

A comparative in vivo study of antioxidant efficacy of cosmetic formulations

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SUMMARY

Antioxidants have been proposed, over the last decade, as functional ingredients for anti aging preparations and to prevent and modulate oxidative skin damages. However, up to date, beside the photo-induced oxidative skin damages model, none *in vivo* protocols have shown sufficient reproducibility for the validation of the above stated claim in a cosmetic finished product. To this end, we have developed a new *in vivo* protocol based on a reactive oxygen specie driven microinflammatory model of oxidative skin damages. The effects of a pre-treatment of three formulations containing different antioxidants, were investigated, on forearm skin of 15 healthy volunteers, and compared to the respective cosmetic bases and controls. Comparative evaluations, using three different methods, were conducted: visual score (VS), narrow-band reflectance colorimeter (DermaSpectrometer from Cortex) using the erythema index (E), and a new software program (DermAnalyzer), by us developed, using the CIE L*a*b* colour space parameters. The efficacy of the antioxidant formulations, was quantified by the decreases of the a* parameter and E index as compared to the control area. The comparative measurements showed that all the antioxidant formulations tested were able to reduce, in different but statistically significant extent, the intensity of skin redness, when compared to control area (p< 0.001). Taking into account the different methodologies upon which the DermalSpectrometer and DermAnalyzer are based, we have demonstrated the good relationships, between E and a*. Therefore, we propose the methyl nicotinate -induced microinflammation model, in conjunction with objective redness measurements, as mean to determine the *in vivo* efficacy of antioxidant ingredients in cosmetic formulations.

INTRODUCTION

Skin is a highly metabolic tissue which possesses the largest surface area in the body, and is also a major candidate and target of damaging free radicals. It is well known that free radicals and reactive oxygen species (ROS) are involved in the mechanism leading to cutaneous damages, such as early ageing, inflammatory disorders and skin cancers. Elaborate and diversified antioxidant mechanisms, of both enzymatic and non-enzymatic nature, protect skin from oxidative damage. Over the last decade antioxidants have been proposed as functional ingredients for anti aging preparations, and to prevent and modulate oxidative skin damages. Although it has been shown that ROS are directly related to skin ageing, methods for evaluating oxidative damage in skin, and the potential efficacy of antioxidant-containing cosmetic products are not been available. However, up to date, beside the photo-induced oxidative skin damages model, any other *in vivo* protocols have shown sufficient reproducibility for the validation of the above stated claim in a cosmetic finished product. Taking this into account, we have developed a protocol that directly allows the evaluation of the protective role of antioxidant-containing cosmetic/pharmaceutical formulations against oxidative stress, and indirectly, the anti-aging efficacy. The protective role of antioxidants has been evaluated by means of a pretreatment with antioxidant formulations, in order to integrate and reinforce the skin physiological defence. To this end comparative evaluations, based on three different methods, were conducted: visual score (VS), narrow-band reflectance colorimeter (DermaSpectrometer from Cortex) using the erythema (E) index and a new software program (DermAnalyzer) developed by us, using the CIE L*a*b* colour space. The efficacy of the antioxidant formulations evaluated, was readily quantified by the decrease of the a* parameter and of the E index compared to the control area.

MATERIALS AND METHODS

Subject and experimental design: in vivo study

Fifteen Caucasian healthy volunteers (10 female and 5 male) between 23-30 years, participated in the study after giving the written consent. The study was approved by the Ethics Committee. The study was performed according to a double-blind and randomised fashion. Seven randomised areas (10cm²) were outlined on the internal side of the forearm of each volunteer. The study was performed in two stages. The first was the pre-treatment phase consisting of the application of 4 mg cm⁻² twice a day for 3 days of each product studied. On the fourth day the areas, were retreated with the products, 30' before the application of the irritant agent. At this time was determined the baseline value of redness of treated areas (T₀). The irritation was induced in each area by the application of paper discs (5mm of diameter) soaked with 15 µl of 0.5 % aqueous solution of methyl nicotinate (99% purity, Fluka Chemie GmbH) for 3'. After removal of the filter disk, excess solution was gently removed using a paper tissue. A circular erythema spot was induced by this treatment. The volunteers rested during the measurements in the laboratory area, with the test sites exposed to room temperature (20-23°C), and the skin colour of each sites was examined at regular times over 90', using the three discussed methods. Measurements were performed before MN exposure (T₀), immediately after MN removal (3'), and at 5, 10, 15, 30, 60, 90 minutes after MN removal.

In vivo colour measurements of erythema

Two kinds of instrumental methods were used to measure the erythema: Deraspectrometer (Cortex Technology, Handsund, Denmark) and DermAnalyzer developed by us.

