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Exploiting micellar environment for simultaneous electrochemical determination of ascorbic acid and dopamine

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Abstract

A simple and reliable method for simultaneous electrochemical determination of ascorbic acid (AA) and dopamine (DA) is presented in this work. It was based on the use of the cationic surfactant cetylpyridinium chloride (CPC) that enables the separation of the oxidation peaks potential of AA and DA. Cyclic voltammetry (CV) as well as pulse differential voltammetry (PDV) were used in order to verify the voltammetric behaviour in micellar media. In the cationic surfactant CPC, a remarkable electrostatic interaction is established with negatively charged AA, as a consequence, the oxidation peak potential shifted toward less positive potential and the peak current increased. On the other hand, the positively charged DA is repelled from the electrode surface and the oxidation peak potential shifts toward more positive potential in comparison to the bare electrode. Therefore, the common overlapped oxidation peaks of AA and DA can be circumventing by using CPC. Parameter that affects the E_{pa} and I_{pa} such as CPC concentration and pH were studied. Under optimised conditions, the method presented a linear response to AA and DA in the concentration range from 5 to 75 $\mu mol\,L^{-1}$ and 10 to 100 $\mu mol\,L^{-1}$, respectively. The proposed method was successfully applied to the simultaneous determination of AA and DA in dopamine hydrochloride injection (DHI) samples spiked with AA.

Keywords: Dopamine; Ascorbic acid; Differential pulse voltammetry; Cyclic voltammetry; Cationic surfactant; Micelles

1. Introduction

The selective determination of dopamine (DA) in the presence of ascorbic acid (AA) has received considerable attention [1]. The concentration of DA, AA and others neurotransmitters in biological samples is changed from species to species, in a wide range, from 10^{-7} to $10^{-3}\,mol\,L^{-1}$ [1]. Hence, selectivity and sensitivity are important in the development of any procedure for the determination of DA, as well as of any neurotransmitter [1]. DA is one of the most significant catecholamines and plays a very important role in the functioning of the central nervous system, as well as in the cardiovascular, renal and hormonal systems [1–4]. Similarly, AA (Vitamin C) has been used for the prevention and treatment of common cold, mental illness, infertility and cancer [2]. In mammalian tissue, AA is present along with several neurotransmitters including dopamine [2,3]. Thus, simultaneous determination of DA and AA is a problem of critical importance in field of neurochemistry and biomedical chemistry [2]. Both, DA and AA, are compounds that can be determined for electrochemical methods based on anodic oxidation [1,2]. However, a major problem is, the oxidation potential for both AA and DA occurs almost in the same potential at unmodified electrodes, which result in overlapped voltammetric responses making their discrimination highly difficult [2,5].

Therefore, a number of chemically modified electrodes have been developed to separate the electrochemical response of DA and AA [6–19]. For instance, self-assembled monolayers of ω-mercapto-carboxylic acid and stearic acid deposited on a gold electrode and on a graphite paste electrode [6,7],

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adsorption of pyrocatechol sulfonephthalein [10] and electrochemical polymerization of *N*,*N*-dimethyl aniline [13] on a glassy carbon electrode were used to shift the oxidation potential of ascorbic acid towards a more positive value, thus, separating its anodic peak from the dopamine ones.

Among other strategies reported to overcome this drawback, a convenient way is to cover the electrode surface with a positive or negative charged permselective membrane. These ion exchange membranes of both cationic and anionic nature have been developed to electrostatically accumulate/trap oppositely charged molecules. Among them, Nafion [20], poly(ester sulfonic acid) and poly(4-vinylpyridine) [21] are the most common. However, there is still an expanding demand for the development of a simple, reliable and efficient methodology for the simultaneous determination of AA and DA [1,2,4].

In the electroanalysis of neurotransmitters, carbon electrodes have been widely used in comparison to the metal electrodes due to its biocompatibility with tissue, having low residual current over a wide potential range and minimal propensity to show a deteriorated response as a result of the electrode fouling [13,22–25].

