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Simultaneous determination of ephedrine-hydrochloride and dopamine at poly 1, 8- diaminonaphthaline derivatives modified platinum electrode

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ABSTRACT

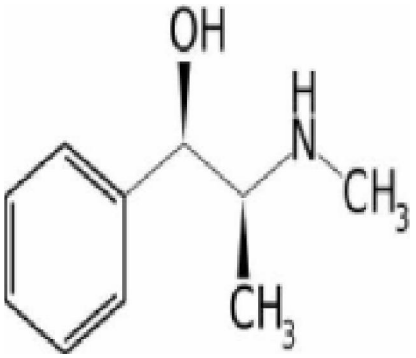
Simultaneous determination of ephedrine hydrochloride (Eph) and dopamine (DA) at poly 8-(3-acetylimino-6-methyl-2, 4-dioxpyran)-1-aminonaphthalene modified platinum electrode (PAMDAN/Pt) was developed at PAMADAN/Pt, the peaks of Eph and DA appear at 0.120 and 0.550 V in pH 3.2. PAMADAN/Pt does not only accelerate the oxidation of Eph and DA, but also enlarges dramatically the peak separation between Eph and DA. The PAMADAN/Pt was successfully applied to detect Eph in pharmaceutical samples and human blood serum.

KEYWORDS

Modified electrode;
Polymer film;
Conducting polymer;
Electro oxidation;
Sensor;
Ephedrine;
dopamine.

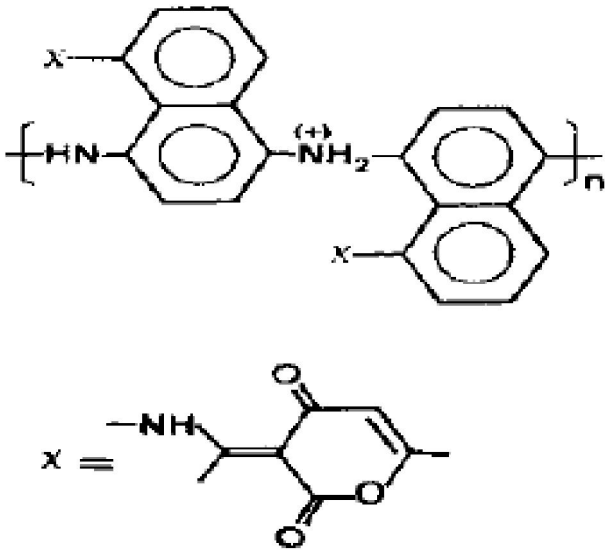
INTRODUCTION

Ephedrine (Eph) (scheme (1)) is a natural alkaloid, obtained by extraction from several plants of the genus Ephedra or by synthesis[1]. Eph has been used for the treatment of asthma, allergic states, catalepsy and myasthenia gravis[2]. Also, Eph has been considered as a prohibited compound by the International Olympic Committee[3] because it is included in the lists of doping substances. Eph and dopamine (DA) are considered as two important catecholamine neurotransmitters present in mammalian central nervous system[4]. The electrochemical detection of Eph at bare electrodes is associated with problems of adsorption and high overpotential. Therefore, Eph has been detected using different methods[5] including electrochemistry at modified electrodes[6-9]. Several analytical methods as fluorescence, spectrophotometry, flow injection and HPLC[10] were reported for the simultaneous determination of Eph and DA.



Scheme 1 : Ephedrine structure.

A survey in the literature indicates that there are only few electrochemical methods for simultaneous determination of Eph and DA[10, 11]. Kang et al.[10] re-



Scheme 2 : Structure proposed for PAMDAN polymer in ref. 21

ported the electrocatalytic response for the simultaneous determination of Eph and DA at 2,3-dimercaptosuccinic acid self-assembled gold electrode.

