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Evaluation of antioxidant properties of dermocosmetic creams by direct electrochemical measurements

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Synopsis

Cyclic voltammetry and linear sweep voltammetry were preliminarily used in order to evaluate the global antioxidant properties of dermocosmetic creams. Experiments were performed by introducing electrodes directly into the creams without any pretreatment of the samples. Current-potential curves showed significant anodic current depending on the antioxidant-containing cream studied. In comparison, little amperometric response was recorded with an antioxidant-free cream base. Aqueous solutions of the corresponding antioxidants showed analogous anodic waves and similar peak potentials. A correlation between the global anodic peak and the presence of the antioxidant species in the cream was made with eleven skin creams, attesting to the reliability of the method. Among the tested electrode materials, platinum gave the best results in terms of electrochemical kinetics and measurement precision (current peak standard deviation less than 5%). Exposure of a depilatory cream to oxidizing agents (e.g., hydrogen peroxide, air, or light) caused a decrease in peak current as expected. This methodology enabled us to evaluate the evolution of the total antioxidant capacity under oxidative stress and gives encouragement to further development of a voltammetric method to quantify cream antioxidant power.

INTRODUCTION

Among body tissues particularly involved in aging, skin constitutes one of the major targets, since it is the most exposed to external oxidant agents: UV light, chemical pollutants, oxygen, etc. The symptoms are quite different, ranging from the early appearance of wrinkles to possible skin cancer caused by excessive exposure to sunlight, and including numerous diseases such as psoriasis (1–3). This process and its consequences represent a crucial health problem and also an exponentially grown commercial

Abbreviations: ABTS: 2,2′-azinobis(3-éthylbenzothiazolinesulfonate). BHA: tert-butyl-4-methoxyphenol. BHT: butyl-hydroxy-toluene. EDTA: ethylenediamine-tetraacetic acid. FRAP: ferric-reducing ability of plasma. MSE: mercurous sulphate electrode. PEG: polyethyleneglycol. ROS: reactive oxygen species. TEAC: Trolox-equivalent antioxidant capacity.

market for the cosmetic industry, since an increasingly aging population takes care of a healthy appearance, trying to "turn back the clock": more than 30% of the personal care market in 1999 concerned the skin care segment, with almost $20 billion worldwide (4).

One of the main causes of aging is a deregulation of the cell respiratory metabolism involving incomplete oxygen reduction with production of superoxide anion $O_2^{\cdot -}$, hydroxyl radical $OH^{\cdot}$, hydrogen peroxide H_2O_2, etc. (5–7). Skin has different antiradical defense systems to regulate these reactive species, namely enzymes and low-molecular-weight antioxidants (8–10). Defection of these preventive mechanisms or excessive production of reactive oxygen species induces so-called oxidative stress (11,12).

Numerous methods are available to determine total antioxidant capacity, based on inhibition reactions in solution involving a specific oxidant reagent like Fe^{3+} (FRAP assay) or ABTS (TEAC assay) (13–16). In this field, electrochemistry appears to be a convenient approach to evaluate the overall antioxidant properties of cosmetics. Recently cyclic voltammetry has been shown to be a suitable electrochemical method to evaluate the global antioxidant capacity of real samples like edible plants, plasma, or wine (17–19): the oxidation signal traduces the ability of the medium to give electrons, i.e., to scavenge the ROS by reducing them.

This paper presents preliminary work allowing the evaluation of the total antioxidant properties of a dermocosmetic product by means of electrochemical techniques performed directly on the bulk of the cream, without any pretreatment of the sample. Cyclic and linear sweep voltammetry were used to show the overall antioxidant capacity of the product. Comparison of the voltammograms with those recorded with antioxidants containing aqueous solutions allowed us to correlate the electrochemical characteristics of the samples with the properties of redox species. Finally, the influence of irradiation and oxygen, as well as the addition of hydrogen peroxide as an oxidant on the global redox status of a depilatory cream, was highlighted.

PRINCIPLES OF VOLTAMMETRY (20)

Cyclic voltammetry has been one of the most frequently used electrochemical methods for more than three decades. The reason is its relative simplicity and its high information content. Cyclic voltammetry is performed using potentiostatic equipment in experimental conditions such as that the only mass transport phenomenon to be taken into account is semi-infinite diffusion. Generally, the working electrode on which the oxidation and reduction reactions are studied is flat so that the mass transport may be considered as unidirectional. The solution contains an electrolyte in large excess compared to the concentration of the electroactive species; the ions of the electrolyte are not involved in the electron transfer reaction at the electrode. This electrolyte decreases the internal cell resistance and enables us to neglect the migration phenomena of the charged electroactive species. There is no forced convection because the electrodes are fixed and the solution is not stirred. Furthermore, the relatively short experimental time scales allow the natural convection to be neglected. The electrode surface area, the volume of the solution, and the concentrations of the electroactive species are such that the experimental determination of the oxidation and reduction current (proportional to the heterogeneous electron transfer rate) change the electroactive species concentration in a negligible way. The waveform of the voltage applied to the working electrode versus the