According to Rusling [26] and Sivagnam and Palaniandavar [27], micellar aggregates (composed by surfactants) may mediate catalytic systems, thus, presenting strong attractive for the development of new methodologies based on the surfactants at the electrode surface. Among these strong attractive, significant change in the redox potential, charge transfer and diffusion coefficients of the electrode processes, stability of electrogenerated intermediates and products derived from electrochemical reactions, as well as in redox events in biological systems can be considered [26–28]. Thus, Szymula and Narkiewicz-Michalek [29] have studied the behaviour of electrochemical oxidation of ascorbic acid in aqueous solution with the surfactants sodium dodecylsulphate (SDS-anionic), sodium-bis(2-ethylhexyl)sulfosuccinate (AOT-anionic), octylphenol ethoxylate (Triton X-100-nonionic) and cetyltrimethylammonium bromide (CTAB-cationic). It was verified that surfactants shift the oxidation peak potential of the ascorbic acid and change the peak current value, mainly due to the surfactant film formed at the electrode/solution interface. Based on this fact, the negative charged ascorbic acid has a tendency to accumulate in the positively charged crown of cationic micelles, which enhances the rate of oxidation and consequently provoke an increase in the peak current. However, the nonionic and anionic surfactants act in an opposite way.

Moreover, surfactants play a very important role in the increase of the solubility of an organic substance, which is either insoluble or sparingly soluble in water [26,27]. Jaiswal et al. [30] reported that the oxidation potential of the Vitamin E (α-tocopherol) was shifted, in cationic surfactant medium, toward more positive value in comparison to the anionic or nonionic surfactants, in mixed solvent systems of surfactant/ethanol/water and surfactant/acetonitrile/water. The simultaneous determination of Vitamin E and ascorbic acid was carried out in the above solvent systems.

Thus, the present work describes a study based on cyclic voltammetry and differential pulse voltammetry techniques, for simultaneous determination of AA and DA in cationic CPC micellar system (cetylpyridinium chloride), using a glassy carbon electrode as a working electrode. Since these compounds exhibit opposite micellar effect their overlapped anodic peaks can be separated in the micellar solution.

2. Experimental

All electrochemical experiments were performed using a potentiostat/galvanostat Autolab® PGSTAT-12 (Eco Chemie B.V., The Netherlands). Experiments were performed in a conventional single-compartment three-electrode cell. A glassy carbon electrode (Metrohm, 2.0 mm in diameter) employed as a working electrode was carefully polished with 0.5 μm alumina slurry on a flat surface, rinsed thoroughly with deionized water, and then sonicated immediately before using in deionized water for 2 min. A platinum wire was employed as an auxiliary electrode. All potentials were recorded in relation to a saturated calomel reference electrode (SCE).

All reagents such as 3,4-dihydroxyphenylamine (dopamine, DA, Sigma–Aldrich), ascorbic acid (AA, Sigma–Aldrich) and cetylpyridinium chloride (CPC, Sigma–Aldrich), were used as received without further purification. Aqueous solutions were prepared with deionized water ($\rho > 18.2\,M\Omega$, Millipore Milli-Q system) and other chemicals used were of analytical grade. All electrochemical experiments were carried out under an atmosphere of high purity nitrogen and at room temperature. For voltammetric experiments, unless otherwise indicated, a 0.1 mol L^{-1} aqueous phosphate buffer ($4.0 \leq pH \leq 8.0$) solution was used as supporting electrolyte. The potential for voltammetric experiments was recorded from −200 to +700 mV.

3. Results and discussion

3.1. Electrochemical oxidation of DA and AA

The cyclic voltammograms recorded with DA at glassy carbon electrode in 0.1 mol L^{-1} phosphate buffer (pH 6.0) in the absence (dashed line) and in the presence of cationic surfactant CPC (solid line) are shown in Fig. 1A. As shown, in the absence of CPC the oxidation of DA takes place at 250 mV and a quasi-reversible cyclic voltammogram is verified. The electron transfer for the oxidation of DA, in the presence of cationic surfactant cetylpyridinium chloride, is rather sluggish presumably due to the electrostatic repulsion of positively charged DA (pK_a 8.92) [1,3,31,32] with the cationic surfactant CPC (Fig. 1A, solid line). Adding CPC to the solution a shift of the anodic peak potential, E_{pa}, toward more positive values and cathodic peak potential, E_{pc},

