Anal. Bioanal. Electrochem., Vol. 7, No. 5, 2015, 623-634

Analytical & Bioanalytical Electrochemistry

2015 by CEE
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Full Paper

Voltammetric Determination of Ascorbic Acid in the presence of Acetaminophen using a Carbon Paste Electrode Modified with Multiwall Carbon Nanotubes and 3-(4'-Amino-3'-Hydroxy-Biphenyl-4-yl)-Acrylic Acid

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Received: 17 June 2015 / Received in revised form: 18 August 2015 / Accepted: 20 August 2015 / Published online: 31 October 2015

Abstract- A carbon paste electrode (CPE) modified with 3-(4'-amino-3'-hydroxy-biphenyl-4-yl)-acrylic acid and multi-walled carbon nanotubes (MWCNTs), was prepared for simultaneous determination of ascorbic acid (AA) and acetaminophen (AC). The electrochemical response characteristics of the modified electrode toward AA and AC were investigated by cyclic voltammetry and square wave voltammetry (CV and SVW). The results showed an efficient catalytic role for the electro-oxidation of AA and AC, leading to a remarkable peak resolution (~320 mV) for two compounds. The mechanism of the modified electrode was analyzed by monitoring the CVs at various potential sweep rates and pHs of the buffer solutions. Under the optimum conditions, the calibration curve for AA was obtained in the range of 1.0×10^{-8}-4.0×10^{-5} M. The proposed method was applied to determination of AA and AC in commercial drugs and in urine samples and the obtained results were satisfactory.

Keywords- Ascorbic acid, Acetaminophen, Simultaneous determination, Carbon nanotube paste electrode

1. INTRODUCTION

L-Ascorbic acid (AA), is an essential nutrient for humans and an important compound from the clinical and the food industrial points of view. AA has been used for the prevention and treatment of many disorders, including atherosclerosis, common cold, Alzheimer's disease, mental illness, infertility and cancer [1]. AA is easily oxidized chemically and electrochemically to L-dehydroascorbic acid (DHA) and partially metabolized to inactive sulfide and oxalic acid, which is eliminated largely by urinary excretion. AA is widely found alongside various biologically and pharmacologically active substances like acetaminophen and other pharmaceutical products as well as in biological fluids [2–5].

Acetaminophen (AC), is a very popular analgesic antipyretic and a nonsteroidal moderate anti-inflammatory drug used to mitigate fever and pain such as headaches, backaches, arthritis and post-operative. It is also an effective substitute of aspirin for patients who cannot use aspirin [6-9]. At normal therapeutic doses, AC is rapidly and completely metabolized in the liver and excreted in the urine mainly as the glucuronide and sulfate conjugates. However, the ingestion of high doses AC can cause the accumulation of toxic metabolites, which may cause fatal hepatotoxicity and nephrotoxicity, in some cases associate with renal failure [10]. The use of a complementary presence of AA intensifies the pharmacological effect of AC, as well as promotes a protective effect with respect to AC hepatotoxicity [11,12]. Therefore the simultaneous determination of AA and AC for quality control analysis (in pharmaceutical formulations) and for medical control (in biological fluids as urine, blood and plasma) is very important.

Nevertheless, very articles on individual determination of the two substances, especially AA, have been published, few articles reported the simultaneous determination of AA and AC in which liquid chromatography, electrophoresis, and spectrophotometry methods were employed for the determination of both drugs in medicinal preparations [13-18]. However, these methods usually need sample pretreatment (e.g., extraction, complex formation) that is time-consuming and grinding. To overcome these faults, electrochemical approaches are used extensively for the especial and sensitive properties such as rapidity, simplicity, wide linear concentration range, low expense of instrument, selectivity, and reproducibility of this technique [19-21]. However, it is well known that the simultaneous determination of both drugs by unmodified electrodes based on carbon or those metallic ones, Hg, Au, Pt suffers from lack of selectivity due to the overlapping oxidation peaks [22].

Carbon paste electrode (CPE) is a particular kind of heterogeneous carbon electrode consisting of mixture prepared from carbon powder (as graphite, glassy carbon and others carbonaceous materials) and an appropriate water-immiscible or non-conducting binder [23,24]. CPEs are widely applicable in both electrochemical studies and electroanalysis thank to their specific advantages, including very low background current (compared to solid graphite or noble metal electrodes), high sensitivity, facility to prepare, low cost, large

potential window and simple surface renewal process [25-27]. Besides the advantageous properties and characteristics listed, a wide variety of modifiers including enzymes, polymers and nanomaterials [28-38] have been used with these versatile electrodes (which resulting in the so-called modified carbon paste electrode).