The electrochemical modification of electrodes with a suitable reagent has been widely used for analytical applications; the resulting electrodes were designed to provide the desired selective sites towards the analytes. Electrochemically modified electrodes have played an important role in the studies of electro catalysis[11, 12], electron transfer kinetics[13], membrane barriers[14], electro organic synthesis[15], etc. Conducting polymer-modified electrodes has been found to improve the electrode sensitivity and selectivity, and to reduce fouling effects in many applications. Recently, conducting polymer modified electrodes were used for the electro catalytic oxidation of several compounds[16] and for the simultaneous determination of the biological materials[17-19].

The present study is aimed to present the electro catalytic determination of Eph in the presence of DA at Poly 8-(3-acetylimino-6-methyl-2,4-dioxpyran)-1-aminonaphthalene/pt modified electrode (PAMDAN/Pt) (Scheme 2). The influence of various experimental conditions such as pH, scan rate and analyte concentration will be discussed. Also, the ability of modified electrode for practical applications as a dynamic sensor for Eph detection under the present experimental conditions for real samples will be explored.

EXPERIMENTAL

Ephedrine-HCl (α-(1-methylaminoethyl) benzylalcohol hydrochloride, $C_6H_5CH[CH(NHCH_3)CH_3]OH.HCl$) used in the present study was supplied from EIPICO pharmaceutical co. (Egypt). The other chemicals, 1, 8-diaminonaphthalene, dehydroacetic acid analytical grade (Aldrich), Lithium perchlorate, acetonitrile, and sulfuric acid were analytical grade. The aqueous solutions were prepared from bi-distilled water. Ephedrine-HCl ampoule 30mg/ml from CID pharmaceutical co. (Egypt) was used. Human serum samples were supplied by the local Hospital Blood Bank and from the investigators.

Cyclic voltammetry (CV) and square wave voltammetry (SWV) were recorded using a potentiostat Model (PST 006) from voltalab-Radiometer analytical with software Model voltaMaster 4. All voltammograms were obtained in a three-electrode electrolytic cell from Bioanalytical system, model C-1A which contains a platinum disc electrode (Pt) of 3.0 mm diameter as working electrode, a platinum coil as counter electrode and Ag/Ag^+ as a reference one. All experiments were conducted at room temperature. The Schiff base 8-(3-acetylimino-6-methyl-2, 4-dioxpyran)-1-aminonaphthalene (AMDAN) was previously prepared and characterized[20].

The electrochemical polymerization of AMADAN at pt-electrode was carried out by scan repletion using CV-method between 0.2V and 0.8V for 20 cycles in 0.1M H_2SO_4.

Human serum samples were obtained by centrifugation of human blood after two hours of intravenous injection at 6000 rpm for 15 min and immediately frozen at -20 ?C until assay. Frozen human serum samples were left on the bench to thaw naturally and were vortex prior to their use[21].

RESULTS AND DISCUSSION

PAMDAN/pt film formation

Figure 1 shows a typical cyclic voltammogram of 10^{-3}M of AMDAN Schiff base in 0.1 M H_2SO_4 aqueous solution at platinum electrode. For the first cycle the monomer electro-oxidizes in an irreversible manner

which give rise to a large anodic peak appeared at 0.5 V. With continuous scanning, a reduction peak at 0.2 V is developed due to the product formed during the first anodic sweep. The first irreversible segment can be attributed to the oxidation of the amino group into the corresponding cation radical[23-27]. On the negative sweep, this peak does not show a complementary reduction peak indicating the existence of a very fast chemical follow-up reaction[23, 24]. On scan repetition, the first oxidation peak at 0.5 V shifts to a more positive direction and diminishes with appearance of a new redox system at 0.28/0.20 V. The limiting currents of this new system increase by continuous cycling which indicates an accumulation of electroactive polymer film on the electrode surface. This growth behavior is in close agreement with those reported previously[22-27].

