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面皰治療製品中維生素 A 酸與化妝品中維生素 B_5 測定方法之研究

Determination of Retinoic Acid in Acne Products and Panthenol in Cosmetics

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Simultaneous determination of retinal, retinol and retinoic acid (all-*trans* and 13-*cis*) in cosmetics and pharmaceuticals at electrodeposited metal electrodes

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關鍵詞：視網醛、視網醇、反式與順式維生素 A 酸、電沉積金屬。

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Simultaneous determination of retinal, retinol and retinoic acid (all-*trans* and 13-*cis*) in cosmetics and pharmaceuticals at electrodeposited metal electrodes

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Abstract

The electrochemical behaviors of retinoids on glassy carbon and electrodeposited metal electrodes were investigated in various aqueous or nonaqueous containing supporting electrolytes, respectively. Retinal, retinol, all-*trans*-retinoic acid (all-*trans*-RA) and 13-*cis*-retinoic acid (13-*cis*-RA) can be separated at the thin-film lead deposited glassy carbon electrode in 0.1 M tetrabutylammonium hydroxide. The electroreduction process is applied for the simultaneous quantitative determination of retinoids in cosmetics and pharmaceuticals. Comparison with results obtained from high performance liquid chromatography shows good agreement. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Retinal; Retinol; Retinoic acid; Electrodeposited metal electrodes

1. Introduction

Research with retinoids and clinical observations in humans sometimes gives apparently contradictory results. Tretinoin (all-*trans*-retinoic acid, all-*trans*-RA) and isotretinoin (13-*cis*-retinoic acid, 13-*cis*-RA) are widely used in treating cystic acne and certain disorders of keratinization. However, tretinoin has been shown to accelerate ultraviolet (UV) radiation-induced carcinogenesis in the skin of hairless mice [1–3] and improve photoaging in human skin [4]. Different molecular forms of vitamin A have different physiological actions. In vision, the functional form is retinal. Retinol is necessary for the growth and maintenance of epithelial tissues and for reproduction [5]. Recently, retinoid therapy has been considered for chemoprevention of sunlight-induced skin cancers in humans [6]. Unfortunately, retinoids are shown to be potent teratogens in animals and humans [7]. Retinol levels might be disturbed during liver and renal diseases. Retinol, the purest form of vitamin A, is proven to be one of the most effective ingredients to reduce the visible signs of aging in cosmetics. But some ordinary retinols can irritate skin. The Chinese cosmetic law allows maximum limits of 0.05% and 2500 IU for retinoic acid and retinol. It is therefore important to measure the levels of retinoids in biological samples and in anti-aging cosmetics.

The analytical methods for retinoic acid determination include colorimetry [8], gas chromatographic-mass spectrometry [9], derivative spectrophotometry [10] and liquid chromatography [7,11–18]. Analytical methods for retinol and retinal have been developed by using high-performance liquid chromatography

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(HPLC) on biological samples [19–31]. However, there were only two methods applied in cosmetics and pharmaceuticals which include spectrophotometry [10] and liquid chromatography [17]. The spectrophotometry is not suitable for commercial samples assay due to other components totally or partially overlapping the analyte signal. With the HPLC methods, all-*trans*-RA could not be separated from the 13-*cis*-RA and other impurities, and often required several chromatographic conditions in order to analyse commercial samples. In this work, the electrochemical behaviors of retinoids using a bare glassy carbon electrode (GCE) and thin-film metal deposited GCE has been investigated in various alkaline solutions by differential pulse voltammetry (DPV). The optimum experimental conditions for the simultaneous determination of retinoid-containing cosmetics and pharmaceutical samples are described in this paper.

2. Experimental

2.1. Apparatus

All electrochemical experiments were performed using an EG&G Princeton Applied Research (Princeton, NJ) 384B and 253 polarographic analyser connected to a Metrohm 628 rotating disk and a Houston Instrument (Austin, TX) Hiplot DMP-40 plotter, respectively. A three-electrode system was employed, consisting of a working electrode (GCE and M^{+n}/GCE), a platinum counter and a saturated calomel electrode (SCE) reference electrode.