A. Reflectance photometry: Deraspectrometer

The DermaSpectrometer (Cortex Technology, Handsund, Denmark) is a hand-held microprocessor controlled reflectance photometer, with a digital readout narrow-band spectrophotometer, that was developed specifically for measurements of erythema/melanine (E/M) index. The light sources of this instrument are two light-emitting diodes with narrow bands of emitted wavelengths. The peaks of the two bands are centered at 568 nm (green light)

and 655 nm (red light). A photodetector measures the light by the skin. After being converted into digital form with a built-in microcomputer, the reflectance in the two bands are transformed into E index and the melanin M index. It was used for the measurement of E index, according to the guidelines provided by the manufacturer. The measures of E index of each treated area at established times, was compared with the E index value of a experimentally non involved skin control area. Diameter of the measuring area are 6mm in diameter, and three measurements were taken of each site, moving the measurements head few centimetres between measurements. The instrument is calibrated using a black and white calibration plate. When placing the probe on the skin surface for the measuring we took care to avoid much pressure on to the skin.

B. DermaAnalyzer

a. Image recording

True colour images of the observed skin area, were taken using a digital photo camera (Nikon Coolpix900). The position of the camera was adjusted, so that the examined areas were in the centre of the images. For illumination two sources of light with an angle of 45°, one on the left and one on the right of the subject, were used to reduce shadows on the border of the arms and to avoid reflections on the skin. All the images were taken under the same constant lightening conditions. For this, the measurements were carried out in a special examination room, which no person entered during the recording. To analyse the development of the erythema response, a series of true colour images was taken in the time over 90'. Measurements were performed before MN exposure (T0), after MN exposure, immediately after MN removal (3'), and at 5, 10, 15, 30, 60, 90 minutes after MN removal.

b. Software

The images were downloaded from the digital camera, archived on the computer hard-disk, and afterwards analysed singularly with the DermAnalyzer. The program lets to choose each treated area simply drawing a line around the zone to be analysed. After this step, the program algorithm developed by us, automatically pulls apart the treated skin from the untreated, and measures the CIE a* components of the red area giving as final result the average CIE a* value for every single zone.

Visual score

Visual score was performed by two dermatologists who assigned to every examined area a score, based on erythema and/or oedema evaluation, according to the European Society of Contact Dermatitis guideline.[1]

The simple scoring system is a follow: 0, no reaction (negative); 0.5, very weak erythema or minute scaling (doubtful); 1, weak erythema, slight oedema, slight scaling and/or slight roughness (weak); 2, moderate degree of erythema, oedema, scaling and/or roughness, or minor degree of erosion, vesicles, bullae, crusting and/or fissuring (moderate); 3, marked degree of erythema, oedema, scaling, roughness, vesicles, bullae, crusting and/or fissuring (strong); 4, as 3, with necrotic area (very strong/caustic).

In vitro colour measurements

The in vitro measurements (mean values of 10 independent measurements) were carried out on selected colours (red) of the standard reference.

In vivo colour measurements

The in vivo measurements (mean values of 5 independent measurements) were carried out on the examined skin regions, treated with different cosmetic formulation, and on the control area (volar part of the forearm), by means of the DermaSpectrometer and DermAnalyzer.

Statistical analysis

Repeated measures one-way analysis of variance (ANOVA) was used for comparison between group, followed by Dunnett's post test. The visual clinical assessment data were analysed with Friedman's test. A p value <0.05 was considered significant.

Study material

The following 5 products were tested:

Product A: Aqua, glyceryl stearate (and) ceteareth-20 (and) ceteareth-12 (and) cetearyl Alcohol (and) cetyl Palmitate, glycerin, cetearyl alcohol dimethicone, dicaprylyl ether, dioctylcyclohexane, caprylic/capric triglyceride, methylparaben, ethylparaben, propylparaben, butylparaben, phenoxyethanol, *magnesium ascorbyl phosphate*, disodium EDTA, sodium dehydroacetate, imidazolidinyl urea, parfum.

Product B: Aqua, glyceryl stearate (and) ceteareth-20 (and) ceteareth-12 (and) cetearyl alcohol (and) cetyl palmitate, glyceryl stearate, cetearyl alcohol, dimethicone, caprylic/capric triglyceride, dicaprylyl ether dioctylcyclohexane, methylparaben, ethylparaben, propylparaben, butylparaben, phenoxyethanol, *retinol palmitate*, *magnesium ascorbyl phosphate*, imidazolidinyl urea, disodium EDTA sodium dehydroacetate.

Product C: Aqua, cocoglycerides, dicaprylyl ether, pentylene glycol, glycerin, stearic acid, cetearyl alcohol, glyceryl stearate, polyglyceryl 10-pentastearate, behenyl alcohol, aluminium starch octenyl succinate, abies pectinata, arginine, *ascorbyl palmitate*, capryloyl glycine, citric acid, disodium EDTA, *Fagus sylvatica*, hydroxypropyl guar, lecithin, parfum, *propagermanium*, sodium stearyl lactylate.

Product D: Cosmetic base of A and B

Product E: Cosmetic base of C

One area was control area, not pretreated with cosmetic formulations, and irritated with MN.

