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Simultaneous Voltammetric Determination of Ascorbic Acid and Its Derivatives in Cosmetics Using Epoxy-carbon Composite Electrodes

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ABSTRACT

We used a voltammetric method to determine the levels of ascorbic acid (AA) and two AA derivatives (magnesium ascorbyl phosphate (MAP) and ascorbyl palimitate (AP)) in cosmetic products. These compounds are believed to affect the process of skin aging and are widely used in skin care products.

An electrode composed of an epoxy resin and graphite powder can be easily fabricated. We studied the effects of solutions' pH and different supporting electrolytes on the performance of a carbon-epoxy electrode which we used as a working electrode to take voltammetric measurements that would quantify the levels of AA, MAP and AP in various cosmetic products effectively. In the voltammogram, anodic peak potentials of AA, MAP and AP were obtained at about 0.35 ± 0.07, 0.81 ± 0.11, and 0.23 ± 0.03 volts in various pH and different supporting electrolytes respectively. Linearity of peaks from plots of current vs. concentration (correlation coefficient $r^2 > 0.997$) was over the range of 10-300 μg/mL for all analytes. The limits of detection (LOD) for AA, AMP and AP were lower than 0.17, 0.46 and 0.09 μg/mL respectively. For the three analytes in cosmetic formulations, the relative standard deviations were below 6.3%, and the range of recovery ratio ranged from 92.2 to 104.5%. Various compounds coexisting in the formulations that were potential sources of organic interferences in voltammetric analysis were explored. Ascorbic acid oxidizes easily to dehydroascorbic acid, but its derivatives are relatively stable. Material instability could result in a significant error in the analytical method. Different approaches were used to inhibit ascorbic acid degradation, and purge of oxygen from the analyzed solution with nitrogen was found to be the most effective method.

Key words: ascorbic acid (AA), magnesium ascorbyl phosphate (MAP), ascorbyl palimitate (AP), cyclic voltammetry (CV), differential pulse voltammetry (DPV), carbon-epoxy electrode, composite electrode, antioxidant, whitening agents

INTRODUCTION

The 76/768/EEC Council Directive sixth amendment to the European Union's rules governing cosmetic products requires cosmetic industries to begin ingredient labeling on cosmetic products. However, as cosmetic products are typically complex formulations of various compounds with varying chemical properties, more sophisticated detection and analysis methods must be developed in order to comply with the new regulations.

While electrochemical methods represent an inexpensive and easily automated approach to testing, their main advantage is the ability to convert analytical information into digital data. Voltammetry, one of the most common electrochemical testing methods, is often used to analyze electroactive components in the samples. The carbon-based electrode is the most common type of electrode used in voltammetric detection, and epoxy carbon electrodes offer relatively high mechanical strength and compatibility. Another advantage of the epoxy-carbon electrodes is that it can be easily fabricated into a variety of formats, including a conventional electrode, a micro- sized composite electrode, or a biosensor[1-6]. However, epoxy-carbon electrodes preparation requires a relatively long cure time ranging from overnight to a full week. This represents a serious drawback for electrode re-preparation and stability of electrodes, especially for biosensors. Therefore, many fast curing epoxy resins have been developed to improve the cure time. The fast curing epoxy resins were reported as beneficial and time-saving in our previous study[7].

It is widely acknowledged that ascorbic acid and its derivatives have important physiological effects on the skin that include melanogenesis inhibition, collagen biosynthesis promotion, and prevention of free radical formation[8-10]. Therefore, these materials are employed extensively in skin care products as antioxidants and as important agents in the treatment of skin pigmentation and aging.

Many methods by which ascorbic acid may be detected and measured in foods and pharmaceuticals (e.g., titration, spectrophotometry[11,12], HPLC[13], potentiometry[14], voltammetry[15,16] and amperometry[17]) have been reported in the literature. However, little has been reported

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on the effective detection and measurement of the AA derivatives, ascorbyl phosphate and ascorbyl palimitate[18-20]. To date, simultaneous voltammetric analysis of these two AA derivatives in cosmetics has not been reported on in literature.