Some reviews based on the use of multiwall carbon nanotubes (MWCNTs) as electrochemical sensor has been very well documented for the determination of electroactive analytes [39,41], due to their extraordinary structural, mechanical, electrical, and electrochemical properties when used as electrodic materials. It has been shown that application of MWCNTs results in special advantages over conventional electrodes, such as high electrical and thermal conductivity, chemical stability, enhanced mass transport (via thin layer diffusion besides the semi-infinite planar diffusion), highly effective surface areas, more adsorption, reactive sites and high tensile strength [42]. All these fascinating properties make MWCNTs as a suitable candidate for the study of a number of biological species.

In the present work, following the idea of searching new methods for AA detection, we describe the preparation of a new electrode composed of 3-(4′-amino-3′-hydroxy-biphenyl-4-yl)-acrylic acid modified carbon nanotubes paste electrode (3,4´AACNTPE). We described initially the preparation and suitability of a 3,4´AACNTPE as a new electrode in the electrocatalysis and determination of AA in an aqueous buffer solution. Then we evaluated the analytical performance of the modified electrode in determination of AA in the presence of AC.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at 25±1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and 3,4´AACNTPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was used for pH measurements.

AA, AC and all of the other reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-9.0. 3,4′-AA was synthesized in our laboratory as reported previously [37]. Multiwall carbon nanotubes were of analytical reagent grade and were obtained from Nanostructured & Amorphous Materials, Inc.

2.2. Preparation of the electrode

The 3,4´AACNTPE were prepared by hand mixing 0.01 g of 3,4′-AA with 0.89 g graphite powder and 0.1 g multiwall carbon nanotubes with a mortar and pestle. Then, ~0.7 mL of

paraffin was added to the above mixture and mixed for 20 min until a uniformly-wetted paste was obtained. The paste was then packed into the end of a glass tube (ca. 3.4 mm i.d. and 10 cm long). A copper wire inserted into the carbon paste provided the electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper.

For comparison, 3,4′-AA modified CPE electrode (3,4′-AACPE) without multiwall carbon nanotubes, multiwall carbon nanotubes paste electrode (CNTPE) without 3,4′-AA, and unmodified CPE in the absence of both 3,4′-AA and multiwall carbon nanotubes were also prepared in the same way.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of 3,4´AACNTPE

3,4´AACNTPE was constructed and its electrochemical properties were studied in a 0.1 M PBS (pH 7.0) using cyclic voltammetry (CV). The experimental results show well-defined and reproducible anodic and cathodic peaks with E_{pa} and E_{pc} of 270 and 130 mV *vs.* Ag/AgCl/KCl (3.0 M) respectively.