The HPLC system consisted of a Model 576 pump (Gasukuro Kogyo, Japan), a Model 7125 injector equipped with a 20 μl sample loop and a Model 502 U spectrodetector. Chromatograms and peak areas were obtained with a SISC Chromatogram Data Integrator. Absorbance measurements were recorded with Cary UV–VIS spectrophotometer (Varian Australia Pty Ltd). Matched quartz cells with 1 cm path length were used to hold all solutions for measurements.

2.2. Reagents

All-*trans*-RA and all-*trans*-retinol were purchased from TCI (Tokyokasei Co., JP) and Acros Organics (Geel Belgium, NJ), respectively. 13-*Cis*-RA and 13-*cis*-retinal were purchased from Aldrich (Milwaukee, WI). All other chemicals were of analytical reagent grade. Samples of anti-acne gel and cream and anti-wrinkle cream were bought from a number of retail outlets in the south of Taiwan.

2.3. Laboratory precautions

All the assay procedures were carried out in a dark room, provided with a red lamp to avoid photodegradation of retinoids. The glassware were protected with tin foil. Flasks containing stock solutions were flushed with nitrogen and stored at −20°C. Standards were stored at −16°C.

2.4. Procedure

2.4.1. Determination of retinoids by DPV

The thin-film metal electrode was produced by the following method, prior to analysis, the glassy carbon electrode (3 mm diameter) was mirror polished sequentially with aqueous suspension of 1.0, 0.5 and 0.05 μm alumina. The electrode was rinsed with deionized water and electrolytically plated with copper (1.0×10^{-3}–2.0×10^{-3} M), mercury (8.0×10^{-4}–2.0×10^{-3} M) and lead (5.0×10^{-4}–2.0×10^{-3} M) metal ions from 10 ml of 0.1 M sulfuric acid, 0.1 M perchloric acid and 0.1 M acetate buffer (pH 4.5), respectively. Plating time was 4 min with a potential scan from −0.8 to 0.0 V at 1500 rpm.

The buffer (pH 8.76) was made by mixing 0.1 M solutions of ammonia and ammonium chloride in deionized water. Briton and Robinson buffer (pH 10.26, 11.78) were prepared by mixing 0.5 M solutions of phosphoric acid, boric acid, acetic acid and 0.2 M sodium hydroxide solution. Comparative tests of various pH and supporting electrolytes were taken for retinoids in a phosphate buffer (pH 2.6 and 6.2), 0.1 M ammonia, Briton and Robinson buffer and methanol or water containing various salts such as sodium perchlorate, lithium perchlorate, tetraethylammonium perchlorate, tetraethylammonium tetrafluoroborate, tetrabutylammonium perchlorate and tetrabutylammonium hydroxide solution.

A 1.0 g amount of cosmetic and pharmaceutical samples was accurately weighed, dissolved in 10 ml of isopropanol and methanol (1:1, v/v) and mixed with vertex treatment for 10 min. After centrifuging, the supernatant was transferred into a 10 ml amberized calibrated flask and made up to a volume with methanol. In order to obtain calibration graphs for the retinoids, 10 ml of supporting electrolyte were pipetted into a voltammetric cell and de-aerated with nitrogen for 4 min before voltammetric measurement. By micropipette, aliquots of 1000 ppm retinoid solution were added. After each addition voltammograms were obtained; the solution de-aerated for 2 min after each addition before obtaining the voltammogram. Quantitative analyses were performed in the differential pulse mode. The potential was set at 0.0 to 0.80 V and −1.0 to −1.8 V versus saturated calomel electrode (SCE) for oxidation and reduction. The pulse height was 50 mV, and the scan rate 4 mV s^{-1} with a film electrode. For sample solution analysis, 1 ml of the solution was pipetted into a 10 ml amberized calibrated flask and diluted to volume with tetrabutylammonium hydroxide solution. This solution was analysed by DPV using the same condition as for calibration graph.