RESULTS

In vivo experiments

Erythema index

Mean values for instrumental recordings of erythema index are shown in Table I; Fig.1 shows the time course of a* value, for each cosmetic formulation evaluated.

As it can be observed from the data, the pre-treatment of the sites with the examined cosmetic products, is significantly effective in the inhibition of MN-induced skin erythema ($p < 0.0001$). Among the three different formulation (A, B, C) tested, the efficacy resulted in the following order: C>B>A, with a significant difference between C and A ($p < 0.05$). As expected, the cosmetic bases were less effective than the corresponding finished products. In particular, E was significantly less effective than the corresponding C product ($p < 0.05$). Differences between individual test sites compared to control area, were calculated with Dunnett's multiple comparison test, and approached significance for A ($p < 0.05$), B and C ($p < 0.01$), but, as one may expect, not for D and E. After exposure to MN, the increase in redness for the control area, compared to the baseline value (T0), were 36.7% after 15' and 73.5% after 30', while the increase for C was 34.1% after 15' and 26.7% after 30'. In the case of preparation B and control an initial decrease in a* value observed has been attributed to an initial vasocompression due to tissue oedema. Moreover, preparations A, B, D and E, also displayed an average value of redness increase of about 45% after 15', and 41.25% after 30'. (Table II). Finally, whereas the treated areas showed a complete recovery of the baseline values, the control area showed a consistent delay in the recovery of initial skin colour; indeed the a* value at T90 was 28% higher than the baseline value (T0).

Table I

	T0	T3	T5	T10	T15	T30	T60	T90
Test site 1 control area (MN)	10.33 (±0.710)	11.63 (±0.73)	15.60 (±0.70)	13.93 (±0.61)	14.13 (±0.57)	17.93 (±0.67)	16.73 (±0.60)	13.26 (±0.58)
Test site 2 (A+ MN)	10.13 (±0.74)	10.83 (±0.75)	13.93 (±1.12)	14.53 (±0.87)	14.96 (±0.85)	14.46 (±0.81)	12.83 (±0.70)	11.13 (±0.60)
Test site 3 (B+ MN)	10.20 (±0.92)	11.30 (±1.02)	13.40 (±0.95)	14.30 (±0.95)	14.56 (±0.86)	14.20 (±0.83)	11.20 (±0.64)	10.56 (±0.65)
Test site 4 (C+ MN)	10.33 (±0.82)	10.50 (±0.917)	11.00 (±0.82)	12.83 (±0.88)	13.86 (±0.79)	13.10 (±0.83)	10.93 (±0.54)	10.10 (±0.61)
Test site 5 (D+ MN)	10.50 (±0.69)	11.16 (±0.96)	14.50 (±0.81)	15.33 (±0.84)	15.10 (±0.68)	14.96 (±0.87)	12.73 (±0.69)	11.53 (±0.65)
Test site 6 (E +MN)	10.40 (±0.83)	10.90 (±0.87)	14.50 (±1.02)	14.83 (±0.92)	15.30 (±0.94)	14.73 (±0.82)	12.73 (±0.77)	11.06 (±0.81)

Means ±SD (n=15), are given for E index measured with the DermaSpectrometer (Cortex) for the five tested areas pretreated with the different formulations and the control area (no pre-treated, and irritated with MN). The values are given as arbitrary units. Higher values reflect increase redness. A, ascorbyl-magnesium phosphate; B, retinyl palmitate and ascorbyl-magnesium phosphate; C, propagermanium; D, bases of A and B; E, base of C. MN: methyl nicotinate. T= minutes