In this report, a differential pulse voltammetry (DPV) with an electrode of a fast cure epoxy-carbon composite was used to analyze the presence of ascorbic acid and its derivatives in cosmetic products. Our epoxy-carbon composite electrode consisted of a homemade electrode using a fast curing epoxy resin[7]. As analyte degradation significantly influences an analytical method's precision and accuracy due to analytes instability (this is especially true for AA, which is much more unstable than its derivatives), we used a variety of approaches to investigate the ability of each to inhibit oxidation. The validity of each of the approaches tried and the effect of the oxidation on the analytical results were discussed. The possible organic interferences occurred between and among various compounds and factors affecting the voltammetric analysis precision were explored.

MATERIALS AND METHODS

I. *Reagents and Solution*

All reagents used were either LC grade or analytical grade. Millipore-Milli Q water was used in all cases. Ascorbic acid, tetrabutyl-ammonium perchlorate (TBAP), potassium nitrate, nitric acid, acetonitrile, and 2-ethylhexyl salicylate were purchased from Sigma-Aldrich (Madrid, Spain). Magnesium ascorbyl phosphate, ascorbyl palmitate, and arbutin were purchased from Tedia (Ohio, USA). A control cosmetic preparation, containing (by weight) 5% of Kowax, 1% of stearic acid, 1% of cetyl alcohol, 3% of sorbital, 0.3% of uniphen P-23, 0.3% of unicide U-13, 0.5% of lipo320, 1% of glycolic acid, 2% of mineral oil, and 85.9% (by weight) of H_2O was formulated in our laboratory. Raw materials for our control cosmetic preparation were purchased from Lipo (USA). Commercial cosmetic product samples were purchased from several retail outlets in Taiwan. A stock solution of AA and its derivatives were prepared in water or methanol in a concentration of 1000 mg/L. Diluted solutions were obtained daily by adding a suitable dilution in supporting electrolyte. Supporting electrolytes were prepared in water or in a mixture of methanol/water (70/30, v/v).

II. *Equipment*

A Princeton Applied Research Model 263 Potentiostat/ Glvanostat was used to perform study measurements in two different modes: the Cyclic Voltammetry (CV) and Differential Pulse Voltammetry (DPV) modes. The working electrode was a carbon-epoxy composite electrode. A platinum counter electrode and an Ag/AgCl reference electrode (from Metrohm) were used. A Bransonic ultrasonic oscillator and Corning pH meter were used.

III. *Procedure*

(I) *Electrodes Preparation*

A graphite powder (180 mg) and epoxy resin of A (12 mg) were mixed thoroughly for 2 min, then an epoxy resin of B (12 mg) was added to the composite and mixed thoroughly for 1 min. A part of the epoxy-carbon resin was pressed manually into the electrode cavity (5 mm diameter, 2 mm depth) in a PP holder and was then smoothened against a clean flat glass plate until a flat surface was obtained. The electrode was cured at room temperature for 30 min. Electrical contact to the cured epoxy-carbon was made by pressing a copper O-Ring (outer diameter: 5 mm; inner diameter: 1 mm; thickness: 2 mm) against a connecting copper wire before pressing the epoxy-carbon resin. To restore the electrode face, we polished it with a 0.5 μm alumina slurry and then washed it with a stream of deionization water between two measurements. When the electrode was prepared multiple times in accordance with the above mentioned procedures, a reasonable reproducibility was obtained with a relative standard deviation (RSD, n = 5) of 15%. Electrodes fabricated in this manner typically had a resistance of approximately 95Ω.

(II) *Control Sample and Real Sample Extraction*

Our control sample was prepared by accurately weighing 3.20 g of the control cosmetic formula and 0.10 g ascorbic acid (or a derivative) into a 20-mL beaker. This mixture was then stirred at room temperature for 1 hr.

We accurately weighed 0.5 g of the control (or real / commercial cosmetic product) sample into a centrifuge tube and added 5 mL of water or methanol. Water or methanol was used as a solvent to extract the ascorbic acid and its derivatives. This centrifuge tube was placed in an ultrasonic oscillator, oscillated for 15 min, and left still for 10 min. The mixture was then centrifuged at 4,300 rpm for 5 min at room temperature and then was filtered through a 0.45 μm membrane. The result was a clear extract ready for the measurement. To remove oxygen from the solutions, a portion of each solution tested was collected and transferred to a test-tube of Pyrex glass and infused with N_2 at a flow rate of approximately 5 mL/min for 1 min. The test tubes were then sealed with a Bakelite screw caps lined with silicone rings.