2.4.2. *Determination of retinoids by HPLC*

Stock solution of standards were prepared by dissolving the appropriate amount of retinoids in methanol. A set of standard solutions were produced by diluting aliquots of the stock solutions with methanol to 10 ml in amberized calibrated flasks. Taking into account about the retinoids content of the anti-acne creams and gels, anti-wrinkle creams and skin nourishing creams, samples (approx. 0.1–0.5 g) of the latter were weighed accurately in a 15 ml beaker, diluted to about 1 ml with dichloromethane and 9 ml methanol, dissolved and centrifugated. The supernatant was transferred into a 10 ml amberized calibrated flasks. An aliquot of the solution was filtered through a 0.45 μm membrane filter prior to HPLC analysis. A Vydoc 201 TP octadecylsilyl C_{18} column (particle size 5 μm, 250 mm×4.6 mm i.d.) was used for reverse-phase HPLC. The mobile phase was methanol at a flow rate of 1.0 ml min^{-1}, the UV detector was operated at 345, 368 and 340 nm for all-*trans*-RA, 13-*cis*-RA and retinol, respectively. By means of the injection value, 25 μl of the prepared sample solution and standard solution was chromatographed under the operating conditions described above. Quantitation was based on the peak area of the sample.

3. Results and discussion

3.1. *Choice of analytical method*

Retinol is a pentaene and may undergo oxidation at glassy carbon electrode (GCE) by a similar mechanism to other conjugated double-bond systems [22]. The oxidation was found to occur in two steps and to be irreversible; the reactant was found to undergo adsorption at the GCE [25]. Retinoids are readily oxidized with GCE, thin-film copper modified GCE and thin-film lead modified GCE in methanol containing sodium perchlorate at potentials greater than 0.9 V versus SCE. Although each retinoid has slight differences in their oxidative formal potential, the anodic scan give two peaks, A and B (Fig. 1a–c), thus, that electrode could not undertake for the simultaneous quantitative determinations of these retinoids in a mixture.

Hydrocarbons in which the double bond is conjugated with an aromatic ring, polynuclear aromatic hydrocarbons, aliphatic hydrocarbons and those containing conjugated double bonds are reducible at the dropping mercury electrode [32]. Reduction of carbon–carbon double bonds may result either in saturation of the double bond or in hydrodimerization; the latter reaction is the most common in compounds which can act as Michael acceptors [33]. The peak potentials of unsaturated hydrocarbons are above −2.0 V. However, the addition of another carbon–carbon double bond to an aliphatic polyene carbonyl compound causes a shift in potential towards less negative potential; that is, the ease of reduction increases with the number of double bonds conjugated with the carbonyl group [32]. Thus, retinoids containing five double bonds conjugated with the carboxyl group giving waves at potentials less negative than unsaturated hydrocarbons. The peak potential of the first wave of retinoids is −1.128 V and the height decreases with increasing pH. The second wave is well defined in retinoids only in solutions more alkaline than pH 8 or greater. Comparative tests of supporting electrolytes such as phosphate buffer (pH 2.6 and 6.2), ammonia (pH 8.76), Briton and Robinson buffer (pH 10.26, 11.78) and tetrabutylammonium hydroxide solution (pH 12.47) were conducted. The height of retinoid wave in solutions of 0.1 M tetrabutylammonium hydroxide was found to be much higher than in the other supporting electrolytes. For analytical purposes