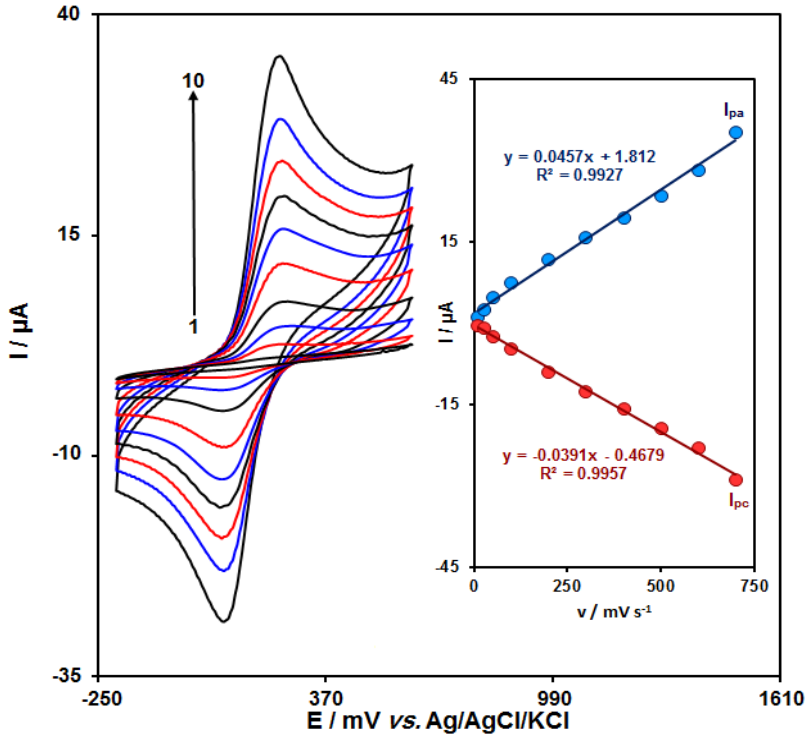

Fig. 1. CVs of 3,4´AACNTPE in 0.1 M PBS (pH 7.0), at various scan rates, numbers 1–10 correspond to 10, 25, 50, 100, 200, 300, 400, 500, 600 and 700 mV s^{-1}. Inset: Variation of anodic and cathodic peak currents versus scan rate

The observed peak separation potential, $\Delta E_p=(E_{pa}-E_{pc})$ of 140 mV, was greater than the value of 59/n mV expected for a reversible system [43] suggesting that the redox couple of 3,4′-AA in 3,4´AACNTPE has a quasi-reversible behavior in aqueous medium. The effect of the potential scan rate (ν) on electrochemical properties of the 3,4´AACNTPE was also studied by CV (Fig. 1). Plots of the both anodic and cathodic peak currents (I_p) were linearly dependent on ν in the range of 10 to 700 mV s^{-1} (Fig. 1 inset), indicating that the redox process of 3,4´AA at the modified electrode are those anticipated for a surface-confined redox couple [43].

3.2. Electrocatalytic oxidation of AA at a 3,4´AACNTPE

Fig. 2 depicts the CV responses for the electrochemical oxidation of 2.5 µM AA at unmodified CPE (curve b), CNTPE (curve d), 3,4´AACPE (curve e) and 3,4´AACNTPE (curve f). Also, curve a shows unmodified CPE in 0.1 M PBS (pH 7.0).

As it is seen, while the anodic peak potential for AA oxidation at the CNTPE, and unmodified CPE are 495 and 560 mV, respectively, the corresponding potential at 3,4´AACNTPE and 3,4´AACPE is ~270 mV. These results indicate that 3,4´AA can act as a good mediator and peak potential for AA oxidation at the 3,4´AACNTPE and 3,4´AACPE shift by ~225 and 290 mV toward negative values compared to CNTPE and unmodified CPE, respectively. However, 3,4´AACNTPE shows much higher anodic peak current for the oxidation of AA compared to 3,4´AACPE, indicating that the combination of multiwall carbon nanotubes and the mediator (3,4´AA) has significantly improved the performance of the electrode toward AA oxidation. In fact, 3,4´AACNTPE in the absence of AA exhibited a well-behaved redox reaction (Fig. 2, curve c) in 0.1 M PBS (pH 7.0). However, there was a drastic increase in the anodic peak current in the presence of 2.5 µM AA (curve f), which can be related to the strong electrocatalytic effect of the 3,4´AACNTPE towards this compound.

The effect of scan rate on the electrocatalytic oxidation of AA at the 3,4´AACNTPE was investigated by linear sweep voltammetry (LSV) (Fig. 3). As can be observed in Fig. 3, the oxidation peak potential shifted to more positive potentials with increasing scan rate, confirming the kinetic limitation in the electrochemical reaction. Also, a plot of peak height (I_p) *vs.* the square root of scan rate ($\nu^{1/2}$) was found to be linear in the range of 6-20 mV s^{-1}, suggesting that, at sufficient over potential, the process is diffusion rather than surface controlled [43].

Fig. 3B shows a Tafel plot that was drawn from points of the Tafel region of the LSV. The Tafel slope of 0.0888 V obtained in this case agrees well with the involvement of one electron in the rate determining step of the electrode process, assuming a charge transfer coefficient of α=0.34 [43].