(III) *Determination of AA and Its Derivatives through A Voltammetric Method*

One milliliter of the extracted solution was transferred into the voltammetric cell, and 9.0 mL of the supporting electrolyte solution was then added. Measurements were carried out at a scan rate of 20 mV/s and in a range of

potential scanning from 0.00 V to +1.20 V. The pulse amplitude was 50 mV in DPV mode. The voltammogram was recorded. The analytes content in the extracted solutions was determined by the standard addition method, used to minimize the matrix interferences.

RESULTS AND DISCUSSION

I. *Choice of Analytical Conditions*

The detection by cyclic and differential pulse voltammetry of ascorbic acid and its derivatives was carried out using a carbon-epoxy electrode. The cyclic voltammograms (Figure 1) showed that all analytes reached anodic peaks during the positive scans from 0.10 to 1.1 V and no cathodic peaks were observed during the negative scans. The result indicated that both these compounds have electroactive function groups in their molecules and that these electrolytic oxidation processes are irreversible reaction. Based on this finding, we used differential pulse voltammetry, which is more sensitive, to quantify the analytes in formulations. The differential pulse voltammogram (not shown) records the anodic peak potentials (E_P) for AA, AP and MAP at 0.36, 0.21 and 0.83 V respectively, and the anodic peak currents (I_p) at 7.2, 2.4 and 3.7 μA respectively. In general, the oxidation peak current was proportional to the concentration of analytes. Accordingly, it can be applied to the determination of analytes' concentration.

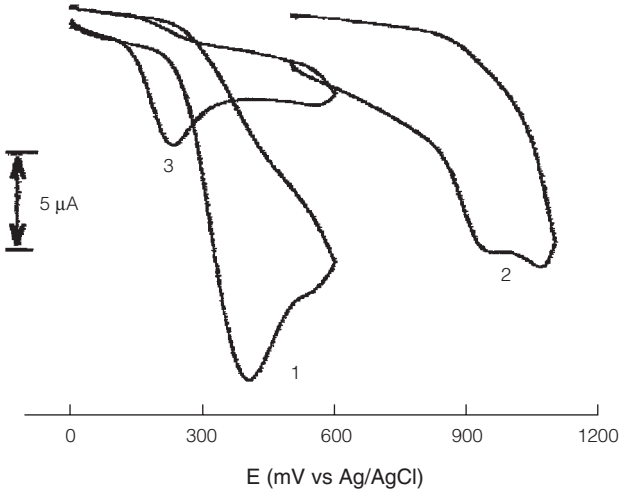

Figure 1. Cyclic voltammograms at a carbon-epoxy electrode for 100 μg/mL: (1) AA and (2) MAP in 0.1 M KNO_3/0.01 M HNO_3 in aqueous solutions, and (3) AP in 0.1 M KNO_3/0.01 M HNO_3 in 70% methanol solution, and the scan rate of 20 mV/s.

In order to obtain the optimum analytical condition for the quantification, two of the major factors which affect the anodic current, pH and supporting electrolytes, are investigated. The electrolytic oxidation behavior of the analytes under a variety of solutions of supporting electrolytes, including the aqueous or methanol/water (70/30, v/v) solution of 0.1 M KNO_3/0.01 M HNO_3, 0.1 M phosphate buffer (pH 4.0-10), and 0.01 M HNO_3 were tested and the results measured were listed in Table 1. It was found that the effect of pH on E_P for all the analytes was significant; their E_P decreases as the pH increases. This means that alkaline medium facilitate the electrolytic oxidation to occur. Especially for AP at pH 10, its E_P is greatly shifted from 100 mv to -179 mv and I_p is largely increased with increasing pH; a possible explanation may be derived from undissociation of AP at various pH value in methanol/water solution, since studies of other electroactive species have shown that dissociation can result in the decrease in magnitude of the original molecular form. On the contrary, I_p for both AA and MAP has the tendency to decrease with increasing pH due to the formation of other forms[21,22]. Obviously, higher pH value seems to be advantageous for the hydrophobic whitening agent such as AP. In relation of supporting electrolyte, I_p of AA in 0.1 M KNO_3/0.01 M HNO_3 medium has extremely increased when compared to the other supporting media, but I_p of MAP has only increased to a little extent. Two major factors which can