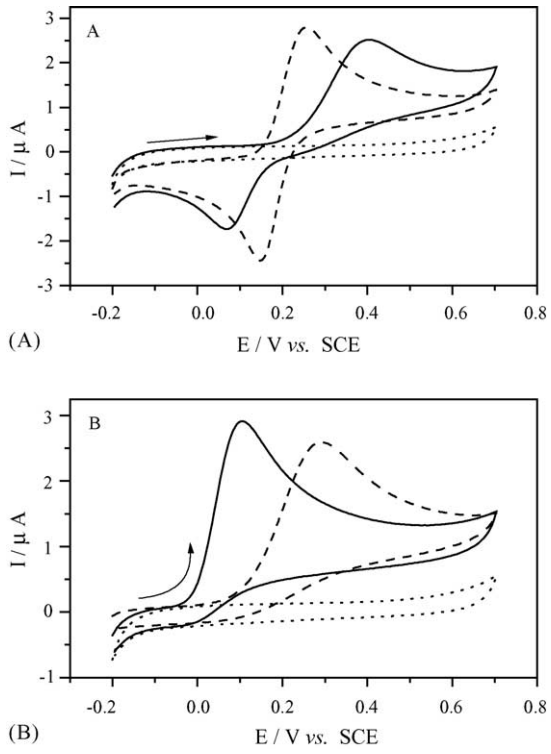

Fig. 1. Cyclic voltammograms of 100 μmol L^{-1} dopamine (A) and 100 μmol L^{-1} ascorbic acid (B) recorded at a glassy carbon electrode in 0.1 mol L^{-1} phosphate buffer (pH 6.0). (Dotted line) blank; (dashed line) in aqueous solution; (solid line) in 2.0 mmol L^{-1} CPC.

at less positive values is observed. In addition, a decrease in both peak currents, I_{pa} and I_{pc} was verified. The separation between anodic and cathodic peak potentials (ΔE_p) in the absence of CPC was found to be 100 ± 2 mV while a value of 320 ± 5 mV in the presence of the cationic surfactant was verified. The very broad cyclic voltammetric response at glassy carbon electrode in the presence of CPC resulting in a higher overpotential is a good indicative of the decrease in the rate of electron transfer [3,33].

The behaviour of AA oxidation at glassy carbon electrode in 0.1 mol L^{-1} phosphate buffer at pH 6.0 in the absence (dashed line) and in the presence of cationic surfactant CPC (solid line) can be observed in Fig. 1B. As shown, the oxidation of AA in the absence of CPC takes places around 280 mV (Fig. 1B, dashed line) and the electron transfer rate is rather sluggish owing to fouling of the electrode surface by the adsorption of the oxidation product of AA [6,32].

It is important to stress that according to the literature, the oxidation of AA involves two protons and two electrons in acid medium [34]. However, in the experimental condition (pH 6.0), which the pH value is higher than the first pK_a (4.17) of AA, the oxidation process involves in fact the loss of single proton and two electrons [6,32]. As the ascorbic acid is in the monoprotonated form, in micellar medium it is established an electrostatic interaction with cationic surfactant CPC, which shift the anodic peak potential, E_{pa}, to less positive values as well as provokes slight increase in the peak current, I_{pa}. As shown in Fig. 1B, the oxidation peak of AA in the presence of CPC, at 100 mV with $E_p - E_{p/2}$ of 63 mV was less broad than that observed in absence of surfactant. Considering the cyclic voltammetry behaviour of AA at glassy carbon electrode in the presence of CPC, it is possible to emphasize that electron transfer reaction is increased.

