c h a r a c -
t e r i z e d in that said concentration of photosensitizing agent or pre-
cursor is from 0.5 to 1.0 %.

23. A therapeutic method as claimed in any of claims 1 to 20 for
treatment of acne vulgaris, acne scars, rosacea, psorieasis, actinic kera-
25 toses, basal cell carcinoma, Bowen's disease or eczema , c h a r a c -
t e r i z e d in that the photosensitizer fluid incorporates the photosen-
sitizing agent or precursor in a concentration from 0.1 to 2.0 % and is
delivered to the target area in from 2 to 36 successive spray doses with
a duration of said regular intervals between 3 and 15 minutes.

30 24. A therapeutic method as claimed in claim 23, c h a r a c t e r -
i z e d in that said concentration of photosensitizing agent or precursor
is from 0.5 to 1.0 %.

25. A liposome photosensitizer fluid for use in the method claimed
in any of claims 1 to 24, c h a r a c t e r i z e d by comprising a nanokol-

old liposomic emulsion having a mean vesicle diameter not exceeding 250 nm, in which a photosensitizing agent or a precursor for a photosensitizing agent is encapsulated in liposome particles.

26. A liposome photosensitizer fluid as claimed in claim 24, characterized in that said concentration of photosensitizing agent or precursor is from 0.5 to 1.0 %.

27. Use of a liposome photosensitizer fluid as claimed in claim 25 or 26 for non-therapeutic photodynamic treatment of a skin target area.

28. Use of a liposome photosensitizer fluid as claimed in claim 26 or 27 for therapeutic photodynamic treatment of a skin target area.

29. Use of a liposome photosensitizer fluid as claimed in claim 26 or 27 for diagnosing a benign or malign dermatologic condition.

30. Use of a photosensitizing agent or a precursor for a photosensitizing agent in the manufacture of a photosensitizer fluid for application in photodynamic treatment of a skin condition, wherein the photosensitizing agent or precursor is incorporated in the photosensitizer fluid with a concentration in the range from 0.1 to 2.0 % and the photosensitizer fluid is delivered to the target area in the form of a number of repeated spray doses during an overall delivery time ranging from 0.25 to 8 hours.

31. Use of a photosensitizing agent or a precursor for a photosensitizing agent as claimed in claim 30, characterized in that said concentration of photosensitizing agent or precursor is from 0.5 to 1.0 %.

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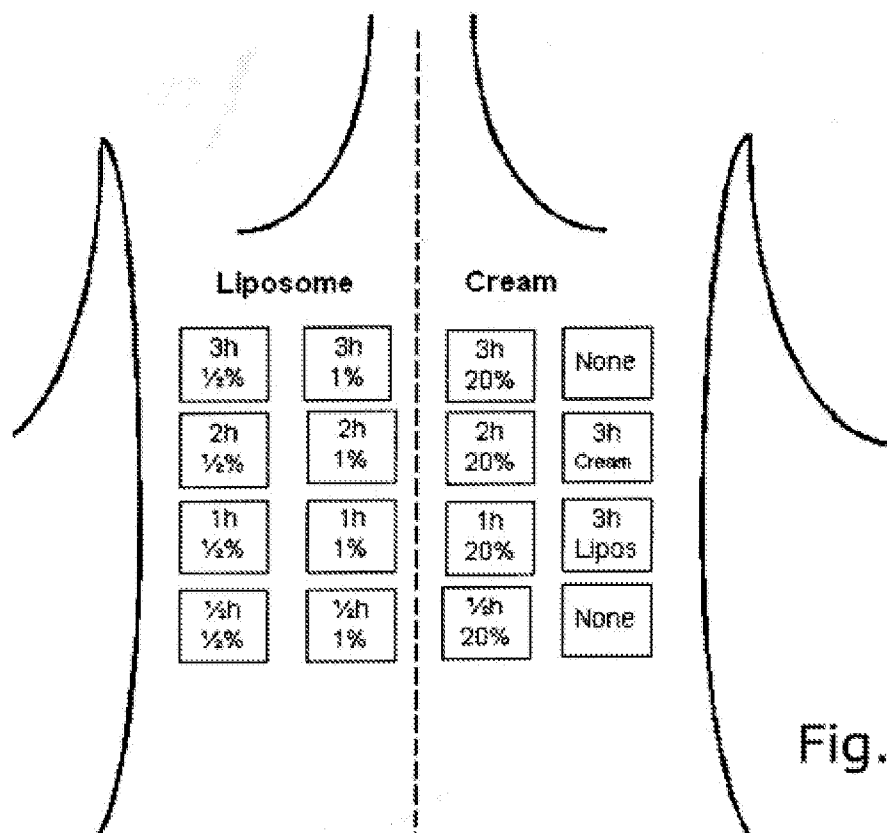