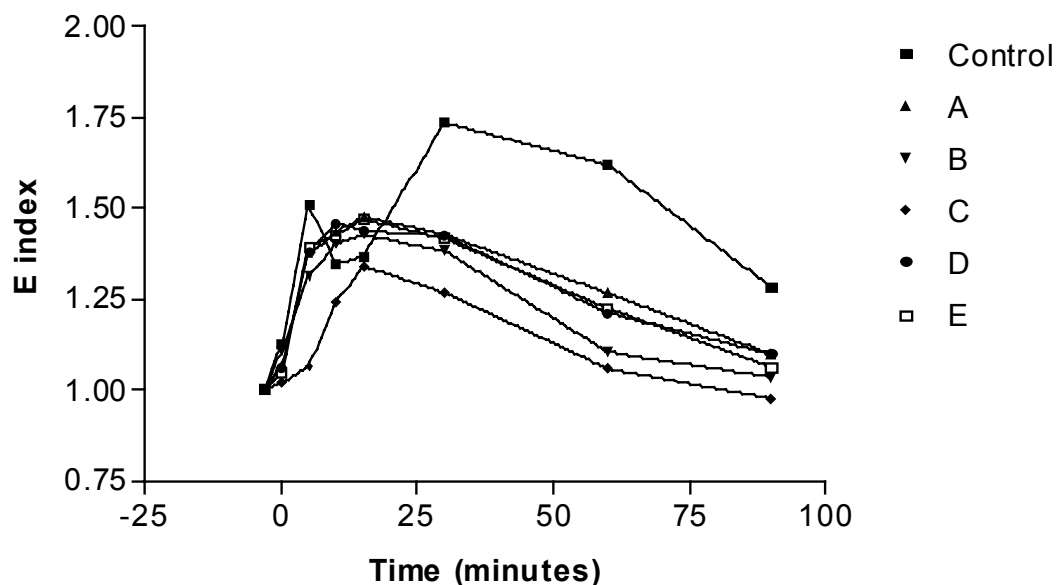


Fig.1. Mean values (n=15), are given for E index measured with the DermaSpectrometer (Cortex) for the five formulations and the control area. The values are given as normalized data. Higher values reflect increase redness. A, ascorbyl-magnesium phosphate; B, retinyl palmitate and ascorbyl-magnesium phosphate; C, propagermanium; D, bases of A and B; E, base of C.

Table II

Time	A	B	C	D	E	Control
15'	36.7	47.7	42.8	34.1	43.8	47.1
30'	73.5	42.7	38.2	26.7	42.5	41.6

Percentage of relative difference [(value after-value before)/value before X 100] from the baseline value (T0) of each examined area, after 15' and 30' from exposition to MN.

DermAnalyzer

Mean values of a* for sites treated with the formulations, vehicles, and controls are shown in Table III; Fig. 2 shows the time course of a* value, for each cosmetic formulation. The data obtained with this technique, confirms the above discussed results of Deraspectrometer. As it can be observed from the data, the pre-treatment of the sites with the examined cosmetic products, was significantly effective in the inhibition of MN-induced skin erythema (p<0.0001). Among the three different formulations tested (A, B, C) the efficacy resulted in the following order: C>B>A. As expected, the cosmetic bases D and E were less effective (p<0.01) as compared to the respective antioxidant formulations A, B and C but, differently from what reported above, also D was significantly different. The differences between individual test sites compared to control area, were calculated with Dunnett's multiple comparison test, and approached significance for A,B,C and E (p<0.01), but not for D. After exposure to MN, the increase in redness for the control area, compared to the baseline value (T0), were 65.7% after 15' and 48.1% after 30', whereas the increase for C was 25.2% after 15' and 24.2% after 30'. (Table IV)

Table III

	T0 (before MN irritation)	T3	T8	T13	T18	T33	T63	T93
Test site 1 control area (MN)	12.40 (±1.07)	13.45 (±1.13)	16.08 (±1.99)	18.03 (±1.92)	20.55 (±1.05)	18.37 (±1.13)	15.83 (±0.95)	14.55 (±0.86)
Test site 2 (A+ MN)	12.16 (±0.87)	12.26 (±1.07)	13.93 (±0.92)	14.78 (±0.71)	15.31 (±0.89)	15.48 (±0.52)	14.25 (±0.52)	12.06 (±0.83)
Test site 3 (B+ MN)	11.83 (±0.62)	11.98 (±0.4)	12.96 (±0.95)	13.96 (±0.45)	14.81 (±0.37)	14.70 (±0.29)	13.71 (±0.37)	11.65 (±0.45)
Test site 4 (C+ MN)	11.56 (±0.44)	11.76 (±0.94)	12.05 (±0.82)	12.55 (±0.74)	13.30 (±0.79)	13.08 (±0.83)	12.65 (±0.63)	11.31 (±0.79)
Test site 5 (D+ MN)	11.93 (±1.16)	13.90 (±0.96)	15.33 (±0.81)	16.00 (±1.26)	16.80 (±0.98)	15.85 (±0.97)	14.53 (±1.09)	12.66 (±1.02)
Test site 6 (E+ MN)	12.35 (±0.97)	13.18 (±0.90)	14.46 (±0.93)	15.78 (±0.76)	16.50 (±0.83)	16.31 (±0.59)	14.35 (±0.87)	13.35 (±0.91)

Means ±SD (n=15), are given for a* parameters measured with the DermaAnalyzer for the five tested areas pretreated with the different formulations and the control area (no pre-treated, and irritated with MN). The values are given as arbitrary units. Higher values reflect increase redness. A, ascorbyl-magnesium phosphate; B, retinyl palmitate and ascorbyl-magnesium phosphate; C, propagermanium; D, bases of A and B; E base of C. MN: methyl nicotinate. T= minutes