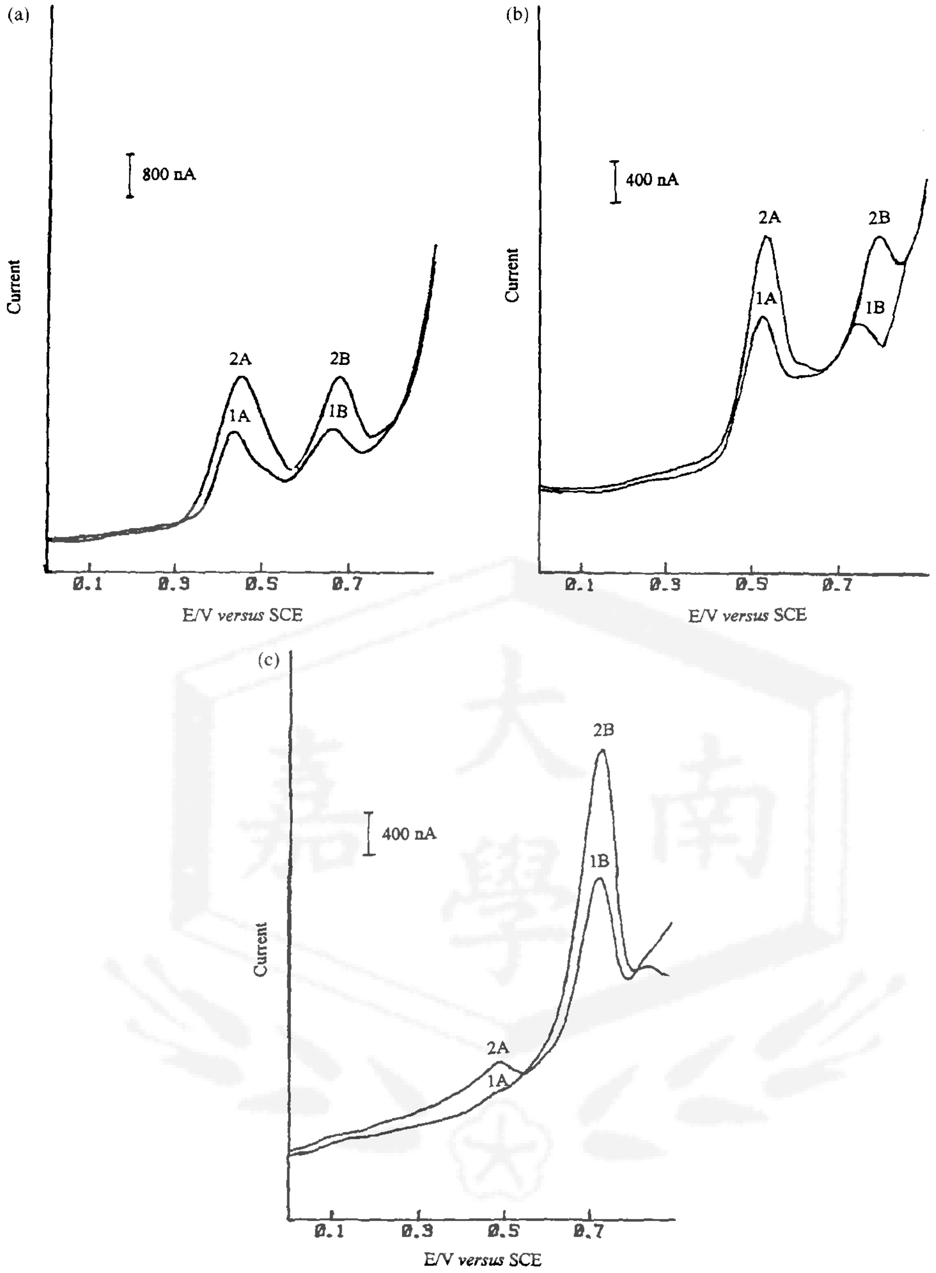

Fig. 1. (a) DPV of all-*trans*-retinoic acid (all-*trans*-RA) in 0.1 M sodium perchorate–99% methanol at a GCE. The current peak values were 1.242 and 0.619 μA at 0.432 and 0.660 V with 20 ppm all-*trans*-RA concentrations, respectively, and 2.112 and 1.323 μA with 40 ppm all-*trans*-RA concentrations, respectively. Scan rate 4 mV s^{-1}; pulse height 0.05 V; (b) DPV of all-*trans*-retinoic acid (all-*trans*-RA) in 0.1 M sodium perchorate–99% methanol at a thin-film copper deposited GCE. The current peak values were 0.838 and 0.348 μA at 0.520 and 0.728 V with 20 ppm all-*trans*-RA concentrations, respectively, and 1.602 and 0.556 μA with 40 ppm all-*trans*-RA concentrations, respectively. Scan rate 4 mV s^{-1}; pulse height 0.05 V; (c) DPV of all-*trans*-retinoic acid (all-*trans*-RA) in 0.1 M sodium perchorate–99% methanol at a thin-film lead deposited GCE. The current peak values were 0.000 and 0.1.171 μA at 0.500 V and 0.724 with 20 ppm all-*trans*-RA concentrations, respectively, and 0.189 and 2.490 μA with 40 ppm all-*trans*-RA concentrations, respectively. Scan rate 4 mV s^{-1}; pulse height 0.05 V.

the best supporting electrolytes for the determination of retinoids is 0.1 M tetrabutylammonium hydroxide solution.