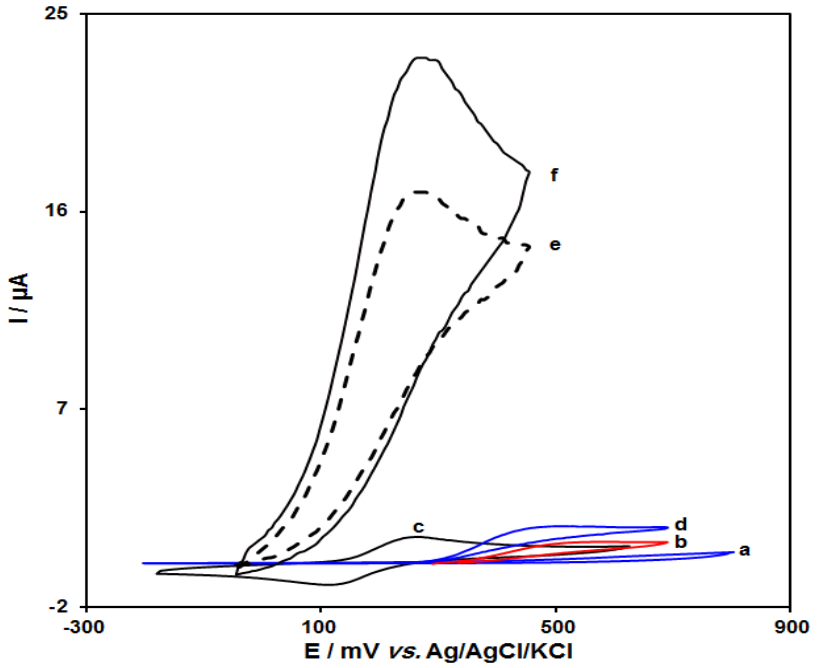

Fig. 2. CVs of (a) unmodified CPE in 0.1 M PBS, (b) unmodified CPE in 2.5 µM AA, (c) 3,4′-AACPE in 0.1 M PBS, (d) CNTPE in 2.5 µM AA, (e) 3,4′-AACPE in 2.5 µM AA, and (f) 3,4´AACNTPE in 2.5 µM AA. Conditions: scan rate of 10 mV s^{-1}; pH 7.0

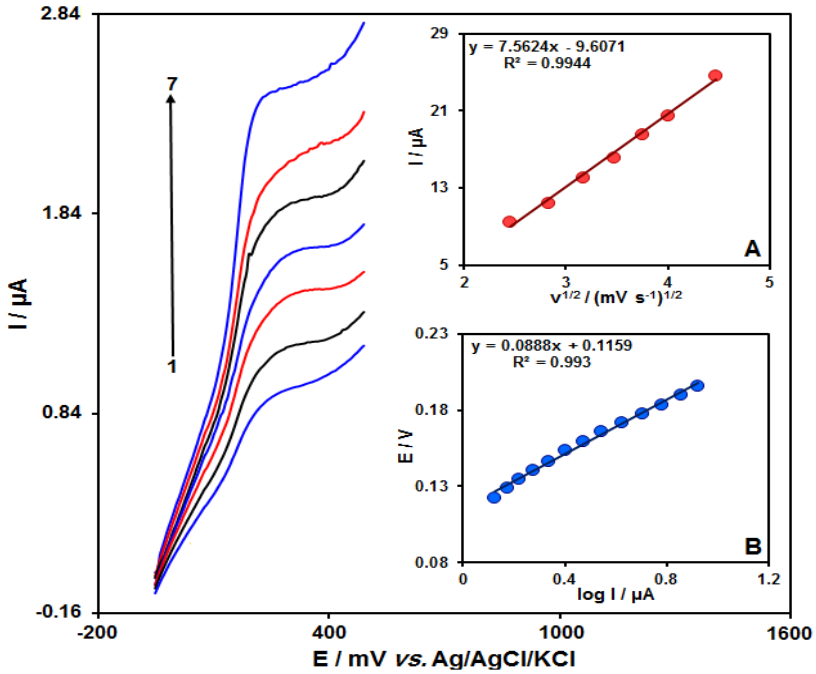

Fig. 3. LSVs of 3,4´AACNTPE in 0.1 M PBS (pH 7.0) containing 1.0 µM AA at various scan rates; numbers 1-7 correspond to 6, 8, 10, 12, 14, 16 and 20 mV s^{-1}, respectively. Insets: Variation of (A) anodic peak current *vs.* $v^{1/2}$ and (B) Tafel plot derived from the LSV at the scan rate of 10 mVs^{-1}.