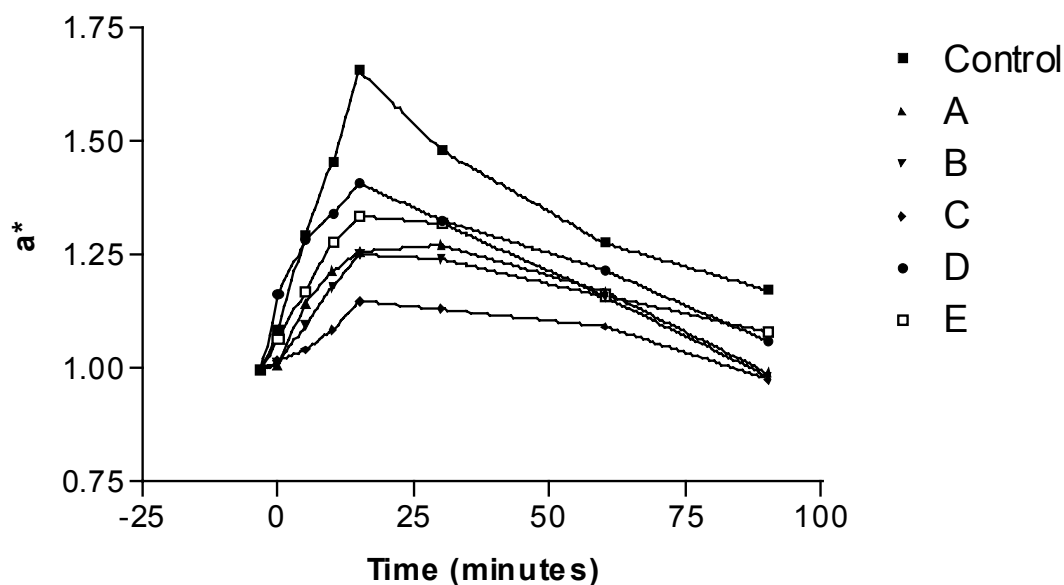


Fig.2. Mean values (n=15), are given for a* parameter measured with the DermaAnalyzer for the five formulations and the control area. The values are given as normalized data. Higher values reflect increase redness. A, ascorbyl-magnesium phosphate; B, retinyl palmitate and ascorbyl-magnesium phosphate; C, propagermanium; D, bases of A and B; E base of C.

Table IV

Time	A	B	C	D	E	Control
15'	25.8	25.2	14.9	40.7	33.6	65.7
30'	27.2	24.2	13.1	32.8	32.1	48.1

Percentage of relative difference [(value after-value before)/value before X 100] from the baseline value (T0) of each examined area with DermAnalyzer, after 15' and 30' from exposition to MN.

Visual score

The time course of the mean values of visual score (VS) for sites treated with the formulations, vehicles, and controls are shown in Fig.3. A statistically significant increase of clinical score was observed in the control area, where was frequently present oedema. Regarding the VS, the analysis with the Friedman test was statistically significant ($p < 0.0002$), but whereas differences between control versus treated areas were clearly appreciable, differences among different formulations were less objectivable. However, also in this case formulation C showed a better pattern as compared to the other formulations. It has to be noted that the degree of erythema never reached the score 3, in any of the tested areas. The maximum score observed was 2 at time 15', but only in 3 subjects.

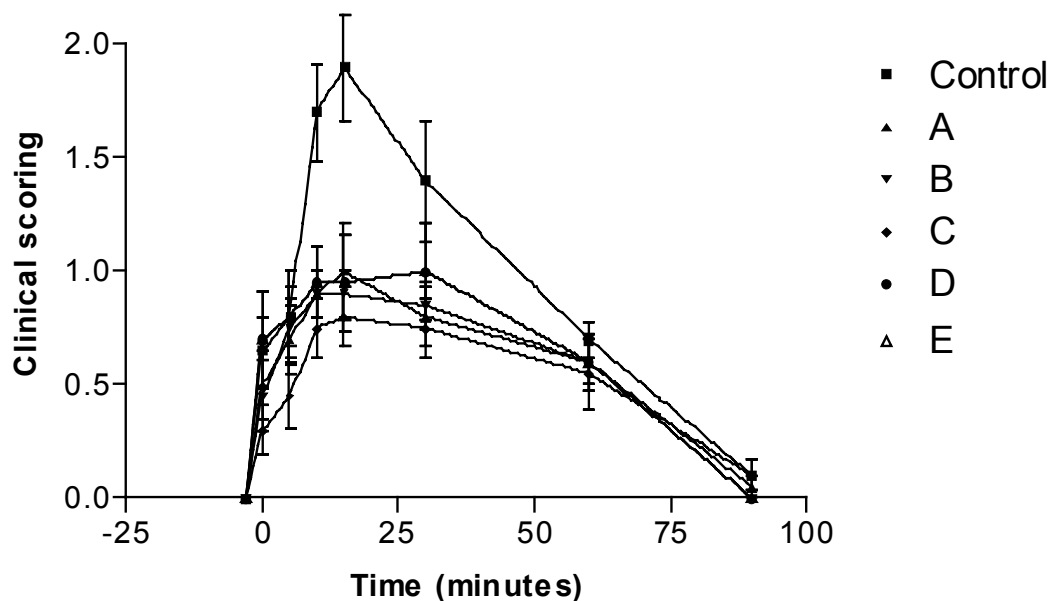


Fig.3. Mean values of clinical scoring \pm SD (n=15), are given for the five formulations and the control area. A, ascorbyl-magnesium phosphate; B retinyl palmitate and ascorbyl-magnesium phosphate; C, propagermanium; D, bases of A and B; E base of C.