The significant shift in the oxidation peak potential as well as the changing of the peak current for both AA and DA after adding CPC can be assigned to the adsorption of the surfactant onto electrode surface, which may change the overpotential of the electrode and influence the electron transfer rate. The formation of micellar aggregates may also influence the mass transport of electroactive species to the electrode [3,34,35]. In the present study, a possible adsorption of the cationic surfactant CPC onto electrode surface may result in a positively charged hydrophilic film with the polar head group towards to the bulk of the solution. This positively charged hydrophilic layer makes difficult the dopamine reaches the electrode surface, and as consequence, the reaction becomes difficult. This micellar effect on the oxidation of DA is basically an electrostatic interaction between the surfactant film adsorbed on the electrode and the protonated dopamine [26,34–36]. On the other hand, the adsorption of the cationic surfactant CPC may lead to an enhancement of the electrostatic attraction between the cationic film and anionic species onto electrode surface, thus, decreasing the overvoltage and increasing the electron transfer reaction [3,13]. In order to prove that the films of CPC adsorbed on the electrode surface is the responsible for the electrochemical behaviour observed in presence of CPC, an investigation preparing the film in a concentrated CPC solution before the measurements was carried out. After immersing the electrode in a 0.1 mol L^{-1} CPC solution during 20 min, and then washed with water, the cyclic voltammogram recorded in presence of AA and DA without CPC in the medium was similar to those obtained in the CPC presence, indicating the CPC film formation on the electrode surface. However, the CPC film adsorbed on the electrode surface is not stable, presumably due to the leaching out (data not shown).

Since AA and DA have similar oxidation potential at most solid electrodes, simultaneous determination of these species is a great problem due to its overlapped signal [2,13,32,37]. Thus, simultaneous determination of AA and DA mixture was carried out by using the cationic surfactant CPC in order to evaluate the selectivity of the present procedure. Fig. 2 shows the cyclic voltammograms of a mixture of AA and DA in 0.1 mol L^{-1} phosphate buffer solution in the absence (solid line) and in the presence (dashed line) of cationic surfactant CPC. A single anodic oxidation peak was observed at 260 mV for the mixture of AA and DA in the absence of CPC (Fig. 2, solid line). On the other hand, two well-defined anodic peaks were observed for the oxidation of AA and DA when CPC was used.

The difference between the peak potentials is about 300 mV, which is enough for the simultaneous quantification

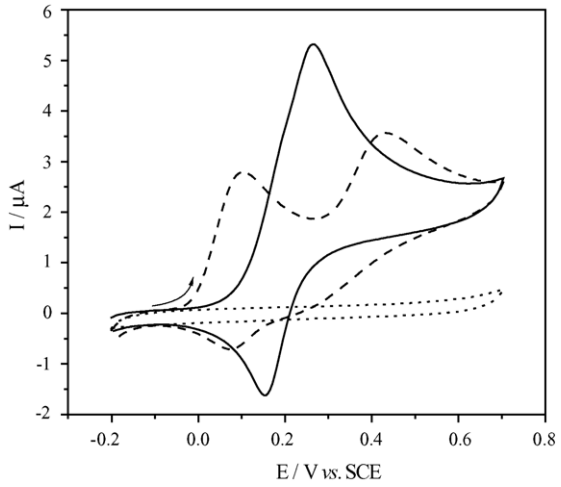

Fig. 2. Cyclic voltammograms of mixtures of dopamine (100 μmol L^{-1}) and ascorbic acid (100 μmol L^{-1}) recorded at a glassy carbon electrode in 0.1 mol L^{-1} phosphate buffer (pH 6.0). (Dotted line) blank; (solid line) in aqueous solution; (dashed line) in 2.0 mmol L^{-1} CPC.

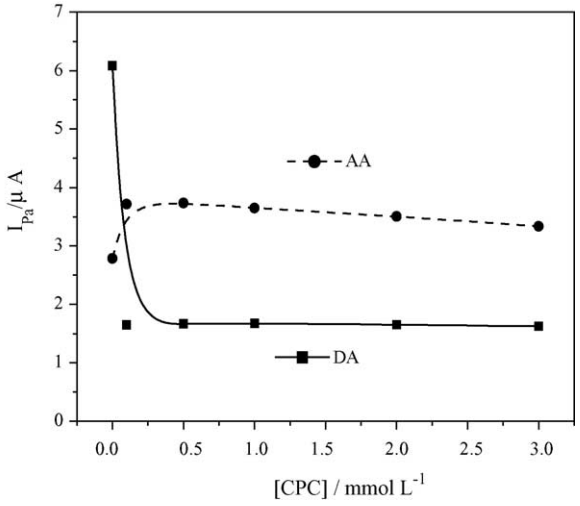

Fig. 4. Variation of anodic peak current (I_{pa}) of dopamine and ascorbic acid at a glassy carbon electrode with the surfactant concentrations. Experimental conditions were the same as indicated in Fig. 2.

of AA and DA, indicating that these species do not interfere each other.