3.3. Chronoamperometric measurements

Chronoamperometric measurements of AA at 3,4´AACNTPE were carried out by setting the working electrode potential at 0.3 V (at the first potential step) and at 0.05 V (at second potential step) *vs*. Ag/AgCl/KCl (3.0 M) for the various concentration of AA in PBS (pH 7.0) (Fig. 4). For an electroactive material (AA in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [43]:

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \quad (1)$$

Where D and C_b are the diffusion coefficient ($cm^2\ s^{-1}$) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I *vs*. $t^{-1/2}$ were employed, with the best fits for different concentrations of AA (Fig. 4A). The slopes of the resulting straight lines were then plotted *vs*. AA concentration (Fig. 4B). From the resulting slope and Cottrell equation the mean value of the D was found to be 1.0×10^{-6} cm ^{2}/s.

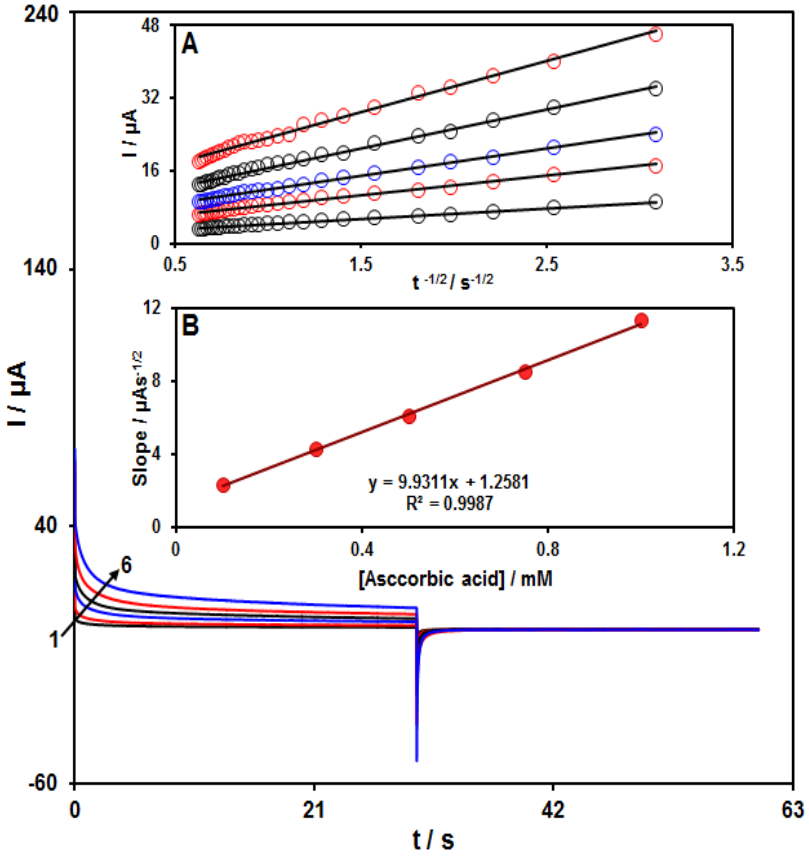

Fig. 4. Chronoamperograms obtained at the 3,4´AACNTPE in the absence (1) and in the presence of 0.1 (2), 0.3 (3), 0.5 (4), 0.75 (5) and 1.0 (6) mM of AA in a buffer solution (pH 7.0). (A) Dependence of I on the $t^{-1/2}.s^{1/2}$ derived from the chronoamperogram data. (B) Plot of the slope of the straight lines against AA concentration

3.4. Calibration plot and limit of detection

The electrocatalytic peak current of AA oxidation at the surface of the 3,4´AACNTPE can be used for determination of AA in solution. Therefore, SWV experiments were performed using modified electrode in 0.1 M PBS (pH 7.0) containing various concentration of AA (Fig. 5).

The plot of peak current *vs.* AA concentration consisted of two linear segments with slopes of 5.3319 and 1.0804 µA / µM in the concentration ranges of 1.0×10^{-8}-7.5×10^{-6} M and 7.5×10^{-6}-4.0×10^{-5} M respectively. Also, the detection limit of AA was obtained 9.5 nM.

