CRL Clinical Research Laboratories, Inc.

Determination of the Protective Effects of Cosmetic Formulations Against UV Exposure.

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Introduction

Overexposure to sunlight and environmental pollutants along with today's lifestyle play a significant role in the aging process. Photoaging, caused by cumulative ultraviolet radiation, damages the elastin and collagen fibers in the deep layers of the skin causing premature aging.

Use of cosmetic products containing sunscreens, antioxidants and moisturizers may help to protect the skin against premature aging. Sunscreens act by absorbing, scattering or reflecting ultraviolet light. Antioxidants such as vitamins A, E and C and beta-carotene may have photoprotective effects such as their ability to scavenge "free radicals." Free radicals have been implicated in many of the undesirable aspects of skin aging, whether due to intrinsic causes or to exposure to UV radiation. Increasingly, scavengers are incorporated in cosmetic formulations to control the undue proliferation of these potentially damaging species. When combined, antioxidants may enhance the photoaging protective effects of sunscreens. Barrier type moisturizers are formulated using a broad range of lipid-water ratios. Occlusive agents, water-in-oil formulations, emollient lotions and humectants provide a protective effect on the lipid barrier.

The method described is a non-invasive technique developed to evaluate the protective effects of a variety of cosmetic products. This method can be used to assess the protective effects of individual ingredients in a formulation or the end product.

Purpose

The objective was to develop an *in-vivo* method for assessing the protective effects of cosmetic products. *In-vivo* tests of free radical scavenger activity have generally been limited to indirect assays, such as the measurement of Thiobarbituric Acid Reactive Substances (TBARS) formed on the skin of irradiated mice. Historically, bleaching of carotenoids have been used to evaluate the auto-oxidation activity of natural products. The relative effectiveness of scavengers of lipid peroxyl free radicals after application to the skin was assessed by measuring the UVA-bleaching of beta-carotene as a function of the energy exposure.

Methods

Healthy male and female subjects between the ages of nineteen (19) and sixty-four (64) years and of Fitzpatrick skin types I and II were selected.

Fitzpatrick skin type classification is determined by measuring luminance (L*) and yellow (b*) components of the skin with a Chromameter. From the L* and b* values, the Individual Typologic Angle (ITA°) is calculated as follows:

$$ITA° = [ArcTan((L^*-50)/b^*)] \times 180/\pi$$

The calculated ITA° relates to the Fitzpatrick skin type classifications by categorizing the resulting angles within a Cartesian plane, delineated by vectors, which correlate with visual classification of skin color type. The inclusion ranges of each vectorial category is listed by skin type below:

Skin Type Classification		ITA° Angle
I	(Very Light)	56° - 90°
II	(Light)	42° - 55°
III	(Intermediate)	29° - 41°
IV	(Tan)	11° - 28°

Subjects who met the Inclusion Criteria signed an Informed Consent in conformity with 21 CFR Part 50: "Protection of Human Subjects" and completed a Panelist Profile/Medical History Form.

Chromameter (Minolta CR-300)

The Chromameter can be used to measure changes in color by expressing the color of measured surfaces numerically in L*a*b* color space. This system is recommended by the CIE (Commission Internationale de l'Eclairage) for skin color assessment. In this color space, L* is the luminance and gives the relative brightness from total black (L* = 0) to total white (L* = 100). The a* value represents the balance between the reds (positive values) and the greens (negative values). The b* value represents the balance between the yellows (positive values) and the blues (negative values). The b* value most closely describes the intensity of the orange color of the β-carotene stain and is in direct correlation with the Color Index, *I*. The Chromameter provides a means by which oxidative damage caused by free radicals and, conversely, the prevention of oxidation by free radical scavengers, can be measured. Free radical oxidation induced by UVA radiation elicits a bleaching of β-carotene stain. The bleaching and prevention of bleaching by a test material can be measured by the Chromameter.

Bleaching is reported as the change in b* versus UVA Irradiation Energy. It can also be expressed in terms of a Color Index, *I*, defined as:

$$I = \frac{b_n^* - b_i^*}{b_o^* - b_i^*} \times 100$$

where, b_n^* is the value of b* measured after *n* J/cm² of irradiation of the area treated with the cosmetic formulation and painted with the β-carotene; b_o^* is the value of b* after application of the β-carotene but before irradiation; and b_i^* is the value of b* before application of the β-carotene. This index compensates for any effects of skin color on b_n^* and b_o^*.

Procedure

The designated inner forearm of impaneled subjects was cleansed with a 70% isopropyl alcohol prep pad (Medium, Dynarex) and allowed to dry. Test sites were selected on the volar surface of the designated forearm of each subject, with each test site defined by the open, central area of a self-adhesive ring (Professional ProFoot Products™, P.P.R. Co., Inc.). The adhesive side of the rings were placed directly on the skin (Figure 1). The rings are used to retain the test material and β-carotene solution as well as to function as a guide for taking measurements with the Chromameter.

Test materials evaluated were an antioxidant (vitamin E), sunscreen (SPF-15), barrier type moisturizer (Petrolatum) and a combination of all three. The test materials were applied to the forearm according to a randomized schedule. Additional sites were treated with a β-carotene control and an untreated control. Treatment of the test sites were randomized for each subject.