In vitro experiments

Sensitivity and range of measurements

To assess the in vitro range of measurements, standard colour charts were used, and for each colour parameter, respectively, the lowest and highest experimental values were noticed. For the E index (DermaSpectrometer) varies from -12 to +69. The corresponding a^* values for the DermAnalyzer were from -46 to +61.

In vitro correlations

Correlations were calculated between related colour parameters obtained with the different instruments. Figure 4 shows the scattergram related to the correlation between the a^* value data and the E index, measured, during the in vitro experiments, by DermAnalyzer and DermaSpectrometer respectively. The correlation between a^* value and the E index was very high ($r = 0.99$).

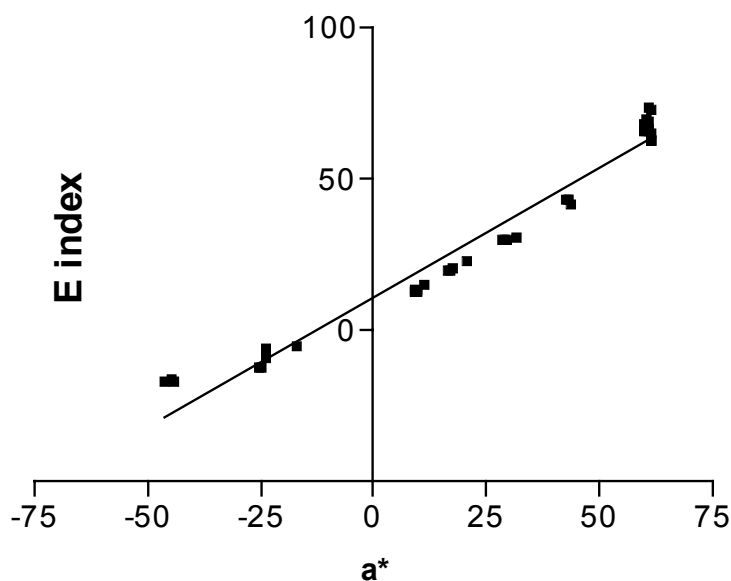


Fig.4 Relationships between the a* value and E index, measured, during the in vitro experiments, by DermAnalyzer and DermaSpectrometer respectively. The correlation between a* value and the E index was very high ($r = 0.99$).

In vivo experiments

Sensitivity and range of measurements

The interindividual range of measurements of skin redness, varied from 10.10 to 17.9, for E index (DermaSpectrometer). The corresponding a* values, calculated with DermAnalyzer, varied from 11.3 to 20.55.

In vivo correlations

The correlation between parameters was calculated by using the values collected over the whole range of measurements of the induced colour changes.

Figure 5 shows the scattergram related to correlation between the a* value data and the E index, measured, during the in vivo experiments, by DermAnalyzer and DermaSpectrometer respectively. The mean correlation value (reported as Total in Fig.5) between a* value and the E index was rather good ($r = 0.8$). This mean value was greatly decreased due to the low coefficient resulting for the control area ($r = 0.6$). This latter result has been attributed to the presence of oedema in the control area, that induces a decrease of the redness value. In our opinion, the limited area examined by DermaSpectrometer does not allow to include into the evaluation the redness surrounding oedematous area. This occurrence is avoided by the DermAnalyser which consider the whole irritated area.