reference electrode is triangular. The voltage varies linearly with time and the slope is referred to as the scan rate. In a few seconds, a current density–potential curve can be obtained, giving information about the energy level necessary to perform the electrode reactions and the rate of these reactions. If the heterogeneous electron transfer rate is high compared to the diffusion rate, this curve presents a peak, whose current is proportional to the concentration of the electroactive species present in solution and to the square root of the scan rate. Another important criterion to characterize the electrode reaction is the value of the peak potential, which is independent of the scan rate if the electron transfer is fast in relation to diffusion. If the product of an electrochemical reduction reaction may be reoxidized during the reverse scan rate, the reduction peak is accompanied by an oxidation peak and the difference between the peak potentials is an indication of the reversibility of the heterogeneous electron exchange reaction. Cyclic voltammetry is a very useful method to elucidate the mechanism of electrode reactions in the case where the heterogeneous electron transfer is accompanied by chemical reactions occurring before or after the electrode reaction. In the present work, cyclic voltammetry is used to compare creams on the basis of the determination of the peak potential, the peak current intensity, and the charge involved in the electro-oxidation process.

MATERIALS AND METHODS

H_2SO_4, NaOH, and thiolactic acid were purchased from Acros; K_2SO_4 and H_2O_2 from Sigma; and KH_2PO_4 and K_2HPO_4 from Merck. Unless otherwise indicated, all solutions were prepared in potassium sulphate solution (0.4 mol/l^{-1}, pH = 11.0).

Creams were either commercially available or made in Pierre Fabre's laboratory. In the later case the samples were gels containing PEG 600, carbopol 980, paraffin, cremophor RH40, sorbic acid, nipagin, sodium hydroxide, and water. Three gels were made: one with pH = 4.86, one with pH = 6.88 and one containing BHT, with pH = 6.95. A depilatory cream from Klorane was chosen as an example. Others creams were studied: a restructuring cream from Nivea Vital; a corrective dermatological cream for wrinkles, Active C, from Laroche-Posay; a depigmenting emulsion, Trio D, from Laboratoires d'Evolution Dermatologique; a whitening day cream from Decleor; an emulsion, Ysthéal +, from Laboratoires Dermatologiques Avène (Pierre Fabre); an epithelial cream, A Derma, from Laboratoires Dermatologiques Ducray, and an after-sun repair balm, Uriage, from Laboratoires Dermatologiques Uriage.

All the electrochemical experiments were carried out in a single compartment cell at room temperature. An airtight cell was used to study the influence of oxidative stress. An air or nitrogen flux was introduced when necessary, and the flow was controlled with a flow meter: Brooks tube (R-2-15-AAA P-072; float: sapphire; scale: 0-5 l/h). The electrochemical manipulations were performed with an Autolab Metrohm potentiostat interfaced to an HP omni-book XE 4500 microcomputer and using the GPES software. The working electrodes were platinum (0.03 cm^2), gold (0.07 cm^2), or vitreous carbon (0.07 cm^2) rotating-disc electrodes. A large-surface-area platinum grid was used as counter electrode. All potentials were measured and expressed in reference to a saturated mercurous sulphate reference electrode $Hg/Hg_2SO_4/K_2SO_{4sat}$ (MSE; E = 0.656 V/SHE) connected to the cell by a Luggin capillary. Before each experiment, the working electrode surface was polished with abrasive paper (262X imperial lapping film sheets)

and rinsed with distilled water. For vitreous carbon, anodic polarization was then imposed at 1 V during one minute in sulphuric acid (H_2SO_4, 0.1 mol/l^{-1}). For the platinum (respectively gold) electrode, cyclic voltammograms were performed in H_2SO_4, 0.5 mol/l^{-1} (resp. 0.1 mol/l^{-1}) at 50 mV s^{-1} between −0.65 V and 0.75 V (resp. between −0.25 V and 1.1 V) until reproducible current density-potential curves (21) were obtained. In all the electrochemical experiments, the potential range was chosen according to the limits of the electroactivity domain of the solvent, in order to avoid the oxidation/reduction of water.