Table 1. The CV results[a] of AA, MAP and AP in various values of pH and electrolyte

Sample \ Buffer / Data		HNO_3[b]	KNO_3[c]	H_3PO_4[d] pH=4	H_3PO_4[d] pH = 6	H_3PO_4[d] pH = 8	H_3PO_4[d] pH = 10
Ascorbic acid	E_p (mV)	433 ± 36	352 ± 38	303 ± 42	280 ± 29	246 ± 33	261 ± 43
	i_p (μA)	3.7 ± 0.07	7.2 ± 0.23	3.8 ± 0.12	3.2 ± 0.21	2.7 ± 0.18	2.0 ± 0.26
	$W_{1/2}$ (mV)	211	278	222	244	278	267
MAP	E_p (mV)	949 ± 21	902 ± 18	842 ± 28	739 ± 26	714 ± 55	692 ± 35
	i_p (μA)	1.5 ± 0.05	2.3 ± 0.11	2.4 ± 0.24	2.1 ± 0.17	0.6 ± 0.26	0.4 ± 0.12
	$W_{1/2}$ (mV)	100	111	100	117	198	200
Ascorbyl palmitate[e]	E_p (mV)	271 ± 23	223 ± 32	100 ± 57	-13 ± 38	-75 ± 64	-179 ± 47
	i_p (μA)	2.6 ± 0.15	3.7 ± 0.18	5.0 ± 0.21	5.3 ± 0.25	5.4 ± 0.21	5.8 ± 0.25
	$W_{1/2}$ (mV)	144	112	133	138	100	100

[a]All results were studied with 3 determinations.
[b]0.01 M HNO_3.
[c]0.1 M KNO_3/0.01 M HNO_3.
[d]0.1 M H_3PO_3+NaOH.
[e]Solvent: 70% methanol (v/v, in water).

affect the difference of magnitude of I_p; one is the existence of strong ionic strength, the other is due to strong acidic medium involved in supporting electrolyte. The effect of ionic strength on I_p is beneficial for AA and MAP. However, MAP in strong acidic medium has lesser voltammetric responses than AA, since strong acidic medium result in inhibiting voltammetric responses due to its high instability that may be easily hydrolyzed to inorganic phosphate[22]. In summary, the above observation clearly established that KNO_3/HNO_3 is a good medium for electrochemical oxidation for AA and MAP which are more difficult to oxidize in other media. Hence, this further confirmed that the use of a high ionic strength is advantageous for fast charge transfer; a rapid transfer results in a better voltammetric response. Accordingly, the 0.1 M KNO_3/0.01 M HNO_3 aqueous solution as a supporting electrolyte is the optimum analytical condition for simultaneous determination of AA and MAP. Regarding the simultaneous determination of AA, MAP and AP, a supporting electrolyte of 0.1M phosphate buffer in the methanol/water (70/30, v/v) solution (pH 4) would be suitable in order to improve the solubility of AP and the resolution between their anodic peaks.

II. *Stability of Analytes*

The ascorbic acid and its derivatives are easily oxidized to dehydroascorbic acid and its corresponding dehydro-derivatives. The occurrence of the oxidation could result in a significant error while an analytical method is applied for quantifying their amounts in cosmetics. Some methods such as the treatment of deoxygenation and the addition of antioxidants used to inhibit the oxidation were reported[23]. In order to ascertain whether a stabilizing method should be selected in advance for a determinate type of analytes, the influence of different methods on the ability of antioxidation is investigated by voltammetric method.