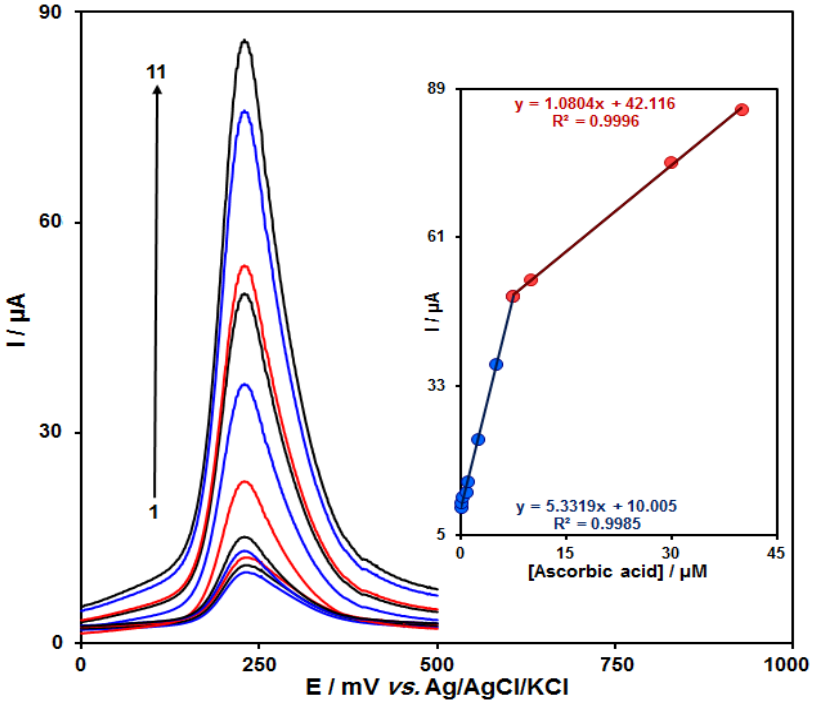

Fig. 5. SWVs of 3,4´AACNTPE in 0.1 M PBS (pH 7.0) containing different concentrations of AA. Numbers 1-11 correspond to 0.01, 0.05, 0.25, 0.75, 1.0, 2.5, 5.0, 7.5, 10.0, 30.0 and 40.0 µM of AA. Insets: The plots of the electrocatalytic peak current as a function of AA concentration in the range of 0.01-7.5 µM (A) and 7.5-40.0 µM (B)

3.5. Simultaneous determination of ascorbic acid and acetaminophen

The determination of AA and AC in mixtures were performed at the 3,4´AACNTPE using SWV by simultaneously changing the concentrations of AA and AC, and recording the SWVs (Fig. 6).

The voltammetric results showed well-defined anodic peaks at potentials of 240 and 560 mV, corresponding to the oxidation of AA and AC, respectively, indicating that simultaneous determination of AA and AC is feasible at the 3,4´AACNTPE as shown in Fig. 6.

The sensitivity of the modified electrode towards the oxidation of AA was found to be 5.2587 μA μM^{-1}. This is very close to the value obtained in the absence of AC (5.3319 μA μM^{-1}, see Section 3.4), indicating that the oxidation processes of these compounds at the 3,4´AACNTPE are independent and therefore, simultaneous determination of their mixtures is possible without significant interferences.