Electrodeposition can be conveniently performed on a cheap substrate like carbon. Electrodeposited metals are extremely pure and highly active. Studies have indicated that the electrodeposition is best obtained under rotating electrode conditions than using stationary electrodes [34]. Since on a rotating electrode, the diffusion layer thickness is considerably lowered which brings about a uniform concentration gradient, for the metal ions resulting in a uniform deposit of the metals. The ionic transfer of the metal ion is quite fast to electrode–electrolyte interface and in many cases the energy of activation for the deposition is lowered by the rotating cathode. The majority of cathodic reactions require an electrode with a high hydrogen overpotential because they take place at a potential negative to the equilibrium hydrogen potential for the electrolyte medium. Mercury and lead have high overvoltage and can be deposited on GCE from the perchlorate and acetate buffer, respectively.

The reduction of retinal, retinol, all-*trans*-RA, 13-*cis*-RA in 0.1 M tetrabutylammonium hydroxide was studied on GCE, thin-film mercury deposited on GCE (TFME) and thin-film lead deposited on GCE (TFLE). It was found that TFME and TFLE gave a better performance than GCE. The voltammetric peak potentials of four retinoids at the TFME were −0.940, −1.472, −1.388 and −1.368 V for retinal, retinol, all-*trans*-RA and 13-*cis*-RA, respectively; thus, that electrode could not perform the simultaneous determination of retinol, all-*trans*-RA and 13-*cis*-RA in a mixture. Moreover the mercury is highly toxic to handle and may lead to toxic materials in the products. However, as can be seen in Fig. 2 the TFLE can improve the separation of the three peaks. In this reduction, three kinds of working electrodes were investigated and results are given in Table 1. The TFLE had lower detection limits than the other electrodes. Therefore, the TFLE was chosen for use in the determination of retinoids in anti-acne and anti-wrinkle samples.

3.2. Reproducibility and accuracy

Fig. 3 shows the results obtained by standard addition of retinol and all-*trans*-RA solution. The peak height of the wave were at −1.326 and −1.520 V with retinol and all-*trans*-RA concentrations, respectively. The analytical curves show good linearity over the range of 5.0–40.0 ppm. For all-*trans*-RA, 13-*cis*-RA and retinol, the regression equations being $y=30.5+66.8x$ (correlation coefficients, $r=0.9991$), $y=40.9+69.1x$ ($r=0.9991$), $y=154+86.9x$ ($r=0.9991$), respectively. The relative standard deviation values were between 2.1 and 4.4%. Fig. 3 shows the DPV of all-*trans*-RA for commercial anti-acne sample spiked with standard solutions.

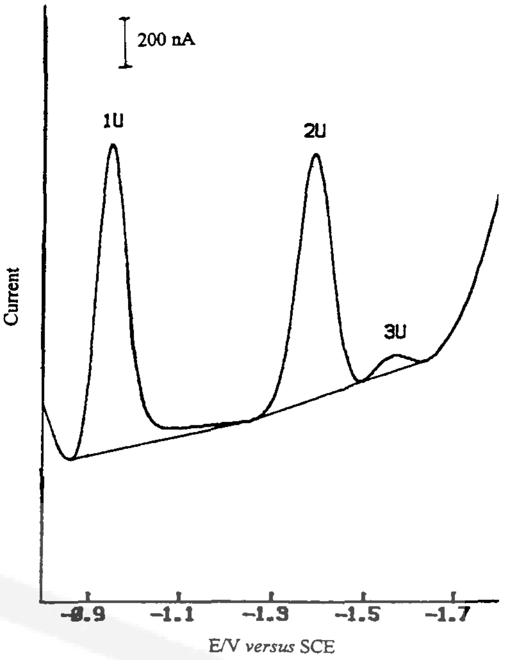

Fig. 2. DPV separation of retinoids at a thin-film lead deposited GCE: 1 U, retinal (peak at −0.952 V); 2 U, 13-*cis*-retinoic acid (peak at −1.392 V); 3 U, all-*trans*-retinoic acid (peak at −1.572 V). Scan rate 4 mV s^{-1}; pulse height 0.05 V. Solution concentration: 0.1 M tetrabutylammonium hydroxide containing 13, 15 and 5 ppm for retinal, 13-*cis*-RA and all-*trans*-RA, respectively.