Prior to application of the test materials or β-carotene, the initial b* parameter was measured using the Chromameter and recorded for all duplicate test sites of the forearm (Baseline). Approximately 2 mg/cm² of each test material was applied to the designated test site and spread manually with a finger cot to ensure even distribution. Sites designated as untreated, with or without β-carotene, remained untreated at this time. The test materials and internal control were allowed to incubate with the skin surface for a period of fifteen (15) minutes. After this incubation period, b* measurements were recorded for all sites (Product Baseline) (Figure 2).

A solution of β-carotene in a mixture of capric and caprylic triglycerides, is applied to all treated sites (Figure 3). The b* parameter is again measured and recorded (β-carotene Baseline).

Procedure (Continued)

For this method, long wavelength UVA (320-420 nm) is used because it is less likely to elicit burning of the skin than UVB irradiation (290-320 nm) and is known to contribute to the formation of free radicals.

The subject's forearm is exposed to approximately 1.0 J/cm² of UVA radiation using a sun lamp, and the b* parameter is again measured by the Chromameter and recorded (Figure 4). This phase of the test method is repeated for five (5) additional energy exposures at 2.0, 3.0, 4.0, 5.0 and 6.0 J/cm² of UVA radiation.

The Color Index is calculated for each of the duplicate test material sites.

Figure 1.

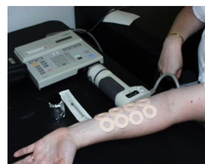


Figure 2.

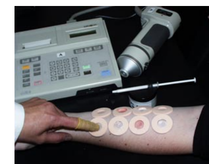


Figure 3.

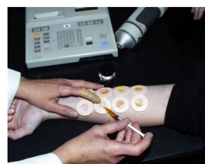
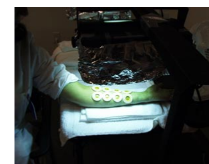


Figure 4.



Preparation of β-Carotene Solution

Thirty (30) milliliters of a saturated solution of beta-carotene in a mixture of capric and caprylic triglycerides (44:55), manufactured by Stepan Company, is measured in a test tube. Approximately 0.70 grams of b-carotene is added to the triglyceride solution and heated over a hotplate to 100°C for approximately two (2) minutes until the mixture becomes a dark orange/red color. The solution is filtered to remove any excess β-carotene and is refrigerated to prevent oxidation. The solution is discarded when evidence of oxidation, changes in color from dark orange/red to light yellow/orange is observed.

Statistical Analysis

Color Index values for sites treated with each test or control material are compared statistically using analysis of variance (ANOVA). Statistical significance exists for all p-values less than or equal to 0.05 at the 95% confidence level. A Dunnett's Test is used to determine the significance of differences between each treatment product and the control at the 95% confidence level.

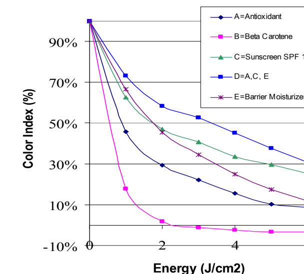
Results

For the purposes of this method development, we chose to evaluate the protective effects of a vitamin (antioxidant), a sunscreen (SPF 15), a barrier-type moisturizer, and a combination antioxidant-sunscreen-barrier moisturizer from the oxidative reaction elicited by UVA radiation. Protective effects of each product were compared to a β-carotene control. The average Color Index Value measured by Chromameter for each product tested at 0, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 J/cm² can be found in Table 1 and Figure 5. All products tested were capable of preventing the bleaching of β-carotene compared to the β-carotene control. However, Product D, the combined antioxidant, sunscreen, barrier moisturizer exhibited a higher Color Index Value than the antioxidant, sunscreen or barrier moisturizer alone. The higher Color Index Value observed for Product D is a consequence of less bleaching of β-carotene. At energy exposures as high as 6.0 J/cm², the combined antioxidant, sunscreen and barrier moisturizer demonstrates a reservoir effect, with properties of free radical scavengers observed without depletion for extended energy exposure.

Table 1. Color Index Value

Energy J/cm2	0	1	2	3	4	5	6
A=Antioxidant	100.00%	45.93%	29.11%	22.11%	15.48%	10.27%	8.56%
B=Beta Carotene	100.00%	17.87%	1.75%	-1.40%	-2.67%	-3.55%	-3.51%
C=Sunscreen SPF 15	100.00%	62.67%	47.03%	40.76%	33.70%	29.70%	25.36%
D=A,C, E	100.00%	73.15%	58.33%	52.50%	45.14%	37.77%	30.67%
E=Barrier Moisturizer	100.00%	66.78%	45.40%	34.46%	24.95%	17.42%	11.93%

Figure 5. Color Index vs. Irradiated Energy



Conclusion

This non-invasive, *in-vivo* method has been demonstrated to be effective in determining a cosmetic product's relative ability to scavenge free radicals by measuring the oxidation of β-carotene. Protective effects of cosmetic formulations can be measured and compared by assessing the UVA bleaching of β-carotene as a function of irradiated energy.

References

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