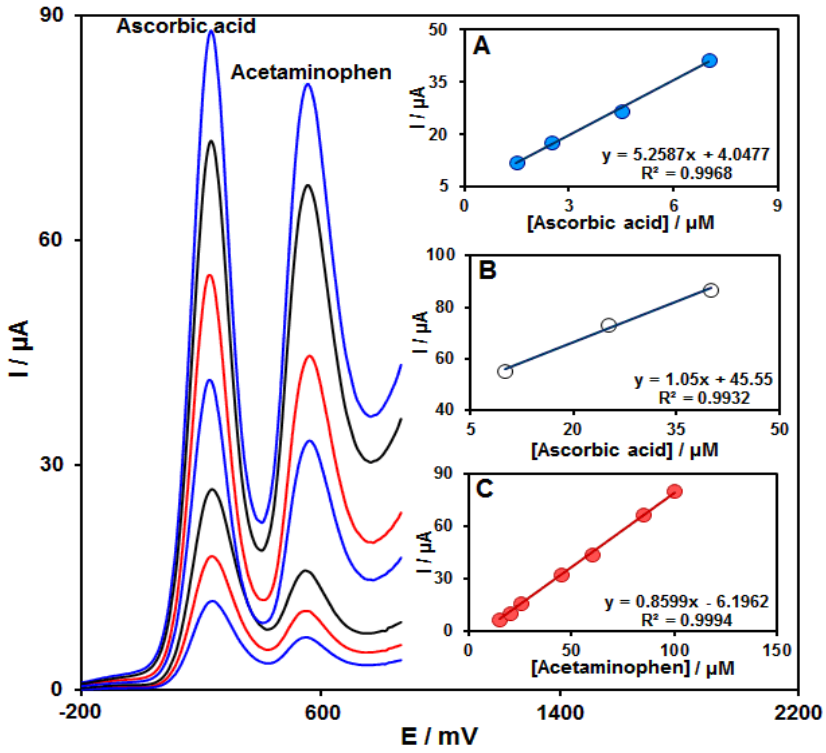

Fig. 6. SWVs of 3,4´AACNTPE in 0.1 M PBS (pH 7.0) containing different concentrations of AA+AC in μM, from inner to outer: 1.5+15.0, 2.5+20.0, 4.5+25.0, 7.0+45.0, 10.0+60.0, 25.0+85.0 and 40.0+100.0 respectively. Insets (A), (B) and (C) plots of Ip *vs*. AA and AC concentrations respectively

3.6. Interferences study

The influence of various substances as compounds potentially interfering with the determination of AA was studied under optimum conditions with 20.0 μM AA at pH 7.0. The potentially interfering substances were chosen from the group of substances commonly found with AA in pharmaceuticals and/or in biological fluids. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error of less than ±5% in the determination of AA. According to the results, glucose, sucrose, lactose, fructose, citric acid, methanol, ethanol, Mg^{2+}, SO_4^{2-}, Al^{3+}, NH_4^+, Fe^{2+}, Fe^{3+},CO_3^{2-}, Cl^- or F^-, alanine,

methionine, phenylalanine, glycine, folic acid (vitamin B_9), saturated starch solution and urea did not interfere with the determination of AA.

3.7. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of AA and AC in AA tablet, AC tablet and urine samples. The results are listed in Table 1. Satisfactory recovery of the experimental results was found for AA and AC. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

Table 1. The application of 3,4´AACNTPE for determination of AA and AC in AA tablet, AC tablet and urine samples (n=5)

Sample	Spiked (μM)		Found (μM)		Recovery (%)		R.S.D. (%)	
	AA	AC	AA	AC	AA	AC	AA	AC
AA tablet	0	0	20.0	-	-	-	3.5	-
	5.0	20.0	25.5	19.9	102.0	99.5	2.9	1.8
	10.0	30.0	29.1	30.9	97.0	103.0	3.1	2.5
	15.0	40.0	35.3	39.4	100.9	98.5	1.7	2.9
	20.0	50.0	39.6	51.2	99.0	102.4	1.9	3.2
AC tablet	0	0	-	12.0	-	-	-	3.1
	7.5	5.0	7.7	16.8	102.7	98.8	1.6	2.4
	17.5	10.0	17.1	22.5	97.7	102.3	2.9	2.3
	27.5	15.0	27.6	26.6	100.4	98.5	2.5	1.8
	37.5	20.0	37.3	33.2	99.5	103.7	3.3	2.9
Urine	0	0	-	-	-	-	-	-
	5.0	15.0	4.9	15.2	98.0	101.3	3.3	1.7
	10.0	35.0	10.1	34.8	101.0	99.4	2.1	1.9
	15.0	55.0	14.9	56.5	99.3	102.7	1.8	2.2
	20.0	75.0	20.5	74.1	102.5	98.8	2.8	3.5

4. CONCLUSION

The results obtained in this work demonstrated the potentiality of the 3,4´AACNTPE modified electrode for simultaneous determination of AA and AC. The modified electrode exhibits highly electrocatalytic activity for the oxidation of AA and AC associated with negative shifts in anodic peak potentials. Thus, large peak separations obtained with this electrode allow it to simultaneously detect these compounds. Moreover, good sensitivity,

high selectivity, low detection limits with the low cost of the sensor, makes this method very suitable for accurate determinations in pharmaceutical and clinical preparations. The proposed method could be applied to the determination of AA and AC in real samples with satisfactory results.

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