3.2. Effect of CPC concentration on DA and AA oxidation

The dependence of surfactant concentration on the E_{pa} and I_{pa} is illustrated in Figs. 3 and 4, respectively. The plateau in the plot of E_{pa} and I_{pa} versus the surfactant concentration demonstrates the saturation of the adsorbed surfactant onto electrode surface. After completing the coverage of the electrode surface, the surfactant can form micelles in the bulk, and consequently, it cannot affect the electrode oxidation process anymore. It was verified that in low surfactant concentrations occur an abruptly change in the E_{pa} and I_{pa} around the critical

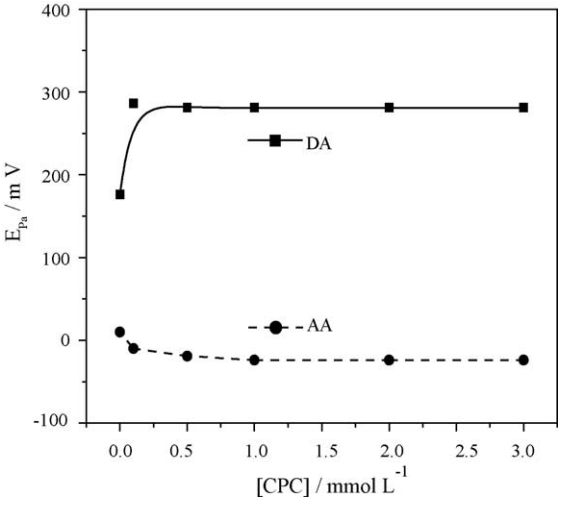

Fig. 3. Variation of anodic peak potential (E_{pa}) of dopamine and ascorbic acid at a glassy carbon electrode with the surfactant concentrations. Experimental conditions were the same as indicated in the Fig. 2.

micellar concentration (CMC) and reach a plateau above the CMC [3,28]. Both, E_{pa} and I_{pa} were dependent on the charge and the concentration of surfactant. It has been well established that the saturated adsorption of surfactants on solid surfaces generally coincides with the CMC of the surfactant [34]. Thus, as shown in Figs. 3 and 4, it is possible to verify that CPC concentrations of 1.0 mmol L^{-1} was enough to a complete saturation of electrode surface with CPC charged species. Hence, this value was chosen for further experiments.

In order to confirm the anti-fouling properties of CPC, 20 cyclic voltammograms were carried out in 100 μmol L^{-1} DA solution in the presence (1.0 mmol L^{-1}) and absence of CPC in the media. DA was chosen due to its stronger production of oxidation products and consequently the fouling of the electrode surface. The electrode response was very stable and showed excellent anti-fouling properties of CPC against the oxidation products of DA, which no decrease was observed even after more than 20 cycles (data not shown). In absence of CPC the surface fouling are notorious at the bare electrode (a decrease of 30% in the response after 20 cycles). The linear relation between I_p and $\nu^{1/2}$ for DA in various scan rates indicates that the process is diffusion-controlled, thus, corroborating the anti-fouling properties of CPC and obviously avoiding the adsorption of DA onto electrode surface.