RESULTS AND DISCUSSION

CYCLIC VOLTAMMOGRAM IN THE CREAMS

Figure 1 shows the experimental device used. Experiments were performed by simply introducing the working, counter, and reference electrodes directly into the creams. A few grams (about 5 to 7 grams; depending on the cream density) were necessary, in order for the electrodes to be in contact with the cream. Figure 2 shows cyclic voltammograms obtained with a platinum electrode introduced directly into two samples, e.g., a depilatory cream from Klorane (pH = 11) containing BHA and thiolactic acid as antioxidants (solid line) and an antioxidant-free base made in Pierre Fabre's laboratory (dashed line). In both cases, the curve presented a conventional shape with essentially no resistive or capacitive current. In the former case, a significant anodic current was recorded with a peak potential close to 0.31 V. Comparatively, only a little amperometric response was obtained with the other cream. The difference can therefore be attributed to the presence

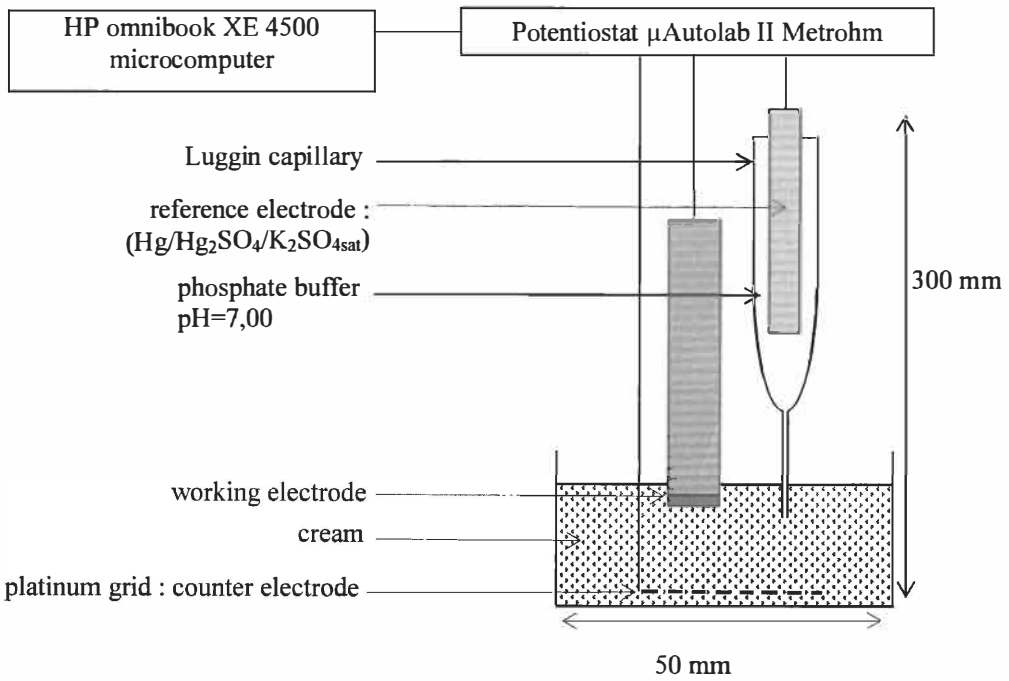

Figure 1. Experimental device used. The three-electrode system was introduced directly into the cream.

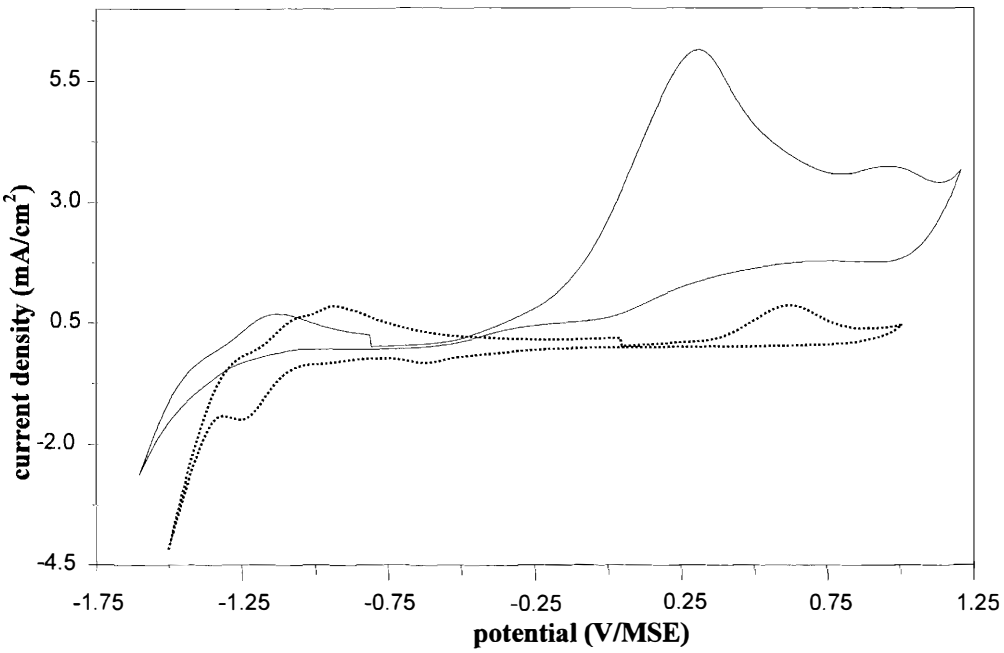

Figure 2. Cyclic voltammograms obtained with platinum electrode introduced into the bulk of the cream: Depilatory cream (—); cream containing no antioxidants (· · · ·). Potential scan rate: 50 mV/s^{-1}.

of antioxidant species in the depilatory cream. Figure 3 shows linear sweep voltammograms performed with the same electrodes immersed in a potassium sulphate solution (pH = 11) containing BHA ($5.5 \cdot 10^{-5}$ mol/l^{-1}) or thiolactic acid ($5 \cdot 10^{-4}$ mol/l^{-1}).