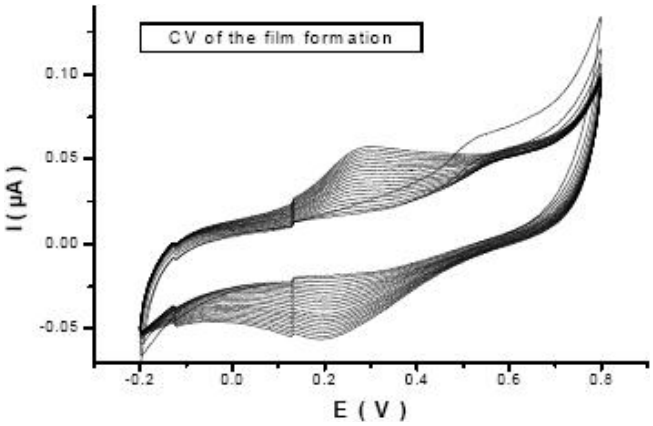


Figure 1 : CVs (20 cycles) corresponding to the electropolymerization of 1.0 mM AMDAN at Pt-electrode in 0.1 *M* H_2SO_4. The electrode potential was scanned at *v* = 100 mV/ s between –0.20 to 0.80 V vs. Ag/ AgCl.

Electro-oxidation of ephedrine-HCl

The electrochemical activity of the modified electrode was tested in both aqueous and non-aqueous media. Figure 2 shows the current response of a redox active site (dashed line) at the modified polymeric film electrode in 0.1 M H_2SO_4 at 0.05 and 0.17 V. The bare pt-electrode shows a nil peak currents at the applied potential range.

The modified electrode was tested in the presence of 10 µM Eph, where significant current responses arose at 0.17 and 0.25 V. This remarkable enhancement in current response provides clear evidence of the catalytic effect of the modified PAMDAN/Pt electrode which acts as a promoter to enhance the electrochemical oxidation of Eph.

A further study was conducted to test the electrocatalytic behavior of the modified electrode towards the oxidation of Eph at various pH values ranged between 1.0 and 7.0. The anodic peak potential for the Eph oxidation was found to shift to more positive potential at lower pH values. The current response decreases with increasing pH values to a large extent indicating a sluggish electron transfer process. Acidic or moderately acidic solutions are the most preferential media at which the polymeric surface film exhibited substantial catalytic activity.

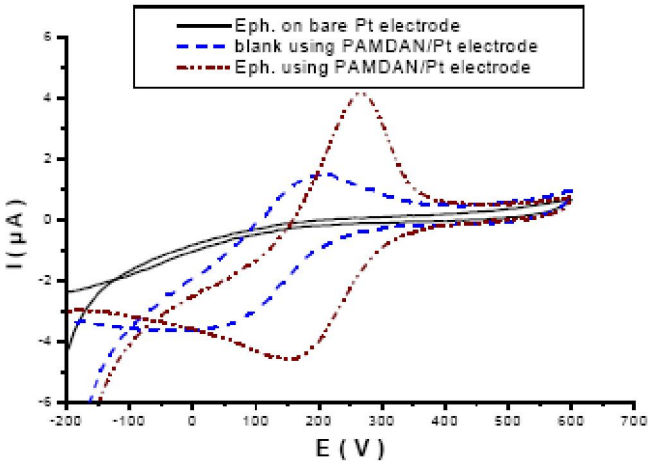


Figure 2 : CVs in the presence of 10 µM Eph recorded at the bare Pt-electrode (solid line) and the PAMDAN/Pt electrode (dash-dotted line) in 0.10 M H_2SO_4 at scan rate of 0.1 V/s. The dashed line CV is the activity of PAMDAN/Pt electrode in the absence of Eph.

Analytical Application

Electrochemical measurements of Eph

Square wave voltammetry (SWV) technique was employed as an active method for the determination of Eph. The electrode potential was scanned between -0.10 V to 0.50 V at scan rate of 0.005V/s. Figures 3 and 4 show the electrochemical oxidation of Eph present in low and high concentration ranges, respectively.