Fig. 1

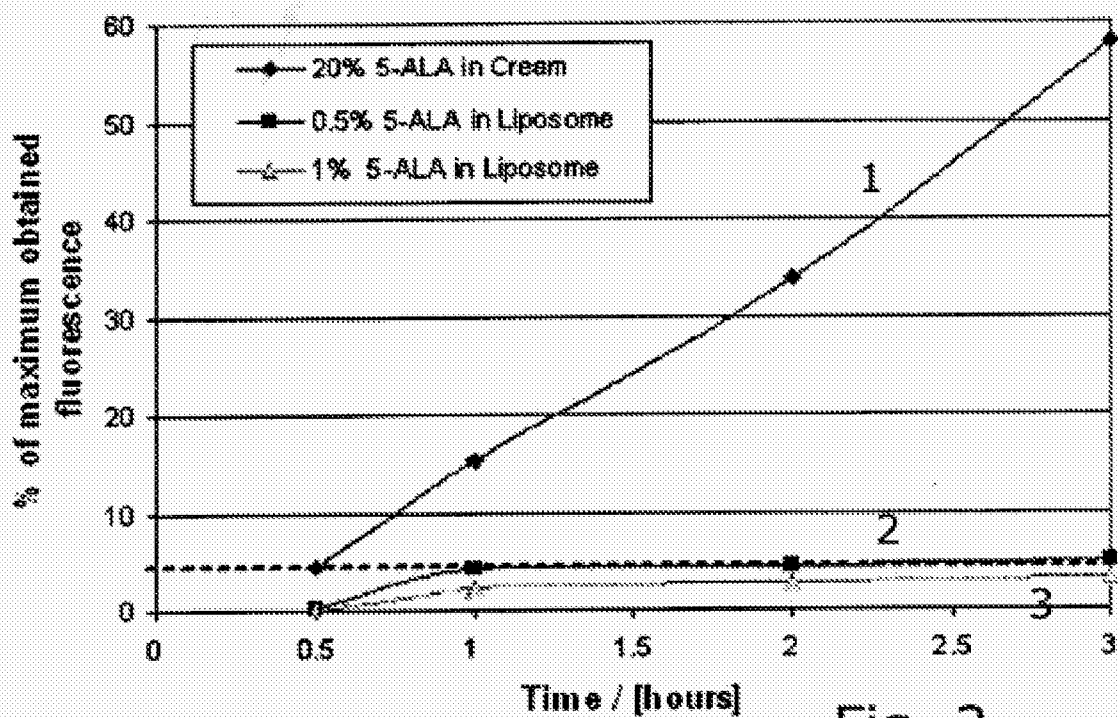


Fig. 2

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