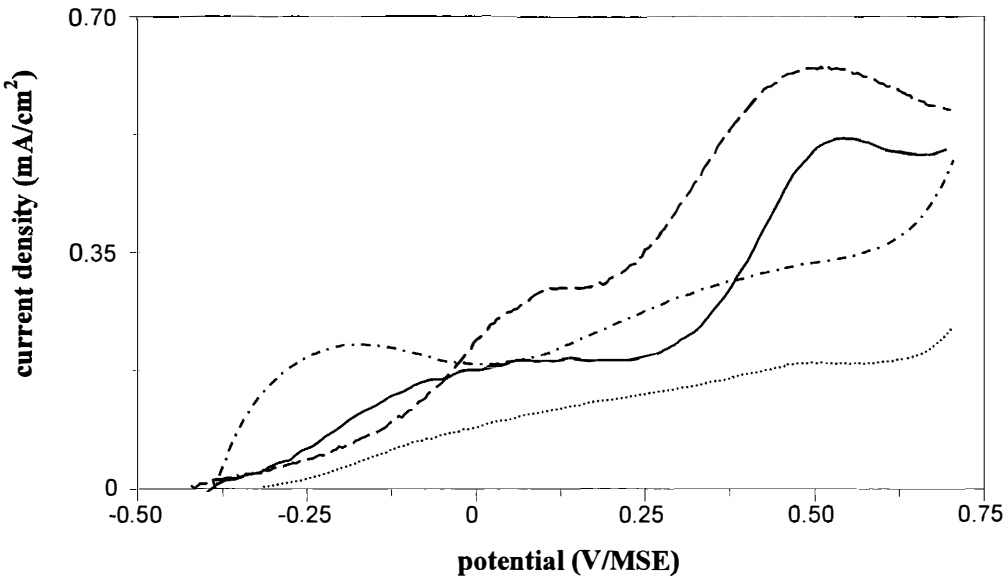

Figure 3. Linear sweep voltammograms obtained with platinum electrode immersed in potassium sulphate solution [0.4 mol/l^{-1}, pH = 11.0 (· · · ·)] containing H_2O_2, $1.96.10^{-3}$ mol/l^{-1} (— · —); thiolactic acid, $5.00 \cdot 10^{-4}$ mol/l^{-1} (– –); and BHA, $5.55 \cdot 10^{-5}$ mol/l^{-1} (——). Potential scan rate: 50 mV/s^{-1}.

The pH was chosen to be in accordance with that of the depilatory cream. Both curves presented an anodic wave. Although the nature and the physicochemical properties of a cream and an electrolytic solution were quite different, comparison of potentials obtained in the two media clearly demonstrated that antioxidant species like thiolactic acid and BHA could be detected in a dermocosmetic cream by a simple electrochemical method. Cyclic voltammetry appeared therefore suitable to evaluate the antioxidant global capacity of creams, similar to what was concluded by Chevion and Chevion (17) and others (18,19) from experiments performed in plasma or plants.

The voltammograms recorded in the depilatory cream (Figure 2, solid line) showed no cathodic current, except that corresponding to the reduction of water appearing at a potential below −1.2 V. Consequently, the cream did not contain any oxidant species that could be detected electrochemically under the adopted operational conditions. In contrast, the curve obtained with the antioxidant-free base (Figure 2, dashed line) revealed several cathodic peaks. In particular, an amperometric response was recorded with a half-wave potential near −0.3 V, corresponding to the reduction of oxygen. Korotkova *et al.* (18) demonstrated the diminution of the oxygen reduction current on a platinum electrode immersed in aqueous solution containing antioxidant species. Actually dissolved oxygen was contained in the cream sample prepared without antioxidant species since an upper potential boundary, i.e., 1.1 V, was chosen in order to avoid oxidation of water into oxygen at the electrode surface during experiments. These last results confirm that cyclic voltammetry is a suitable method to reveal directly the presence of antioxidant species in the cream and to evaluate its antioxidant properties without any pretreatment of the sample.

Table I shows the electrochemical characteristics of cyclic voltammograms obtained in the depilatory cream by using different electrode materials. Although antioxidant species are generally studied with a carbon electrode (22,23), the best results were obtained in our case with platinum. Not only the peak current was higher, but also the peak potential presented the lowest value, indicating that the electron transfer kinetics between platinum and the electroactive species was faster. For each electrode, the experiment was repeated at least six times. Between each experiment, the electrode surface was regenerated (see Materials and Methods) and a new cream sample was used. In all cases the standard deviation was at most 10% for the peak current and 70 mV for the peak potential.