The voltammetric behavior versus time of AA, AMP and AP in the solution without any treatment of inhibiting oxidation was measured. The relationship between the magnitude of the anodic peak current of AA and time of storage in various conditions was estimated. The longer the time for a significant change, the more stable AA is. Figure 2A shows the different extents of the degradation with time for the analytes with 100 μg/mL amount in solution. Among the analytes, there is much less change for MAP and AP until 30 hr but a rapid reduction for AA within 30 hr. The lack of stability of AA is attributed to the formation of DHA due to the occurrence of oxidation[22]. This could lead to a poor accuracy at the voltammeric determination without the treatment of anti-oxidation, we therefore examined the voltammetric behavior of different antioxidants and the ability of oxidation inhibition by various method including the treatment of N_2 purge in the same analytical solution. A series of antioxidants, such as sodium thiosulphate, sodium metabisulphite and sodium

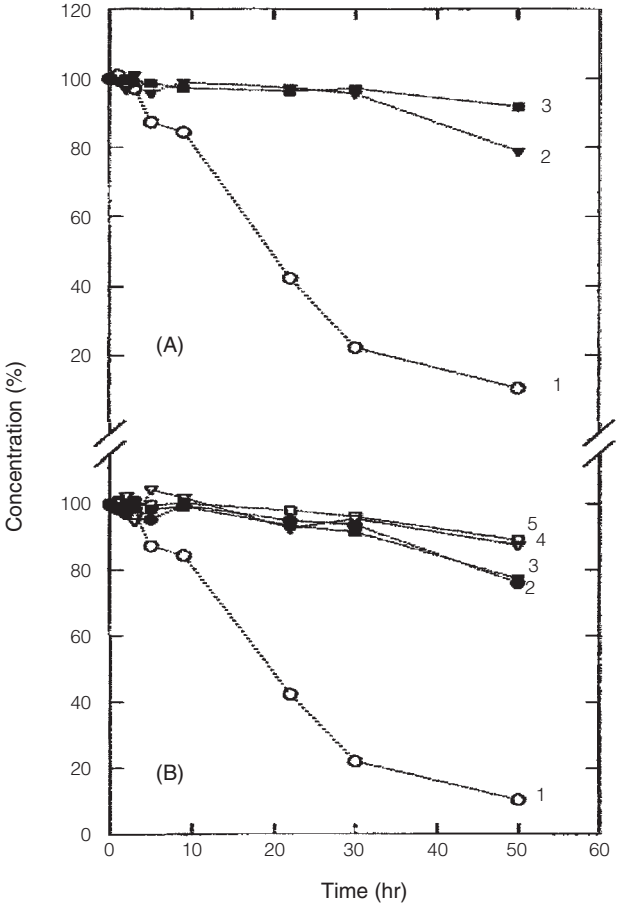

Figure 2. (A) The change of the peak-current obtained from DPV (stability) of 100 μg/mL: (1) AA, (2) MAP and (3) AP with time in 0.1 M H_3PO_4 (pH = 4) in 70% methanol solution. (B) The effect of various conditions, (1) non-purging, (2) addition of 0.04% $Na_2S_2O_3$, (3) addition of 0.04% Na_2SO_3, (4) addition of 0.04% $Na_2S_2O_5$ and (5) purging with N_2, on the peak-current of 100 μg/mL AA in 0.1 M H_3PO_4 (pH = 4) in 70% methanol solution.

sulphite, were individually incorporated into the electrolyte and were measured. Undesired voltammetric response, the anodic peak potentials of sodium thiosulphate, sodium metabisulphite and sodium sulphite in differential pulse voltamograms (not displayed) were observed at 0.76, 0.84 and 0.97 V respectively. The anodic peak potential (E_P) of AA, AP and MAP appears at 0.35 ± 0.07, 0.23 ± 0.03 and 0.81 ± 0.11V respectively. In the case of AA, it is satisfactory that the use of these antioxidants tested did not cause interference. In the case of AP, however, these antioxidants were less soluble in electrolytic solution due to the hydrophobic nature of AP. The anodic peak potential of MAP was close to the antioxidants, therefore overlapping of MAP and these antioxidants were likely to occur. Even though the above results indicate that the voltammetric method is not suitable for simultaneous quantifying the concentration of AA and its derivatives while these antioxidants exist in analytical solution, the suitability of these antioxidants as stabilizer from individual AA analysis viewpoints is to be observed, as shown in Figure 2B. All the