Table 1
Detection limits of retinoids at glassy carbon electrode (GCE), thin-film mercury deposited GCE (TFME) and thin-film lead deposited GCE (TFLE)

Electrode	All-*trans*-RA (ppm)	13-*Cis*-RA (ppm)	Retinol (ppm)	Retinal (ppm)
GCE	40.30	34.15	13.49	8.34
TFME	5.37	6.00	2.51	2.10
TFLE	1.51	2.16	1.34	0.76

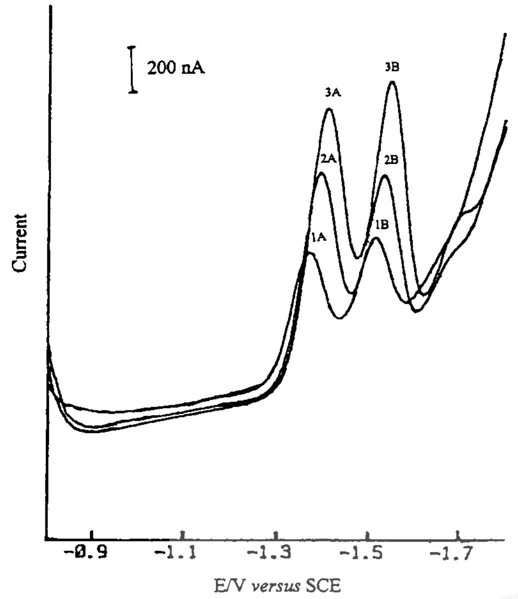

Fig. 3. The DPV recorded to produce analytical curves for retinol(A) and all-*trans*-RA)(B) in 0.1 M tetrabutylammonium hydroxide at a thin-film lead deposited GCE. The current peak values were 0.388 and 0.301 μA at −1.316 and −1.520 V with 5 ppm of retinol and all-*trans*-RA concentrations, respectively. 0.657 and 0.520 μA with 10 ppm of retinol and all-*trans*-RA concentrations, respectively, and 0.900 and 0.765 μA with 15 ppm of retinol and all-*trans*-RA concentrations, respectively. Scan rate 4 mV s^{-1}; pulse height 0.05 V.

Recovery tests were carried out on cosmetic and pharmaceutical products to evaluate the reproducibility and accuracy of the proposed DPV method. Three cosmetic and pharmaceutical products were spiked with the amounts reported in Table 2 and subjected to the whole procedure. As shown in Table 2, excellent recoveries and precision were observed (recoveries ranging from 98.8±1.5 to 102±0.5%).