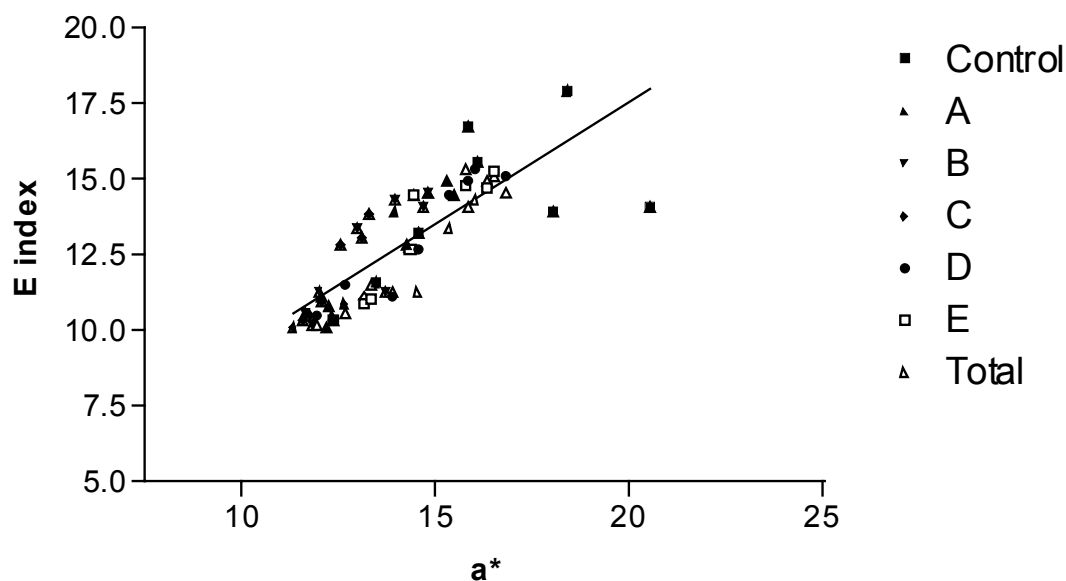


Fig.5 Relationships between the a^* value and E index, measured, during the *in vivo* experiments, by DermAnalyzer and DermaSpectrometer respectively. The total correlation between a^* value and the E index was very high ($r = 0.8$).

DISCUSSION

In skin, free radicals can be generated by UV-irradiation or chemical insults, and up to date, the most widely method to assess the antioxidant/radical scavenging activity of a compound is the photo-oxidative-induced skin erythema. [2] Taking this into account, we have developed a protocol that directly allows the evaluation of the protective role of antioxidant-containing cosmetic/pharmaceutical formulations against oxidative stress, and, indirectly, the anti-aging efficacy. The protective role of antioxidants has been evaluated by means of a pretreatment with antioxidant formulations, in order to integrate and reinforce the skin physiological defence. The oxidative skin damage is a rapid event, and antioxidants prevent this occurrence only when present in adequate concentration at the site of action, and when the oxidative damage take place. [3] Our *in vivo* study consists in the generation of reactive oxygen species by MN induced micro inflammation; this in turn, stimulates cyclooxygenase and the prostaglandins synthesis. [4] This cascade trigger the generation of endoperoxides, thus leading to the amplification of the original stimulus, by production of additional ROS. The relationship between skin ageing and inflammation is at the basis of the microinflammatory model of skin ageing [5][6]. This postulates peroxidation of skin cellular lipids, induced by endogenously or exogenously generated free radicals, as the promoter of a subclinical inflammatory state. ROS are believed to be involved in many inflammatory skin disorders, such as those constitutively produced in epidermal keratinocytes, and can be induced by chemical irritants. [7] We used, in this study, the intensity and duration of skin redness generated by MN, to assess the efficacy of functional ingredients, included in cosmetic formulations to prevent and modulate skin oxidative damages. This nicotinic acid ester, after topical application, rapidly crosses the skin and elicits a distinctive erythema, the intensity of which can be monitored by means of non-invasive instrumental techniques. Traditionally, erythema has been assessed visually by trained observers, using predetermined arbitrary scales, but this approach can be criticized as subjective, of poor reproducibility, lacking in sensitivity, and highly variable between observers. In consequence, instrumental methods have been strongly promoted and do indeed offer several advantages, not least their objectivity. In order to measure objectively the skin redness, various colour measuring devices have been developed [8] and in this paper we propose DermAnalyzer, an effective colour analysis method developed by us. Data obtained with this latter method, were compared with those collected by clinical visual observation and narrow band reflectance colorimeter that detect E index. Our method provide an objective, accurate, quantitative, and

cost-effective way that overcome the drawbacks of traditional instrumental methods used to assess the skin colour. The characteristics of this method are: a) the possibility to achieve a digitalised picture of the skin, suitable for successive re-evaluation, and b) a quantitative evaluation, of the mean redness value of the whole area considered. The traditional chromameter, instead, only consider a limited area (generally a circular surface of about 0.5cm²) thus impairing repeatability of the measure. Moreover, in such cases when measures are complicated by the presence of side effects (i.e. oedema) these latter features consent anyway to achieve a data suitable for statistic analysis. Taking into account the different methodologies upon which the Deraspectrometer and DermAnalyzer are based, we have evaluated the degree of correlation between a* and E*, when measured by the means of the instruments here described. Previous studies [9] demonstrated the relationship between a* and E, and our results confirm the positive correlation between these parameters with both *in vitro* (r=0.99) and *in vivo* experiments (r= 0.8). In this study we have chosen some of the most frequently employed antiageing ingredients, such as magnesium-ascorbyl-2-phosphate, retinyl palmitate.[10] Moreover, a particular germanium based ingredient (β -biscarboxyethyl germanium sesquioxide, INCI name: propargermanium) has attracted our attention in view of the very specific antioxidant properties.[11] In our *in vivo* antioxidant model, among the above cited antioxidants, the more effective formulation was indeed that containing propargermanium. This result has been univocally confirmed by all evaluation methods considered. The irritation pattern of the control area can be adequately assessed using the VS, and by a reflectance parameter such as a* (DermaSpectrometer and DermAnalyzer), but in the regards of the other tested formulations, the VS lacked of sensitivity. The tested formulations containing magnesium ascorbyl phosphate (A) and a combination of this latter antioxidant ingredient with retinyl palmitate (B) resulted less effective than the formulation containing propargermanium (C), however their efficacy was statically significant compared to the control area. In our opinion, the less efficacy of the antioxidant ingredients derivatives of vitamin C and retinol compared in this case to propargermanium, is due to the necessity of enzymatic activity for conversion within the skin into the corresponding active form. Moreover, an optimal percutaneous absorption of these antioxidant vitamins, to achieve the adequate effective quantity in the skin may also play an important role.[12]