3.3. Effect of pH on DA and AA oxidation

The voltammetric behaviours of AA and DA, in the presence of cationic surfactant CPC, were evaluated in 0.1 mol L^{-1} phosphate buffer at different pH values. All peak potentials of AA and DA shifted toward more negative values by increasing the solution pH (4–8). This behaviour can be explained due to the deprotonation step involved in all oxidation process that is facilitated at higher pH value [38]. Although both anodic peaks for AA and DA shift toward less

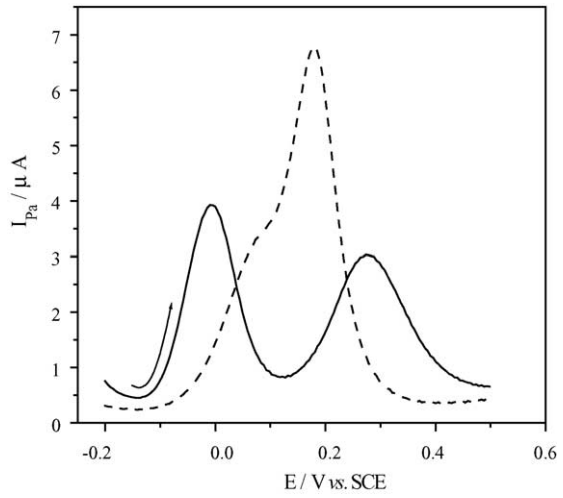

Fig. 5. Differential pulse voltammograms (DPV) for the mixture of a solution of 100 μmol L^{-1} AA and 100 μmol L^{-1} DA in 0.1 mol L^{-1} phosphate buffer (pH 6.0): (dashed line) in the absence of CPC and (solid line) in the presence of 1.0 mmol L^{-1} CPC. Potential amplitude of 50 mV. Step potential of 5 mV.

positive values increasing the pH solution, the oxidation peak of AA shift less than that observed for DA. The difference (ΔE_{DA-AA}) between AA and DA peak potentials decreases with the pH increase. This feature is of paramount importance since at pH 6.0, a difference of 320 mV is achieved. As can be observed in Fig. 2 (solid line), AA and DA can be completely separated allowing simultaneous determination of AA and DA in a mixture [15].

Furthermore, AA has not shown any catalytic effect in the DA oxidation since AA oxidizes before DA. Thus, 0.1 mol L^{-1} phosphate buffer at pH 6.0 was chosen as the supporting electrolyte in this work.

3.4. Simultaneous determination of AA and DA

According to the achieved results from cyclic voltammetry, CPC may be successfully used for the simultaneous determination of AA and DA at the glassy carbon electrode. In this way, differential pulse voltammograms of coexisting species AA and DA were performed aiming to develop a new analytical method. Fig. 5 displays the differential pulse voltammograms of AA and DA in aqueous solution buffered at pH 6.0 with phosphate buffer. As expected, in the absence of cationic surfactant CPC, it was verified an interference in the AA and DA determination which is indicated by an overlap of anodic peaks (Fig. 5, dashed line), thus, disabling the simultaneous determination of AA and DA. However, as observed in Fig. 5 (solid line), the measurements performed in the presence of CPC allows a remarkable separation of AA and DA peaks. The anodic peak potential, E_{pa}, for AA was shifted toward more negative values (−10 mV), whereas for DA, it was shifted toward more positive values (+310 mV). Taking into account, the separation of anodic peak potential between AA and DA in CPC micelles is 320 mV at pH 6.0, which is more than enough to allow selective detection of the two compounds.

In order to verify the performance of the method for the separation of the peak potentials of AA and DA even in high concentration, it was recorded differential pulse voltammograms at different concentrations ranging from 15 to 90 μmol L^{-1} of AA and DA in phosphate buffer solution at pH 6.0 (Fig. 6). As observed, a remarkable separation is still clear with the increase of AA and DA concentrations. Hence, calibration curves constructed for AA and DA were linear for a wide range of concentrations (5–75 μmol L^{-1} for AA and 10–100 μmol L^{-1} for DA) with correlation coefficients of 0.9973 and 0.9998, respectively. Detection limits was found to be 1.5 and 2.0 μmol L^{-1}, respectively, for AA and DA. The precision ($n = 7$) assessed as relative standard deviation (R.S.D.), for AA and DA were, respectively, 2.76 and 2.29% for 20 μmol L^{-1} concentration and 0.30 and 0.62% for solution containing 70 μmol L^{-1}.