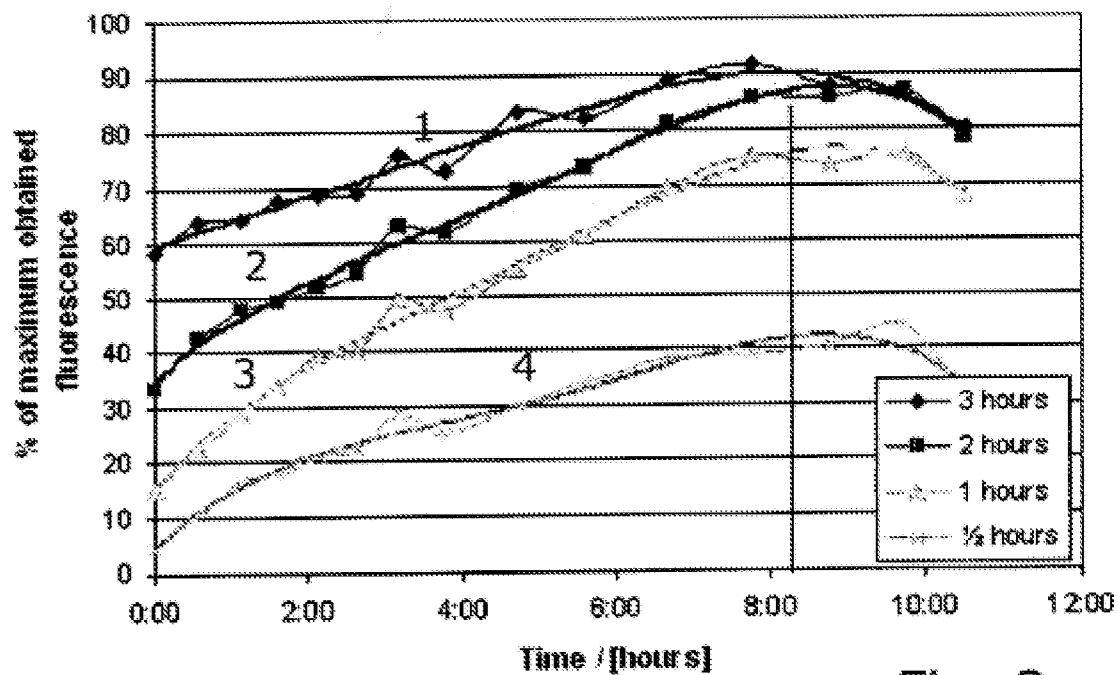


Fig. 3

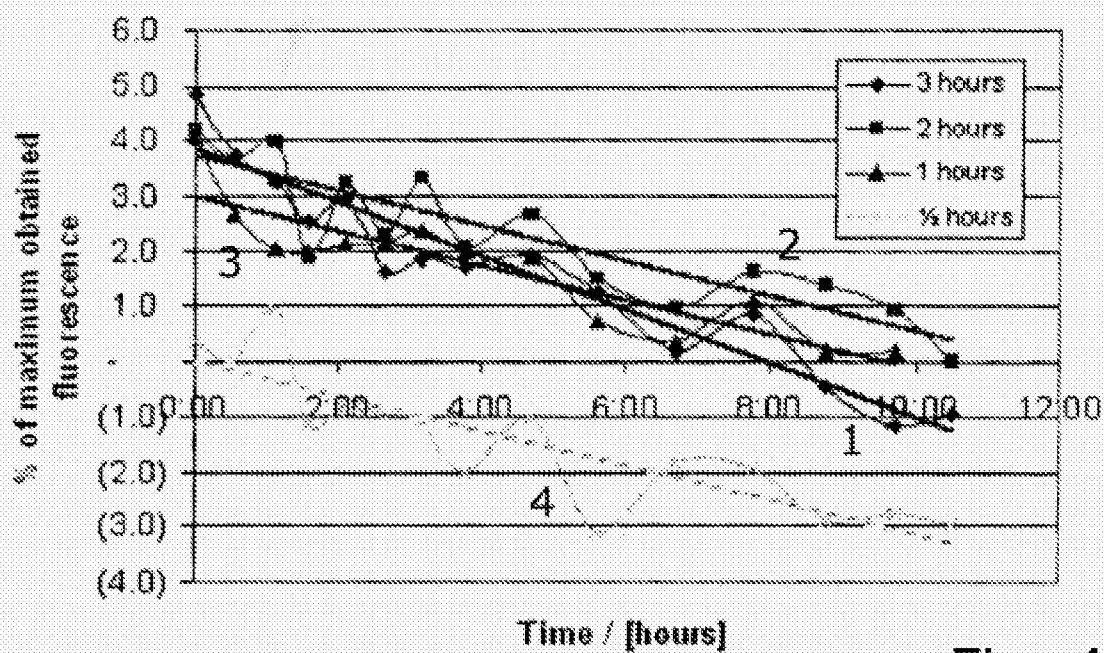
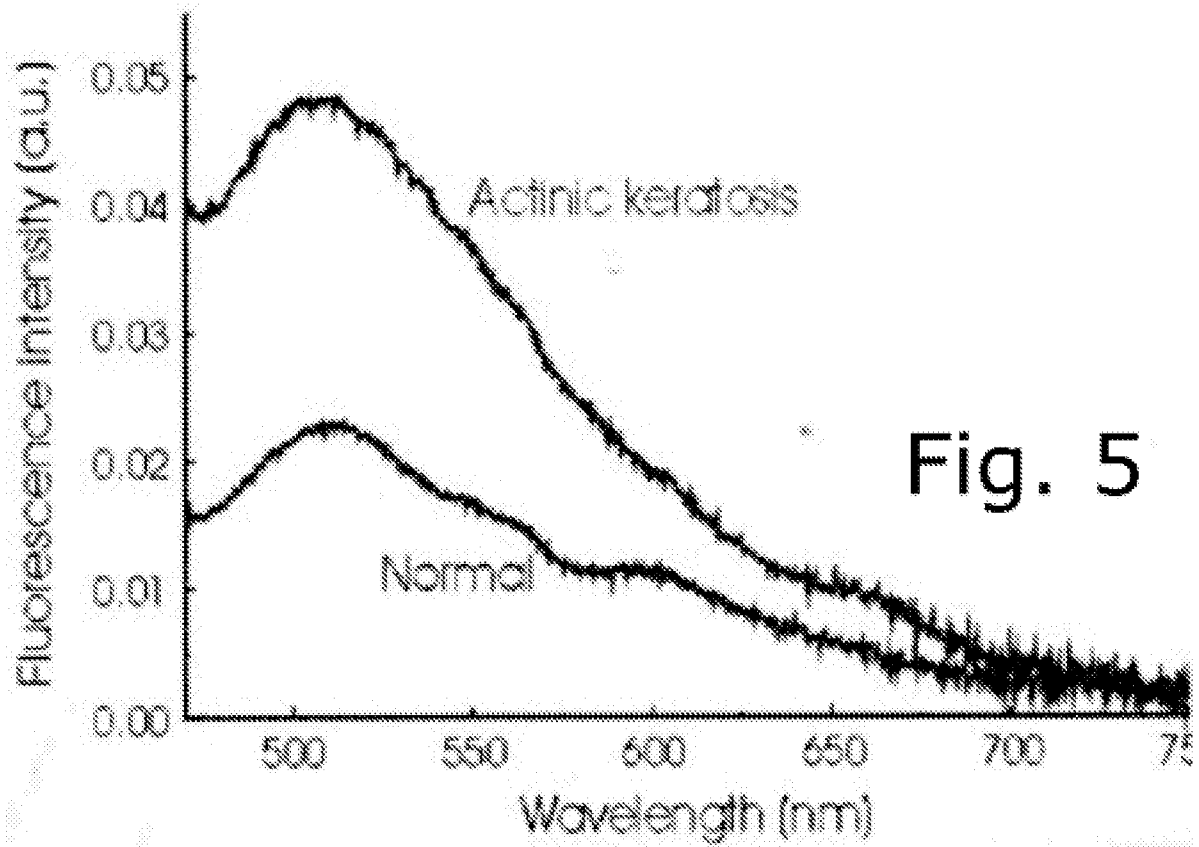