CONCLUSIONS

As stated above, one of the major concern in the application of dermo-cosmetic preparations, based on the antioxidant claim, is the lack of reliable, fast and effective *in vivo* methods for claim substantiation. In this paper we have presented a new approach, to assess the *in vivo* antioxidant efficacy of cosmetic formulations. This is based on the capability of antioxidants to counteract the skin redness following a MN-induced microinflammatory stimulus. The degree of skin redness was comparatively evaluated by three different techniques: visual clinical assessment, DermaSpectrometer, that detect the E index and DermAnalyzer, a new software for skin colour analysis, developed by us, using the CIE L*a*b*. Our results have clearly demonstrated that a micro-oxidative model of skin ageing, is a valuable approach to evaluate and discriminate the efficacy of different antioxidant cosmetic formulations. Moreover, the comparative study, also consented us to further validate DermAnalyzer as an easy to use, technique for skin redness evaluation, endowed with good repeatability. Indeed, although Deraspectrometer and DermAnalyzer are based on different methodologies, we have demonstrated the good relationships, between E index and a*, the parameters normally employed for evaluating the degree of erythema. In conclusion, we believe our approach of value in the determination of the *in vivo* efficacy of antioxidant dermo-cosmetic formulations. Finally, and additional advantage consists in the concomitant application of the DermAnalyzer program which, in comparison to the traditional colour measuring devices, presents the following advantages: suitable for colour determination of the whole treated area, visualization and storage of the skin area considered for successive re-evaluations and low costs.

REFERENCES

1. Tupker, R.A., Willis, C., Berardesca, E., Lee, C.H., Fartash, M., Agner, T., Serup, J. Guidelines on sodium lauryl sulphate (SLS) exposure tests. A report from the Standardisation Group of the European Society of Contact Dermatitis. *Contact Dermatitis*. 37, 53-69 (1997)
2. Aquino, R., Morelli, S., Tomaino, A., Pellegrino, M., Saija, A., Grumetto, L., Puglia, C., Ventura, D., Bonina, F. Antioxidant and photoprotective activity of a crude extract of *Culcitium reflexum* H.B.K. leaves and their major flavonoids. *J Ethnopharmacol*. 79, 183-191(2002)
3. Dreher, F., Denig, N., Gabard, B., Schwindt, D.A., Maibach, H.I. Effect of topical antioxidants on UV-induced erythema formation when administered after exposure. *Dermatology*. 198, 52-55 (1999).
4. Hibatallah, J., Carduner, C., Poelman, M.C. In-vivo and in-vitro assessment of the free-radical-scavenger activity of Ginkgo flavone glycosides at high concentration. *J Pharm Pharmacol*. 51, 1435-1440 (1999)
5. Khodr, B., Khalil, Z. Modulation of inflammation by reactive oxygen species: implications for aging
6. Giacomoni, P.U., Declercq, L., Hellems, L., Maes, D. Aging of human skin: review of a mechanistic model and first experimental data. *IUBMB Life*. 49, 259-263 (2000)
7. Fuchs, J., Zollner, T.M., Kaufmann, R., Podda, M. Redox-modulated pathways in inflammatory skin diseases. *Free Radic Biol Med*. 15, 337-353 (2001)
8. Clarys, P., Alewaeters, K., Lambrecht, R., Barel, A.O. Skin colour measurements: comparison between three instruments: the Chromameter, the DermaSpectrometer, and the Mexameter. *Skin Res Technol*. 6, 230-238 (2000)
9. Shriver, M.D., Parra, E.J. Comparison of narrow-band reflectance spectroscopy and tristimulus colorimeter for measurements of skin and hair colour in persons of different biological ancestry. *Am J Phys Anthropol*. 112, 17-27 (2000)
10. Lupo, M.P. Antioxidants and vitamins in cosmetics. *Clin Dermatol*. 19, 467-473 (2001)
11. Wakabayashi, Y. Effect of germanium-132 on low-density lipoprotein oxidation and atherosclerosis in Kurosawa and Kusanagi hypercholesterolemic rabbits. *Biosci Biotechnol Biochem*. 65, 1893-1896 (2001)
12. Pinnell, S.R., Yang, H., Omar, M., Monteiro-Riviere, N., DeBuys, H.V, Walker, L.C., Wang, Y., Levine, M. Topical L-ascorbic acid: percutaneous absorption studies. *Dermatol Surg*. 27, 137-42 (2001)