Adopting this protocol, eleven creams were studied. The conductivity of the samples, the peak potential and current density, and the amount of charge consumed in the anodic part of the curve were measured. The results are listed in Table II. First, electrochemical measurements were possible, provided the conductivity differed from zero. Second, an

Table I
Influence of Electrode Material on Anodic Peak Current Density and Potential of Cyclic Voltammogram Obtained in Depilatory Cream

Electrode material	i_{peak} (mA cm^{-2})	E_{peak} (V/MSE)	Number of measurements
Platinum	6.0 ± 0.3	0.29 ± 0.04	6
Vitreous carbon	5.2 ± 0.3	0.38 ± 0.06	10
Gold	5.4 ± 0.6	0.42 ± 0.07	7

All electrodes were cleaned as described in Materials and Methods.

Table II
Values of Conductivity and Anodic Peak Current Density, Charge, and Potential Recorded with 11 Antioxidant-Containing Creams or Free Bases

Cream	Antioxidant species	Conductivity (10^{-3} Ω^{-1}/cm^{-1})	Charge (mC)	Reproducibility	
				Potential (V/ESM)	Current density i_p (mA/cm^{-2})
Cream (pH = 4.86)[1]	No	1.5	0.16 (4%)[3]	0.71 ± 0.02	0.78 (4%)
Cream (pH = 6.88)[1]	No	3.1	0.18 (6%)	0.59 ± 0.02	0.81 (4%)
Cream (pH = 6.95)[1]	BHT	3.3	0.21 (16%)	0.60 ± 0.02	0.95 (13%)
Depilatory cream (Klorane)	Thiolactic acid, BHA	3.4	3.52 (3%)	0.29 ± 0.04	6.0 (5%)
Anti-aging cream (Nivea Vital)	Ascorbic acid, BHT, tocopheryl acetate	1.6	2.3 (16%)	0.34 ± 0.07	3.1 (13%)
Corrective dermatological care for wrinkles (Active C)	Ascorbic acid	0.0	0.0	No voltammogram recorded	No voltammogram recorded
Depigmenting emulsion (Trio D[LED])	Ascorbic acid polypeptide, tocopherol	8.6	0.23 (25%)	0.70[2]	0.6 (30%)
Whitening day cream (Decleor)	Tocopheryl acetate	0.4	0.24 (16%)	0.91 ± 0.06	0.51 (13%)
Ysthéal emulsion	BHT retinal, tocopheryl glucoside	0.9	0.35 (16%)	0.87 ± 0.03	0.9 (15%)
Epithelial cream (A Derma)	Retinol, tocopheryl acetate	0.1	0.133 (5%)	1.20[2]	0.25 (4%)
After-sun repair balm (Uriage)	No	3.7	0.24 (9%)	0.70 ± 0.03	0.76 (12%)

[1] Creams made in Pierre Fabre Laboratory (see Materials and Methods).
[2] No peak potential was observed. The standard deviation was calculated from the current at the indicated potential, where a significant anodic wave was observed.
[3] Values in parentheses represent the standard deviation, evaluated with at least six samples.

anodic peak current was recorded with most of the samples. The corresponding peak potential was correlated to the presence of one or several antioxidant species. Furthermore, the amount of charge recorded with the antioxidant-containing creams (e.g., 3.52 mC for the depilatory cream) compared to that obtained with the free bases (less than $2 \cdot 10^{-4}$ C) makes evidence in the first case of a global antioxidant power. Finally, the protocol gave a good reproducibility, with a standard deviation of the peak current density less than 15% in most cases. All these results consequently attest to the reliability of the method.

The influence of the potential scan rate on the electrochemical data was also investigated. The evolution of the peak current density recorded with the depilatory cream is proportional to the square root of the scan rate: $i_p = 25.11/r^{1/2} + 0.1644$. $R^2 = 0.997$. This linear variation suggested that the electrochemical reaction is mass transport controlled (20). Likewise, a direct proportionality was established between the peak potential and the logarithm of the scan rate: $E_p = 0.123/\ln r + 0.71$, $R^2 = 0.97$. These results were in accordance with the Randles, Sevcik, Nicholson, and Shain equation (20) in the case of an irreversible electrochemical system and were analogous to those commonly observed in an electrolytic solution. It might therefore be concluded that the electrochemical response recorded in the cream was not disturbed by subsequent ohmic drop.