Other interesting study was carried out to evaluate the influence of different AA and DA concentrations on separation effectiveness of peak potentials. For this task, DA concentration was fixed at 40 μmol L^{-1} and AA concentration was increased from 40 to 100 μmol L^{-1} (Fig. 7A). In the same way, the influence of high DA concentration on the separation of the peak potential of AA was checked by increasing the DA concentration from 40 up to 100 μmol L^{-1} setting the AA concentration at 40 μmol L^{-1} (Fig. 7B). According to results, it is possible to note that the outstanding usefulness

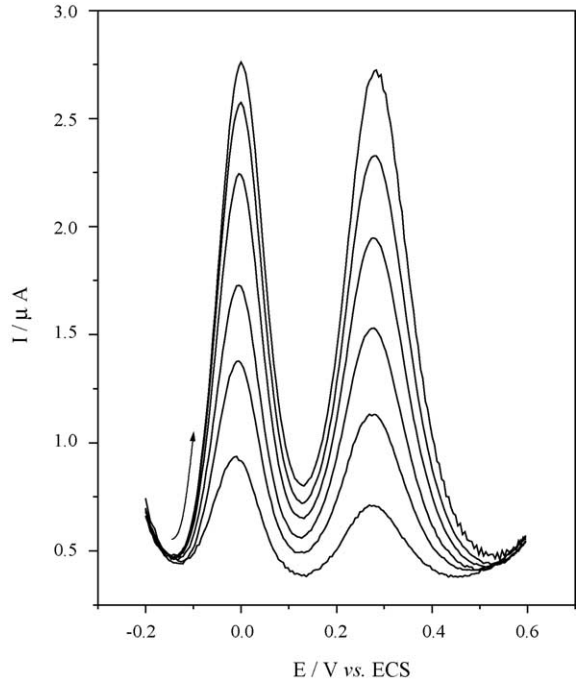

Fig. 6. Differential pulse voltammograms of AA and DA in 0.1 mol L^{-1} phosphate buffer (pH 6.0) containing 1.0 mmol L^{-1} CPC. Concentrations of both AA and DA are simultaneously changed. [AA] = [DA]: (a) 15 μmol L^{-1}, (b) 30 μmol L^{-1}, (c) 45 μmol L^{-1}, (d) 60 μmol L^{-1}, (e) 75 μmol L^{-1} and (f) 90 μmol L^{-1}.

Table 1
Recovery results obtained for simultaneous AA and DA determination in medicine samples

DA labelled concentration (mg mL^{-1})	Added concentration (mg mL^{-1})	DA found[a] (mg mL^{-1})	Recovery (%)
5	0	4.90 ± 0.26	98.0
	5	10.61 ± 0.57	107.2
	15	19.20 ± 0.23	96.5
AA spiked (mg mL^{-1})	**Added concentration (mg mL^{-1})**	**AA found[a] (mg mL^{-1})**	**Recovery (%)**
3.50	0	3.44 ± 0.14	98.3
	3	6.68 ± 0.28	103.7
	9	13.35 ± 0.26	107.3

[a] Results are expressed as mean value ± S.D. based on three replicate. Confidence interval: 95%.