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methods tested including the treatment of N_2 and antioxidants contribute to the inhibition of oxidation in AA. For all the cases, no change in amount of AA was observed for 10 hr, but after 10 hr, the amount of AA decreased slightly; the time required for degradation of 10% concentration both in the case of $Na_2S_2O_3$ and Na_2SO_3 was about 30 hr, and for N_2 methods or $Na_2S_2O_5$ was about 50 hr. Clearly, compared to the antioxidants used, the deoxygenation by purging with N_2 has some advantages, e.g. non-interference, higher efficacy, and more convenient for simultaneous quantification of the analytes in cosmetic formulates. Thus, the purging method should be recommended in the holding of stability of AA and its derivatives.

III. *Validation*

In order to evaluate the characteristics of the analytical method, the regression calibration curves, reproducibility and recovery were studied using differential pulse voltammetry in the presence of single analyte. The excellent linearity (correlation coefficient r^2) was more than 0.997 over the range 1-300 μg/mL for AA or AP except for MAP which the range was 10-500 μg/mL. The limits of detection were calculated by three times the standard deviation of the background currents divided by the slope of the calibration curves obtained from peak heights. The calculated result showed that the LODs of AA, AP and AMP were less than 0.17, 0.09, 0.46 μg/mL respectively. The relative standard deviations and recoveries of the three analytes in cosmetic formulations were studies. The results showed that the relative standard deviations were less than 6.3% and the desirable recoveries, ranged from 92.2 to 104.5% were observed. From the results, we can conclude that this method can be used to determine the quantity of these compounds.

IV. *Interference*

The voltammetric method employed to determinate AA and its derivations was established, however it is also well known that this method suffers from practical difficulty mainly related to the high overlapping of the cathodic peak of analytes with some compounds in preparations. Namely, the existence of interfering species in real samples will give a poor voltammetric response; the accuracy and precision of assay is to be strongly reduced. Table 2 listed the reasonable concentration of some interfering species to be coexisted in cosmetic products. Theses analytes are dissolved in electrolyte by 5 ppm level. It shows clearly that the interference of each compound on the tested whitening agents has a significant difference in reasonable concentration. In the case of salicylic acid, interference was not observed. By contrast, AA was interfered when the content of BHT is more than 5 ppm; MAP and AP were interfered when BHT exceeds 500 ppm. The presented data showed identical interference of arbutin and hexachlorophene on the individual analytes; the determination of MAP will be invalid in the present of the two compounds due to their higher overlap peak with MAP, but AA and AP can be measured in the presence of reasonable concentration. Similar influence on the analytes exist between tricolcarben and triclosan; as shown in Table 2, both of the tricolcarben and triclosan did not interfere on the measurement of AA and AP, and MAP can be measured in the presence of the reasonable concentration of 200 ppm tricolcarben as well as of 100 ppm triclosan. Thus, the voltammetric method could be used to perform the simultaneous determination of the analytes under reasonable concentration of each interfering species.

V. *Simultaneous Detection*

The simultaneous detection of the mixture of AA, AP and MAP was also studied. Figure 3 recorded the corresponding voltammogram of AA, MAP and AP mixture in a concentration of 100 μg/mL. Well separated peaks of MAP from others were observed, but there were also some overlapping between the peaks of AA and AP. Under optimal selection of a base line such as the dash line in the voltammogram, the good linearity ($r^2 > 0.992$) between 10-180 μg/mL and precision (RSD < 7.2%) was obtained even though the overlap could interfere the simultaneous determination of AA and AP. Clearly, in the absence of the hydrophobic AP the corresponding voltammogram of

Table 2. The effect of various interfering compounds commonly present in real samples

		AA (5 ppm)	MAP (5 ppm)	AP (5 ppm)
Interfering compounds	E_{peak} (V)	Reasonable concentration[a]	Reasonable concentration	Reasonable concentration
Salicylic acid	1.21	NI[b]	NI	NI
Arbutin	0.79	100	MBA[c]	NI
BHT	0.59	5	500	500
Hexachlorophen	0.85	100	MBA	NI
Triclocarban	1.15	NI	200	NI
Triclosan	1.10	NI	100	NI

[a]Reasonable concentration (ppm): when the content of the interfering compounds in solution is less than the reasonable concentration, no interference occurs during determining the analytes.