Fig. 4

3/4**Fig. 5**

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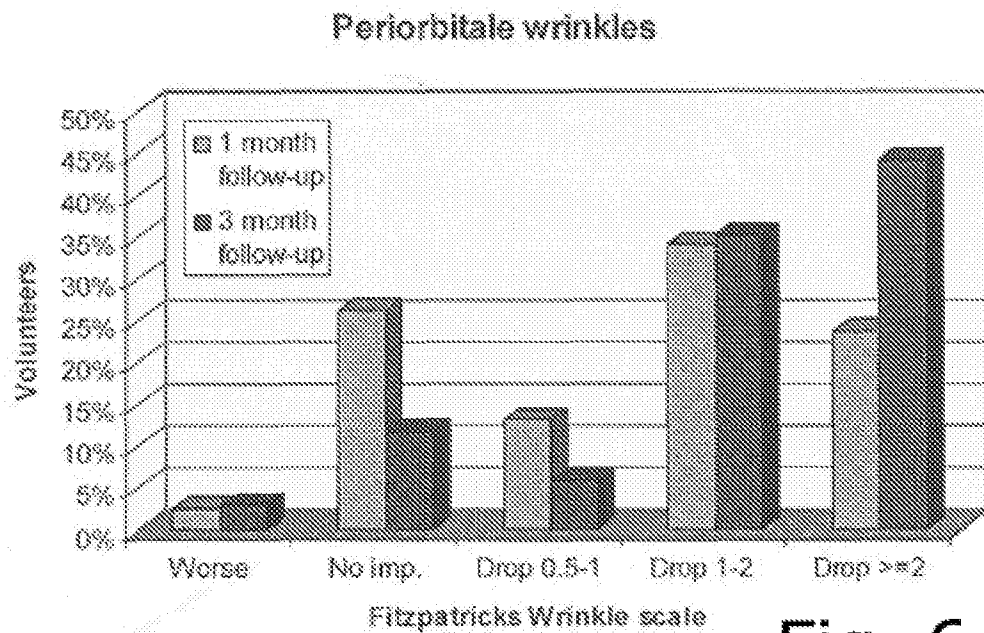