In both Figures, the anodic peak for Eph oxidation obtained at 0.12 V vs. Ag/AgCl and increased subsequently with increasing [Eph]. The regression data for the relationship between the peak current (I_p) and [Eph] for both data points presented in Figures 3 and 4 are given in TABLE 1. It is important to under line that the data presented in the TABLE does not only demonstrate the electrode sensitivity but suggest the construc-

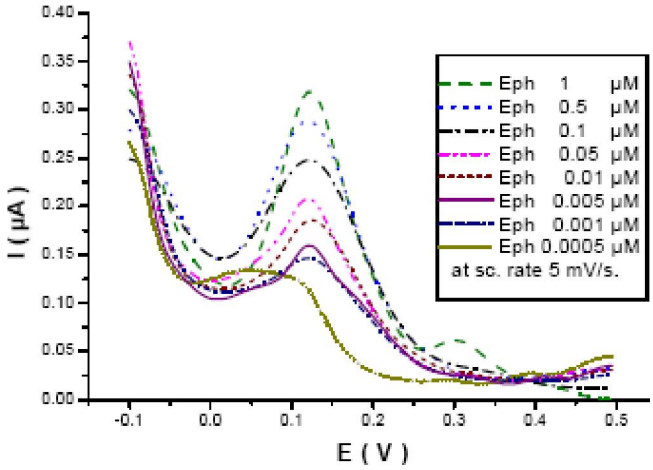


Figure 3 : Square wave voltammograms of diluted solutions of Eph in 0.1 M H_2SO_4 aqueous solution at PAMDAN/Pt modified electrode at scan rate 5 mV/s.

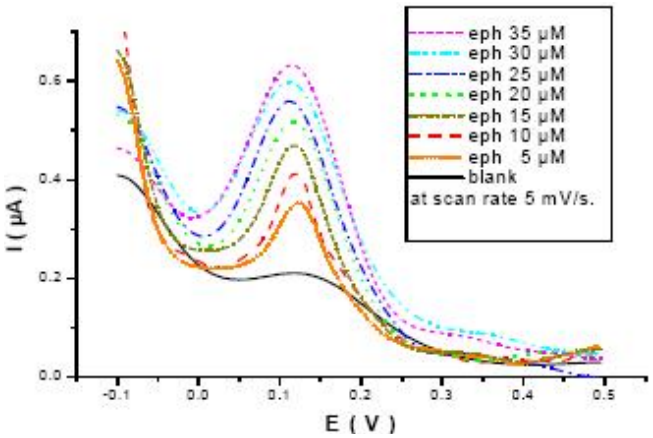


Figure 4 : Square wave voltammograms of different concentrations of Eph in 0.1 M H_2SO_4 aqueous solution at PAMDAN/Pt modified electrode at scan rate 5 mV/s.

TABLE 1 : Shows the regression data for the linear relationship between the peak currents as a function of [Eph].

	Figure 3	Figure 4
Slope	0.1655	0.0096
Intercept	0.1769	0.3145
Correlation coefficient (R^2)	0.7451	0.9807
Detection limit (DL)	0.0378	0.0159

tion of robust sensing surface.

Simultaneous determination of Eph and DA

The polymeric film was evaluated for its ability as an electro-catalyst for the simultaneous determination of DA and Eph. To achieve such important task a selection of buffering system and pH-scale is of great demand. It was shown above that Eph underwent a substantial oxidation in acidic or moderately acidic media. In a similar manner,

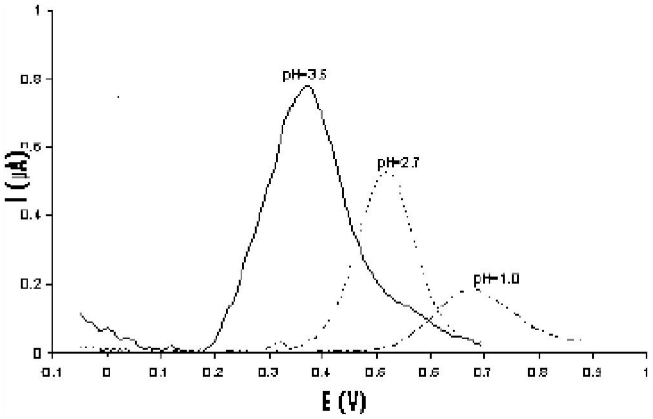


Figure 5 : Square wave voltammograms of 0.01 M DA in different pH values at PAMDAN/Pt modified electrode.