[b]NI: no interference.

[c]MBA: must be absent due to the anodic peak potential (E_P) close to that of MAP.

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mixture of AA and MAP in the concentration range of 30-300 μg/mL exhibited a good separation of anodic potential. The result showed that the linear relationship ($r^2 > 0.996$) between anodic peak and concentration of AA and MAP can be obtained simultaneously, and their calibration sensitivity is similar with the detection of single analyte.

VI. *Analysis of Cosmetic Products*

The epoxy-carbon electrode was applied to the measurement of AA, MAP and AP in four commercially available cosmetic products. They are whitening cream (cosmetic No.1), C20 whitening gel (cosmetic No.2), whitening powder (cosmetic No.3) and peel lotion (cosmetic No.4) respectively. A method of extra-added was used for the study of the determination of AA and AP in commercial available cosmetic products. These analytical results are given in Table 3. Except for the cosmetic No.4, which exhibited a relatively low % of label value for AA due to its instability in cosmetics, the recovery (% of label) of AA, AMP, or AP for the other commercial cosmetics were satisfactory with values ranged from 94% to 105%.

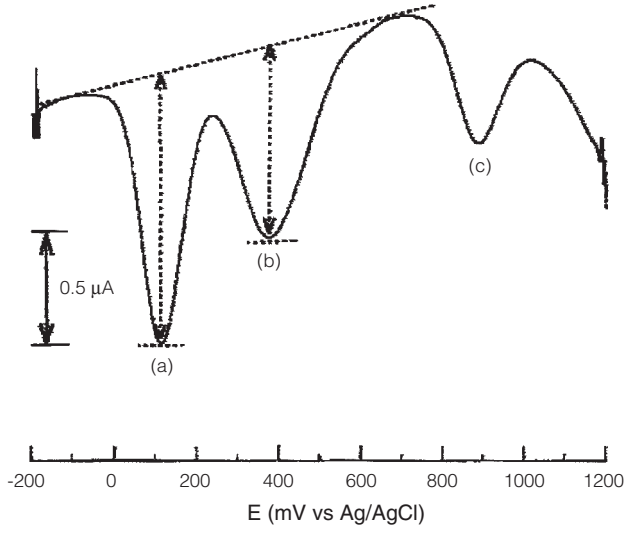

Figure 3. Differential pulse voltammograms obtained for the concentration of 50 μg/mL of the mixture of AA, MAP and AP in the 0.1 M KNO_3/0.01 M HNO_3 in 70% methanol solution and scan rate 20 mV/s. Peak (a) is for AP; Peak (b) is for AA; Peak (c) is for MAP.

CONCLUSIONS

This study had demonstrated that the voltammetric method with the epoxy-carbon composite electrodes can be applied to the simultaneous analysis of AA and its derivates in cosmetics with excellent sensitivity and selectivity. The reliability and stability of the epoxy-carbon composite electrodes offers a good possibility for extending the technique in routine analysis of AA and its derivates in cosmetic products. A convenient purging method for stabilizing ascorbic acid in analytical procedure has been recommended. The interference of some compounds often contained in various cosmetics was studied. However, it is necessary to research more extensively in the application of real sample analysis, because many influences such as efficiency of extraction, coexistence and other interferences may be serious and complicated.

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Table 3. Assay results of the three analytes in commercial cosmetic products

Sample	Analytes	Label claim (%, w/w)	Found (%, w/w)	% of label
No.1	MAP	0.08	0.076 ± 3.2%	95
	Ascorbyl palmitate	0.5	0.51 ± 3.5%	96
No.2	MAP	0.70	0.74 ± 2.8%	105
No.3	Ascorbic acid	20	18.8 ± 5.3%	94
	MAP	3	3.1 ± 2.3%	103
No.4	Ascorbic acid	0.2	0.12 ± 4.3%	60
	Ascorbic acid	0.2[a](1.2)	0.31 ± 2.5%	96
	Ascorbyl palmitate	0.5[a]	0.52 ± 3.6%	104

[a]Externally add to products.

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