ADDITION OF HYDROGEN PEROXIDE AS OXIDANT

Different volumes of a hydrogen peroxide (H_2O_2) solution, added with a micropipette, were manually mixed with a sample of the depilatory cream until the mixture appeared homogenous. This species was added in order to simulate the action of a strong oxidant on the redox properties of the cosmetic product. Schöberl and Wiesner (24) showed that hydrogen peroxide oxidized thiolactic acid with kinetics depending on the concentration of H_2O_2. Figure 4 shows the evolution of the cyclic voltammogram recorded in the cream as a function of the amount of hydrogen peroxide added. Cyclic voltammograms were recorded immediately after the addition of hydrogen peroxide. When the concentration of H_2O_2 was less than 25 µmol per gram of cream, the cyclic voltammogram of the cream was roughly unchanged. In this case, the whole quantity of hydrogen peroxide was consumed by antioxidants. For concentrations of H_2O_2 ranging from 25 to 500 µmol/g, the anodic peak current at 0.31 V gradually decreased, indicating a consumption of the antioxidants. At the same time, a second anodic peak appeared at a potential close to 0.9 V. This new current cannot be attributed to the presence of an excess of hydrogen peroxide in the cream. As a matter of fact, the peak potential did not correspond to that classically recorded for the oxidation of H_2O_2 on a platinum electrode. Figure 3 clearly shows that H_2O_2 oxidation began at around −0.4 V. Moreover, Figure 4 reveals no current corresponding to the reduction of H_2O_2. This new anodic response can rather be attributed to the presence of one or several products resulting from the previous electrochemical and/or chemical oxidation of the antioxidant species. Finally, for concentrations of H_2O_2 more than 1000 µmol/g, the cyclic voltammogram presented the electrochemical characteristics of hydrogen peroxide, i.e., an anodic current with peak potential close to −0.25 V and a cathodic wave appearing from −0.5 V. The whole antioxidant species were then oxidized, and hydrogen peroxide was no longer consumed. It was verified that both amperometric responses increased with further successive additions of hydrogen peroxide. All these results highlight the efficiency of cream to

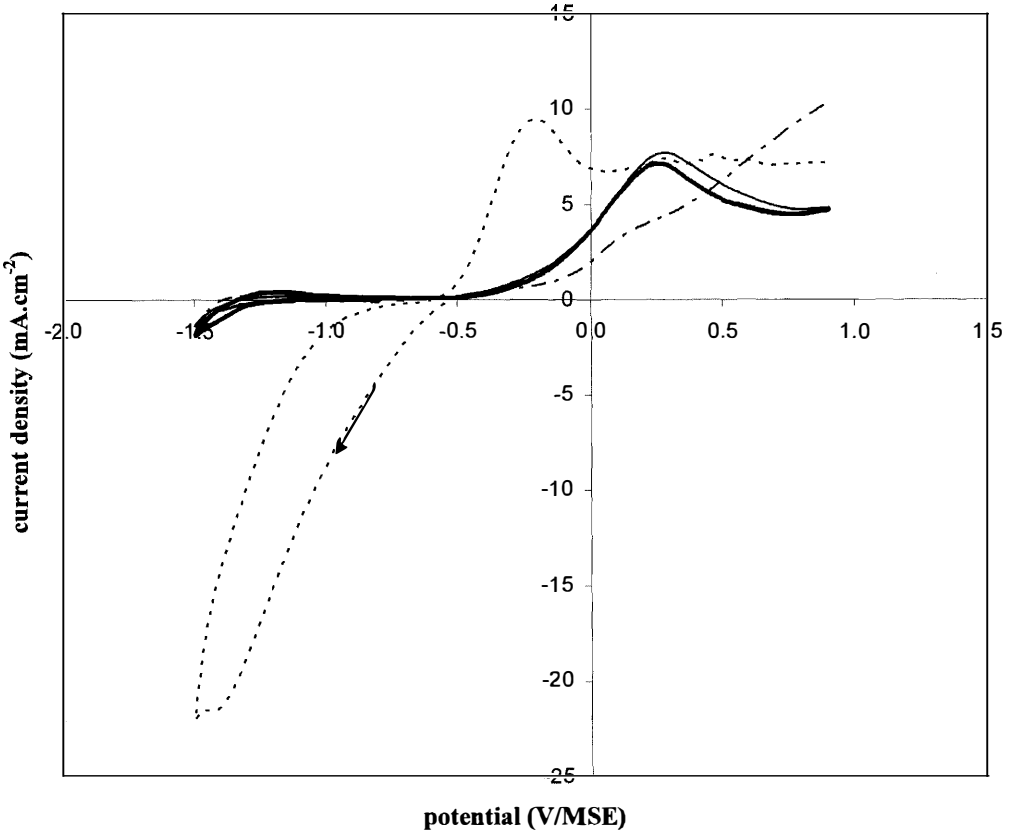

Figure 4. Linear sweep voltammograms obtained with platinum electrode introduced in the bulk of the depilatory cream containing 0 (——), 10 (—), 361 (— · —), and 1246 (- - -) µmol of H_2O_2 per gram of cream. Potential scan rate: 50 mV/s^{-1}.

scavenge significant amounts of hydrogen peroxide as a reactive oxygen species. They also point to the possibility of further developing the method in a way to measure quantitatively the amount of reduced species that show efficient antioxidant activity, expressed by an equivalent of hydrogen peroxide added to consume them.