the selection of proper pH solution for DA oxidation may homogenize the experimental conditions for both species. Figure 5 shows the SWV for the oxidation of 0.01M DA in different pH media. The catalytic activity of PAMADAN/Pt towards DA oxidation enhanced at pH = 3.5 which will shift to more negative potential compared with those at lower pH values.

The ability to selectively determine DA and Eph has been a major goal of electroanalysis research due to their coexist in the extracellular fluid of the central nervous system and serum. Therefore, to examine the simultaneous response of Eph and DA at PAMDAN/Pt electrode, the SWV of simultaneously changing the concentration of mixture of DA and Eph was recorded at pH 3.2 (Figure 6). At the PAMADAN/Pt, the peaks of Eph and DA appear at 0.12 and 0.55 V in pH 3.2. The larger separation of the peak potentials allows

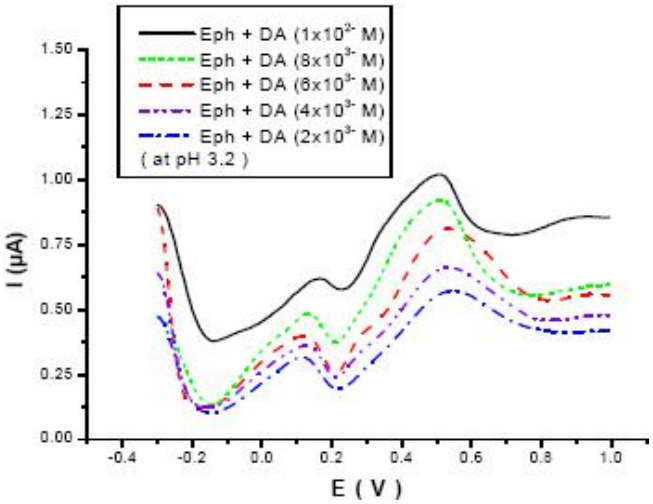


Figure 6 : Square wave voltammograms of simultaneous determination of different Eph and DA concentrations at PAMDAN/Pt modified electrode.

simultaneous detecting of them in their mixture solution. The PAMADAN/Pt displayed excellent electrochemical catalytic activities towards the oxidation of Eph and DA.

The above obtained results, demonstrate that PAMADAN/Pt not only accelerates the oxidation of Eph and DA, but also enlarges dramatically the peak separation between Eph and DA. On the other hand, the remarkable peak current enhancement and the fall of oxidation over-potentials, undoubtedly, testify to the electrocatalytic properties of the PAMADAN/Pt in the oxidation of these compounds. In conclusion, PAMADAN/Pt greatly improves the determining sensitivity of Eph and DA. The enlarged separation of the anodic peak potential, coupled with the increased peak current, renders simultaneous determination of Eph and DA feasible.

Real Sample Analysis

Detection of ephedrine hydrochloride in its pharmaceutical formulations and in the human blood serum sample