Fig. 6

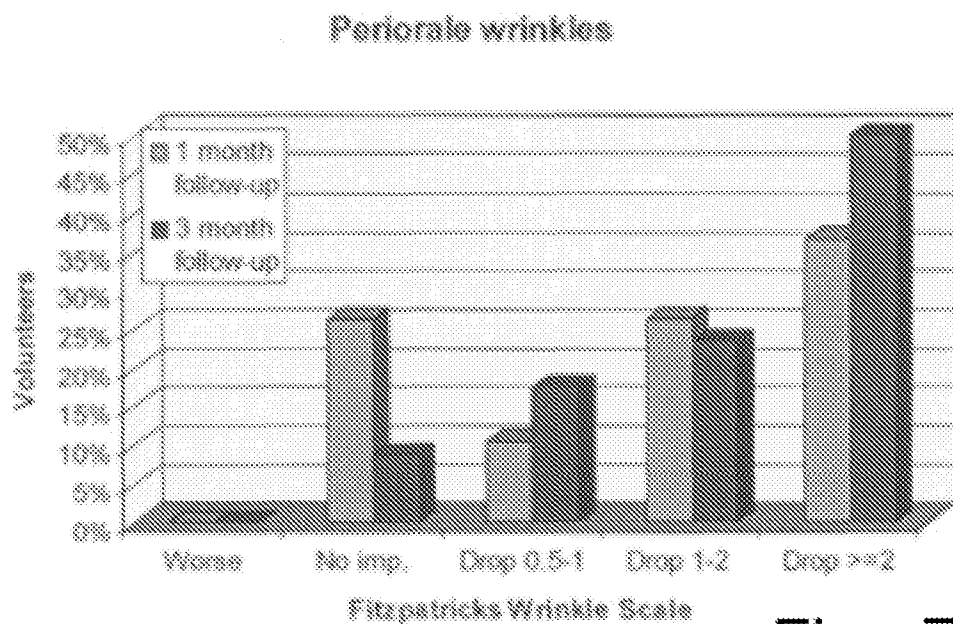


Fig. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2007/050029

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K41/00 A61P17/00 A61P17/10 A61K31/195 A61K8/44
A61Q19/08 A61Q9/00

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 A61Q

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 practical, search terms used)

EPO-Internal, 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 03/086460 A (CANDELA CORPORATION ET AL.) 23 October 2003 (2003-10-23) cited in the application paragraphs [0001], [0009], [0011] - [0013], [0024] - [0027], [0030] - [0032], [0036], [0041], [0047], [0052], [0055], [0056] examples 1-18 claims 1-33	1-31
Y	US 2003/125388 A1 (GANDER ET AL.) 3 July 2003 (2003-07-03) claims 1,8,10,19,22-25 paragraphs [0002], [0009], [0016], [0017], [0021] ----- -/--	1-31

☒ Further documents are listed in the continuation of Box C.

☒ 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 document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which 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

15 November 2007

Date of mailing of the international search report

23/11/2007

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INTERNATIONAL SEARCH REPORT

International application No

PCT/DK2007/050029

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 2002/099094 A1 (ANDERSON) 25 July 2002 (2002-07-25) claims 1,8,14,21,25,26,29 paragraphs [0034], [0055], [0065] - [0068] -----	1-31

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 1-20, 23-26, 29 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claim 28 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK2007/050029

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/DK2007/050029

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 03086460	A	23-10-2003	AU 2003230808 A1	27-10-2003
US 2003125388	A1	03-07-2003	AU 4055601 A	07-08-2001
			CA 2399405 A1	02-08-2001
			DE 10003620 A1	02-08-2001
			WO 0155092 A2	02-08-2001
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			JP 2003526637 T	09-09-2003
US 2002099094	A1	25-07-2002	US 2005143466 A1	30-06-2005