INFLUENCE OF OXYGEN AND NATURAL LIGHT

In order to evaluate the stability of the cream, the influence of two sources of "natural" oxidative stress—oxygen in air and natural light—on its antioxidant properties was studied. An air or a nitrogen flux was maintained in the electrochemical cell containing the cream sample, and the experiment was performed in the dark or in daylight. Cyclic voltammograms were performed at the surface of the cream, and the anodic peak current was recorded as a function of time. Figure 5 represents the evolution of the anodic peak current (ip) compared to the initial peak current (ip_0) for the different experimental conditions.

First, results revealed in all cases a general decrease in the ratio ip/ip_0 with time. This evolution was observed even when the cream was protected from light and oxygen. In

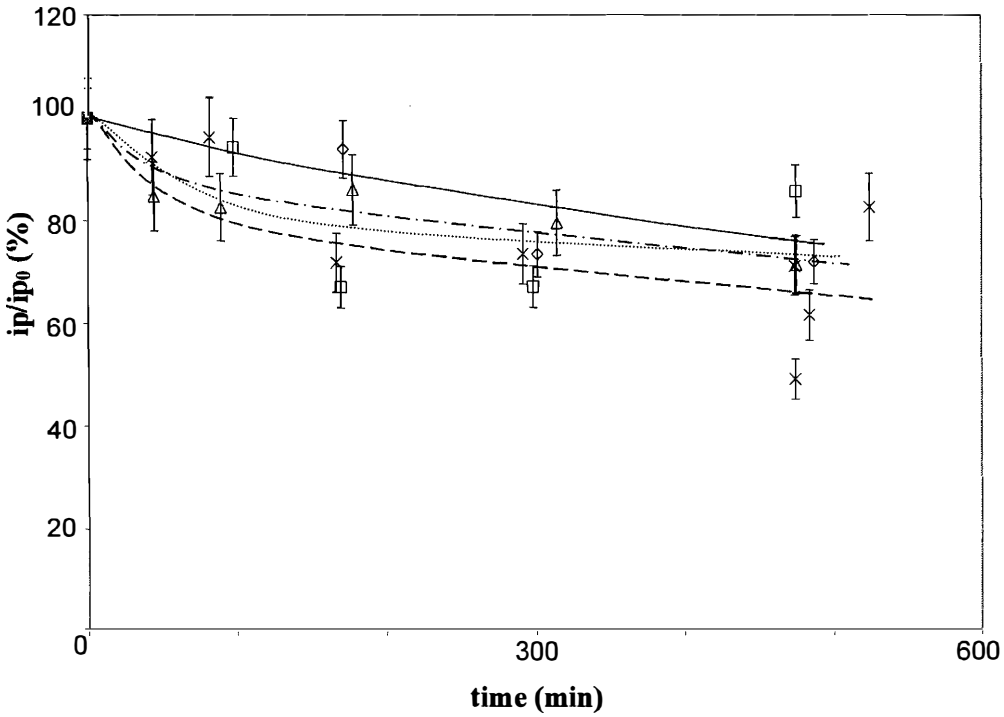

Figure 5. Influence of natural light and/or air on the global antioxidant capacity of a depilatory cream. Evolution of the ratio (peak current density)/(initial peak current density) as a function of time (working electrode: platinum disk; potential scan rate: 5● mV/s): — ◇; exposed to nitrogen and protected from light; · — · △, exposed to air and protected from light; . . . □, exposed to nitrogen and light; – – x, exposed to air and light.

this latter case, the decrease of 20% in the peak current density after 500 min cannot be due to oxidative stress. It can better be assumed that the surface of the cream dried, resulting in the modification of the diffusion properties and a decrease in the electrochemical reaction rate. However, this phenomenon, if correct, remained the same for all the conditions studied because the flux was controlled by a flow meter (flow: 1 l/h). Second, the comparison of the different curves highlights an influence of oxygen and light on the antioxidant properties. An important decrease in the antioxidant properties was observed when the cream was exposed to oxidative stress; the fastest decrease was recorded for the creams exposed to daylight. The loss of the antioxidant properties of the cream exposed to light and nitrogen was 30%; in the case where the cream was exposed to oxygen only, it was 25% (compared to 20% when the cream was protected from oxidative stress). Consequently, daylight and oxygen played an important role in the loss of the antioxidant properties, but the influence of daylight appeared more significant. However, the most important decrease was observed when the cream was exposed to both factors (air and daylight) simultaneously: 40% of its antioxidant properties was lost after 500 min. Otherwise, the modification of the cream properties was mainly situated at the surface of the cream (data not shown).