The cyclic voltammetry (CV) and square wave voltammetry (SWV) were applied to determine the concentration of Eph in both pharmaceutical forms and human blood serum samples. Figure 7 shows the SWV for a diluted concentration of 30 mg/ml of Eph ampoule (0.003 mg/ml) and one ml of human blood serum after two hours of intravenous administration of 30 mg/ml Eph ampoule. The concentration of Eph ampoule and the human blood serum sample concentration were calculated. The concentration of Eph ampoule was 29.5 mg/ml (*0.15* M). The concentration of 1 ml of human blood serum after two hours of intravenous administration of 30 mg/ml Eph ampoule was 0.0093 mg/ml (***47*** μM)). HPLC technique was applied to confirm the results obtained by CV and SWV. Chromatograms of Eph standard (for calibration) and the tested pharmaceutical samples of Eph were shown in Figure 8. The concentration of Eph ampoule was ***31.5*** mg/ml (*0.16* M). The concentration of 1 ml of human blood serum after two hours of intravenous administration of 30 mg/ml Eph ampoule was 0.0103 mg/ml (52 μM)). From the above results, it was found that HPLC results are comparable to those obtained in both CV and SWV techniques, proves that the use of PAMDAN/ Pt electrode as sensor for Eph is applicable, since it is sensitive and accurate method. Also, it is available and easy to operate. The limit of the linear response range for Eph was found to be 0.005 μM (0.001μg/ml) in both CV and SWV techniques, while the limit of detection of Eph by using HPLC technique not exceed than 0.005 μg/ml. This indicates that CV and SWV techniques are sensitive and give an accurate results more than other techniques such as HPLC[28, 29].

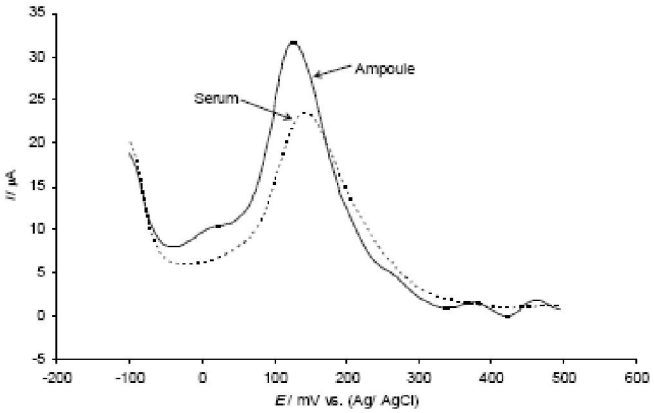


Figure 7 : Square wave voltammograms of 0.003 mg/ml of Eph in its pharmaceutical forms (Eph ampoule) and the human blood serum sample after two hours of taking Eph ampoule 30 mg/ml at PAMDAN/Pt modified electrode at scan rate 0.005 V/s in 0.1 M H_2SO_4.

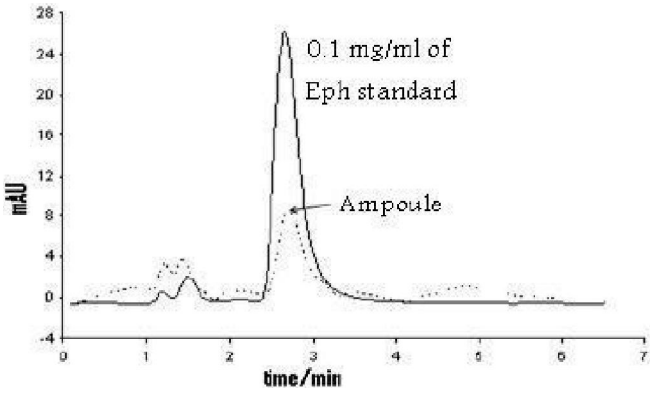


Figure 8 : Chromatographic response of 0.1 mg/ml of Eph standard (solid line) and 30 mg/ml Eph ampoule diluted to 0.02 mg/ml (dotted line).

CONCLUSIONS

The PAMDAN/Pt was developed and applied for the determination of Eph and DA in acidic solution. The anodic peak for Eph oxidation obtained at 0.12 V vs. Ag/AgCl and increased subsequently with increasing the concentration of Eph. The modified electrode showed excellent sensitivity and selectivity and fast re-

sponse. The utility of the proposed method was demonstrated by the determination of Eph in human blood serum. The superiority of the method over other voltammetric methods for the determination of Eph and DA lies in the fact that the PAMDAN/Pt electrode is able to achieve a very low detection limit of Eph and DA. The present work offers a good possibility for extending the proposed method to clinical and forensic analysis of Eph and DA in biological samples.

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