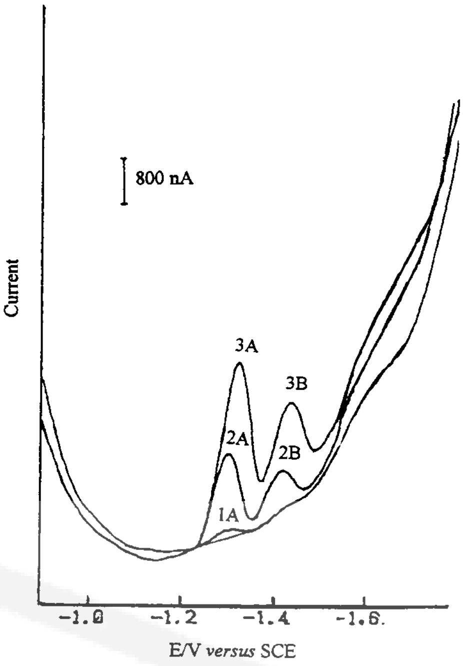

Fig. 4. DPV for retinol and 13-*cis*-RA traces from commercial anti-wrinkle cream at thin-film lead modified GCE: The current peak values were 0.117 and 0.000 μA at −1.308 and −1.436 V with 0 ppm of retinol and 13-*cis*-RA concentrations, respectively, 0.717 and 0.272 μA with 20 ppm of retinol and 5 ppm 13-*cis*-RA concentrations, respectively, and 1.260 and 0.535 μA with 40 ppm of retinol and 10 ppm 13-*cis*-RA concentrations, respectively. Scan rate 4 mV s^{-1}; pulse height 0.05 V.

3.3. Application to cosmetic and pharmaceutical products

The proposed DPV method was applied to the determination of all-*trans*-RA, 13-*cis*-RA and retinol in cosmetic and pharmaceutical products. A representative DPV voltammogram of a commercial anti-wrinkle

Table 2

Recovery of retinoids added to commercial anti-acne and anti-wrinkle creams and gel by DPV at thin-film lead electrode[a]

	All-*trans*-RA				13-*Cis*-RA				Retinol			
	Added (ppm)	Found (ppm)	Recovery (%)	R.S.D. (%)[b]	Added (ppm)	Found (ppm)	Recovery (%)	R.S.D. (%)	Added (ppm)	Found (ppm)	Recovery (%)	R.S.D. (%)
Cream A	5.00	4.98	98.8	1.6	–	–	–		–	–	–	
Cream B	–	–	–		–	–	–		10.00	10.15	102	0.5
Gel	–	–	–		5.00	5.17	1.03	5.0	–	–	–	

[a] Number of determinations (N=3).

[b] R.S.D.: relative standard deviation.

Table 3
Analytical results for the determination of all-*trans*-retinoic acid, 13-*cis*-retinoic acid and retinol in commercial anti-acne creams and gels and anti-wrinkle creams

Cosmetics and pharmaceuticals	Concentration (w/w, %)[a]					
	All-*trans*-retinoic acid		13-*Cis*-retinoic acid		Retinol	
	DPV	HPLC	DPV	HPLC	DPV	HPLC
Anti-acne creams						
1	0.0596 (5.0%)[b]	0.0575 (5.0%)	–	–	–	–
2	0.0475 (4.5%)	(0.5%)	–	–	–	–
3	0.0160 (2.2%)	0.0183(0.6%)	–	–	–	–
Anti-acne gels						
1	0.0247 (3.8%)	0.0249 (5.0%)	–	–	–	–
2	–	–	0.0576 (5.0%)	0.0577 (1.6%)	–	–
Vanishing creams						
1	0.0450 (5.0%)	0.0477 (0.2 %)	–	–	–	–
2	0.0287 (0.4%)	0.0285 (1.1 %)	–	–	–	–
Anti-wrinkles						
1	–	–	–	–	0.0756 (4.2%)	0.0736 (0.7%)
2	–	–	–	–	0.0863 (2.7%)	0.0873 (1.0%)
3	–	–	–	–	0.1108 (1.7%)	0.1136 (1.4%)

[a] Number of determinations (N=5).

[b] Relative standard deviation.

cream is shown in Fig. 4. Analytical results are given in Table 3. These results agreed with those obtained by a high performance liquid chromatographic method. All-*trans*-RA, 13-*cis*-RA and retinol were detected at three different wavelengths 345, 368 and 340 nm, respectively by HPLC. However, DPV method involved for single or simultaneous multiple determinations of retinoids. The single determination of the HPLC procedure should make the interference lower than DPV for the commercial products. Therefore, the relative standard deviation with DPV was higher as compared with those obtained with HPLC.

4. Conclusions

A thin layer of metals deposited on the cathode surface is sufficient to make the cathode effective and the thickness of the deposit can be easily adjusted. Electrodeposited metals have other advantages as compared to cast electrodes.

The DPV procedures described here are applied directly to the analysis of cosmetic and pharmaceutical samples without the need for separation of all-*trans*-RA from the 13-*cis*-RA and other impurities.

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