CONCLUSION

Cyclic voltammetry and linear sweep voltammetry are reliable methods to evaluate the

effective global antioxidant power of dermocosmetic creams. The protocol allowed a simple, rapid, precise and direct determination of the redox properties of eleven antioxidant-containing or antioxidant-free creams without any pretreatment of the sample. The method made it also possible to highlight the evolution of the antioxidant capacity under oxidative stress. This preliminary study gives opportunity to further develop the method in a way to quantitatively determine the cream antioxidant titer.

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REFERENCES

(1) P. U. Giacomoni, L. Declercq, L. Hellemans, and D. Maes, Aging of human skin: Review of a mechanistic model and first experimental data, *IUBMB Life,* **49,** 259–263 (2000).
(2) A. Favier, Le stress oxydant: Intérêt de sa mise en évidence en biologic médicale et problèmes posés par le choix d'un marqueur, *Ann. Biol. Clin.,* **55,** 9–16 (1997).
(3) W. A. Pryor, Oxy-radicals and related species: Their formation, lifetimes and reactions, *Ann. Rev. Physiol.,* **48,** 657–667 (1986).
(4) M. S. Reisch, Turning back the clock, *Chem. Eng. News,* **6,** 15–19 (2000).
(5) A. R. Cross and O. T. G. Jones, Enzymic mechanisms of superoxide production, *Biochim. Biophys. Acta,* **1057,** 281–298 (1991).
(6) B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine* (Oxford University Press, Oxford, 1989).
(7) B. Halliwell, Reactive oxygen species in living systems: Source, biochemistry and role in human disease, *Am. J. Med.,* **91,** 14S–22S (1991).
(8) J. J. Thiele, F. Dreher, and L. Packer, Antioxidant defense systems in skin, *J. Toxicol., Cutan. Ocul. Toxicol.,* **21,** 119–160 (2002).
(9) A. Bast, G. R. M. M. Haenen, and C. J. A. Doelman, Oxidants and antioxidants: State of the art, *Am. J. Med.,* **91,** 2S–13S (1991).
(10) H. Sies, Oxidative stress: From basic research to clinical application, *Am. J. Med.,* **91,** 31S–38S (1991).
(11) B. Halliwell and S. Chirico, Lipid peroxidation: Its mechanism, measurement, and significance, *Am. J. Clin. Nutr.,* **57,** 715S–725S (1993).
(12) C. G. Cochrane, Cellular injury by oxidants, *Am. J. Med.,* **91,** 23S–30S (1991).
(13) R. L. Prior and G. Cao, In vivo total antioxidant capacity: Comparison of different analytical methods, *Free Rad. Biol. Med.,* **27,** 1173–1181 (1999).
(14) C. A. Rice-Evans, Measurement of total antioxidant activity as a marker of antioxidant status in vivo: Procedures and limitations, *Free Rad. Res.,* **33,** S59–S66 (2000).
(15) A. Janaszewska and G. Bartosz, Assay of total antioxidant capacity: Comparison of four methods as applied to human blood plasma, *Scand. J. Clin. Lab. Invest.,* **62,** 231–236 (2002).
(16) M. A. M. Shehata, S. M. Tawakkol, and L. E. A. Fattah, Colorimetric and fluorimetric methods for determination of panthenol in cosmetic and pharmaceutical formulation, *J. Pharm. Biomed. Anal.,* **27,** 729–735 (2002).
(17) S. Chevion and M. Chevion, Antioxidant status and human health: Use of cyclic voltammetry for the evaluation of the antioxidant capacity of plasma and of edible plants, *Ann. N.Y. Acad. Sci.,* **899,** 308–325 (2000).
(18) E. I. Korotkova, Y. A. Karbainov, and A. V. Shevchuk, Study of antioxidant properties by voltammetry, *J. Electroanal. Chem.,* **518,** 56–60 (2002).
(19) P. A. Kilmartin, H. Zou, and A. L. Waterhouse, A cyclic voltammetry method suitable for characterizing antioxidant properties of wine and wine phenolics, *J. Agr. Food Chem.,* **49,** 1957–1965 (2001).

(20) A. J. Bard and L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications* (Masson. Paris, 1983), pp. 239–271.
(21) J. O. M. Bockris, B. E. Conway, S. Sarangapani, and E. Yeager, *Comprehensive Treatise of Electrochemistry* (Plenum Press, New York, 1984).
(22) E. M. Strochkova, Ya. I. Tur'yan, I. Kuselman, and A. Shenhar, Simultaneous voltammetric determination of uric and ascorbic acids in urine, *Talanta,* **44,** 1923–1928 (1997).
(23) Y. Ni, L. Wang, and S. Kokot, Voltammetric determination of butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate and tert-butylhydroquinone by use of chemometric approaches, *Anal. Chim. Acta,* **412,** 185–193 (2000).
(24) A. Schöberl and M. Wiesner, Oxidation of thiolactic acid with hydrogen peroxide, *Ber. Dtsch. Chem. Ges. B,* **65,** 1224–1226 (1932).