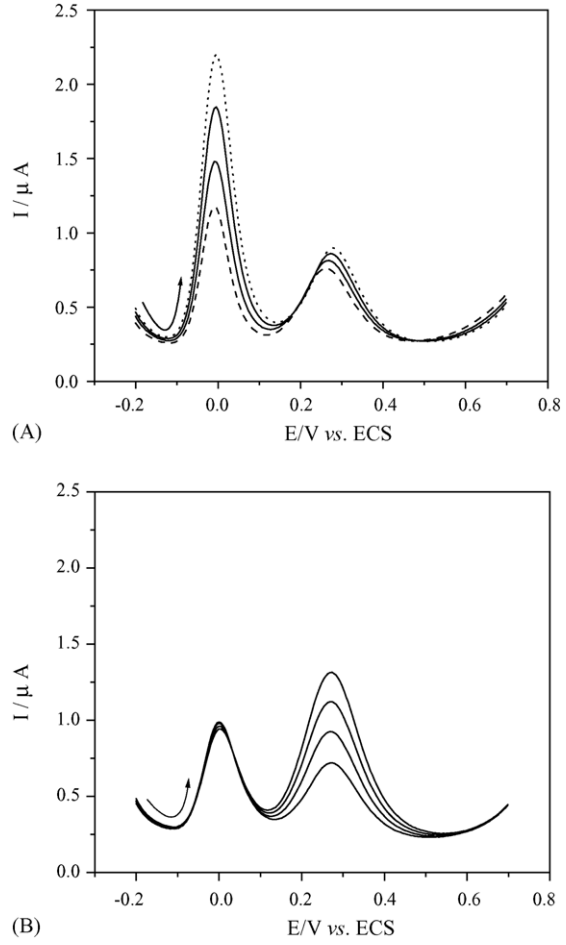

Fig. 7. Profile of differential pulse voltammograms obtained at different concentrations of AA and DA. (A) Concentration of AA ranging from 40 to 100 μmol L^{-1} and DA fixed at 40 μmol L^{-1}; (B) concentration of DA ranging from 40 to 100 μmol L^{-1} setting the AA concentration at 40 μmol L^{-1}. Conditions: 0.1 mol L^{-1} phosphate buffer (pH 6.0) containing 1.0 mmol L^{-1} CPC.

of micellar environment for separation of AA and DA peak potentials even in different proportions.

3.5. Determination of DA in medicine samples

An aliquot of 2 mL of dopamine hydrochloride injection (DHI) solution (standard content of 5 mg mL^{-1} DA, 10 mL per injection) (Revivan®, Zambon Group) was diluted to 10 mL with water and 15 μL of this solution was added in an electrochemical cell and the volume was made up to 5 mL with 0.1 mol L^{-1} phosphate buffer at pH 6.0. The standard addition technique was used for DA determination in DHI sample. The anodic peak current was measured at +310 mV using differential pulse voltammetry. The results achieved by proposed method (5.01 ± 0.16 mg mL^{-1}) are in good agreement (confidence interval of 95%, $n = 6$) with that value labelled in the sample (5.0 mg mL^{-1}), thus, indicating the feasibility of the method for DA determination in sample.

3.6. Mixture analysis of AA and DA in medicine samples

In order to verify the performance and feasibility of the method for simultaneous analysis of AA and DA in medicine samples, dopamine hydrochloride injection (DHI) solution (standard content of 5.0 mg mL^{-1} DA, 10 mL per injection) (Revivan®, Zambon Group) was spiked with known amount of AA. In this sense, 1.89 mL of DHI solution as well as 2.5 mL of standard solution of AA (3.5 mg mL^{-1}) were transferred to calibrated flasks of 10 mL. Afterwards, 20 μL were added into the electrochemical cell containing 1.0 mmol L^{-1} of CPC whose volume was made up to 5 mL with 0.1 mol L^{-1} phosphate buffer solution at pH 6.0. The differential pulse voltammograms were recorded and the anodic peak currents were measured at −10 and 310 mV for AA and DA, respectively. The standard addition technique was employed for AA and DA determination. Accuracy of the proposed method was checked by using addition and recovery tests. As listed in Table 1, the results for AA and DA concentration obtained by proposed method corroborate to those values in the samples. Moreover, good recoveries (96.5–107.3%) with confidence interval of 95% were obtained for the analysed samples. These results show a good performance of the method for simultaneous AA and DA determination.

4. Conclusions

A novel approach for the utilization of cationic surfactants in electroanalytical applications is described in this work. The positively charged CPC adsorbed onto the electrode surface

control the electrode reactions of AA and DA that differ in their net charge. The oxidation of AA is facilitated at the positively charged surfactant CPC in solutions by electrostatic interaction of anionic AA with the CPC. On the other hand, DA, being positively charged, is repelled from the positively charged CPC in solution, shifting its electrooxidation potential toward more positive values. Other important point is the anti-fouling properties of CPC allowing the use of the electrode for long time with the same response. Considering to the analytical features of the method, it is possible to conclude that it presents advantageous characteristics when compared to those methods based on chemically modified electrode mainly due to simplicity since it is used a glassy carbon electrode without modification as well as due to low analytical cost considering the negligible cost of the surfactant. Reasonable values found in recovery tests corroborate to the applicability of the developed method for simultaneous quantitative determination of AA and DA in medicine samples.

Acknowledgments

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