

Determination of epinephrine in the presence of uric acid and folic acid using nanostructure-based electrochemical sensor

M. Mazloun-Ardakani^{*,a}, H. Beitollahi, B.B.F.Mirjalili, A.Akbari

Department of Chemistry, Faculty of Science, Yazd University, Yazd, I. R. Iran

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**Corresponding author:*

E-mail address:

mazloun@yazduni.ac.ir

Phone: +98 351821 1670

Fax: +98 351 8210644

Abstract

Fabrication and electrochemical characterization of a sensor for the determination of epinephrine (EP), uric acid (UA) and folic acid (FA) is described. The sensor was prepared using carbon paste electrode (CPE) modified with 3,4-dihydroxybenzaldehyde-2,4-dinitrophenylhydrazone (DDP) and carbon nanotubes (CNTs), which makes the modified electrode highly sensitive for the electrochemical detection of these compounds. Cyclic voltammetry (CV) at various scan rates was used to probe the fabrication and characterization of the modified electrode. In order to characterize these new modified electrode, the electroanalytical response was evaluated for EP performing cyclic voltammetry, differential pulse voltammetry and chronoamperometry experiments. Under the optimum pH of 7.0, the oxidation of EP occurs at a potential about 215 mV less positive than that of the unmodified CPE. Differential pulse voltammetry (DPV) of EP at the modified electrode exhibited two linear dynamic ranges with a detection limit (3σ) of 70 nM. DPV was used for simultaneous determination of EP, UA and FA at the modified electrode, and quantification of EP in some real samples by the standard addition method.

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1. Introduction

Carbon paste electrode (CPE) is a special kind of heterogeneous carbon electrode consisting of mixture prepared from carbon powder and a suitable water-immiscible or non-conducting binder [1–3].

The use of carbon paste as an electrode was initially reported in 1958 by Adams [4]. In afterward researches a wide variety of modifiers [5–16] have been used with these versatile electrodes. CPEs are widely applicable in both electrochemical studies and electroanalysis field to their advantages such as very low background current (compared to solid graphite or noble metal electrodes), facility to prepare, low cost, large potential window, simple surface renewal process and easiness of miniaturization [17–19]. Besides the advantageous properties and characteristics listed before, the feasibility of incorporation different substances during the paste preparation (which resulting in the so-called modified carbon paste electrode), allow the fabrication of electrodes with desired composition, and hence, with pre-determined properties [20,21].

Since the discovery of carbon nanotubes (CNTs) in 1991 [22], numerous investigations were focused on the studies of their properties and applications [23–25]. Because of the special tube structure, CNTs possess several unique properties such as good electrical conductivity, high chemical stability and extremely high mechanical strength [26,27]. In addition, the subtle electronic behavior of CNTs reveals that they have the ability to promote electron-transfer reaction and have a high electrocatalytic effect when used as electrode materials [28,29]. All these fascinating properties make CNTs as a

suitable candidate for the modification of electrodes [30,31].

Epinephrine (EP) is important neurotransmitters in mammalian central nervous systems [32], and it exists in the nervous tissue and body fluid in the form of large organic cations. The changes of its concentration may result in many diseases [33]. Thus, a quantitative determination of EP concentration is significant for developing nerve physiology, pharmacological research and life science. There are some methods applied for the determination of EP. As an electroactive device, it can also be studied via electrochemical techniques. Some reports showed the electrochemical response of EP on different kinds of electrodes [34–38].

Uric acid (2,6,8-trihydroxypurine, UA) is the primary product of purine metabolism [39]. Physiological UA serum levels range from 41 to 88 mgmL^{-1} and urinary excretion is typically 250–750 mg per day [40]. Its abnormal concentration level in a human body may be symptoms of several diseases, such as gout, hyperuricaemia, and Lesch-Nyhan syndrome. Leukemia, pneumonia, and so on are also associated with enhanced urate levels [41]. So it is desirable to have a simple and direct method for monitoring the concentration of UA in biological fluids.

Several chronic diseases (for example, gigantocytic anemia, leucopenia, mentality devolution, psychosis, heart attack, and stroke), especially those concerned with malformation during pregnancy and carcinogenic processes, are related to the deficiency of folic acid (FA) [42] which is a water-soluble vitamin. Since FA is detected in biological fluids at very low concentration, i.e. $0.003\mu\text{gmL}^{-1}$ (for pancreatic cancerous patients) [43], a highly specific and

sensitive assay is required. Among the different methods for this purpose, electrochemical methods are found to be very promising [44–46].

Therefore, in the present work, we describe the preparation of a new electrode composed of CNPE modified with DDP (DDP-CNPE) and investigate its performance for the electrocatalytic determination of EP in aqueous solutions. We also evaluate the analytical performance of the modified electrode for quantification of EP in the presence of UA and FA.

2 Experimental

2.1 Materials and apparatus

Cyclic voltammetry (CV), differential pulse voltammetry (DPV) and chronoamperometry measurements were performed with an Autolabpotentiostat/galvanostat (PGSTAT-302 N, Eco Chemie, The Netherlands) equipped with General Purpose Electrochemical System (GPES) software. The electrochemical cell was assembled with a conventional three electrode cell: an Ag/AgCl/KCl (3.0 M) reference electrode, a platinum wire counter electrode, and the modified DDP-CNPE working electrode. All experiments were carried out at room temperature. A Metrohm 691 pH/Ion Meter was used for pH measurements.

All solutions were freshly prepared with double distilled water. EP, UA, FA and all other reagents were of analytical grade from Merck (Darmstadt, Germany). Multiwalled carbon nanotubes (purity more than 95%) with o.d. between 5-20 nm, i.d. between 2-6 nm, and tube length 1-10 μm were purchased from plasma Chem. The phosphate buffer solutions (PBS) were prepared from orthophosphoric acid and its salts in the pH range of 2.0–11.0.

2.2. Synthesis of 3,4-dihydroxybenzaldehyde-2,4-dinitrophenylhydrazone

For preparing of the title compound, 0.27 g (2 mmol) of 2,4-dihydroxybenzaldehyde, 0.4 g (2 mmol) of 2,4-dinitrophenylhydrazine and 0.3 g of 37% $\text{BF}_3 \cdot \text{SiO}_2$ were placed in a mortar and thoroughly mixed for 5 minutes. The resulting mixture was dissolved in chloroform and filtered under vacuum. The obtained orange-red solid was crystallized from ethanol. FTIR (KBr, cm^{-1}): 3493, 3286, 1605, 1510, 1445, 1416, 1325, 1269, 1170, 1132, 821, 739. $^1\text{H-NMR}$ (400 MHz, CDCl_3): δ = 5.62 (s, 1H), 6.93 (d, $J=8.4$, 1H), 6.99 (dd, $J=8.4$ and 2 Hz, 1H), 7.42 (sbr, 1 H), 8.16 (d, $J=9.6$, 1H), 8.3 (dd, $J=9.6$ and 3 Hz, 1H), 8.46 (s, 1H), 9 (d, $J=2$ Hz, 1H).

2.3. Preparation of the electrode

The DDP-CNPEs were prepared by dissolving 0.01 g of DDP in $\text{C}_2\text{H}_5\text{OH}$ and hand mixing with 0.89 g graphite powder and 0.1 g CNTs 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, DDP modified CPE electrode (DDP-CPE) without CNTs, CNT paste electrode (CNPE) without DDP, and unmodified CPE in the absence of both DDP and CNT were also prepared in the same way. Fig. 1 shows SEM images of the CNPE (Fig.1A) and CPE (Fig.1B). It can be clearly seen that carbon nanotubes dispersed in the paste homogeneously.

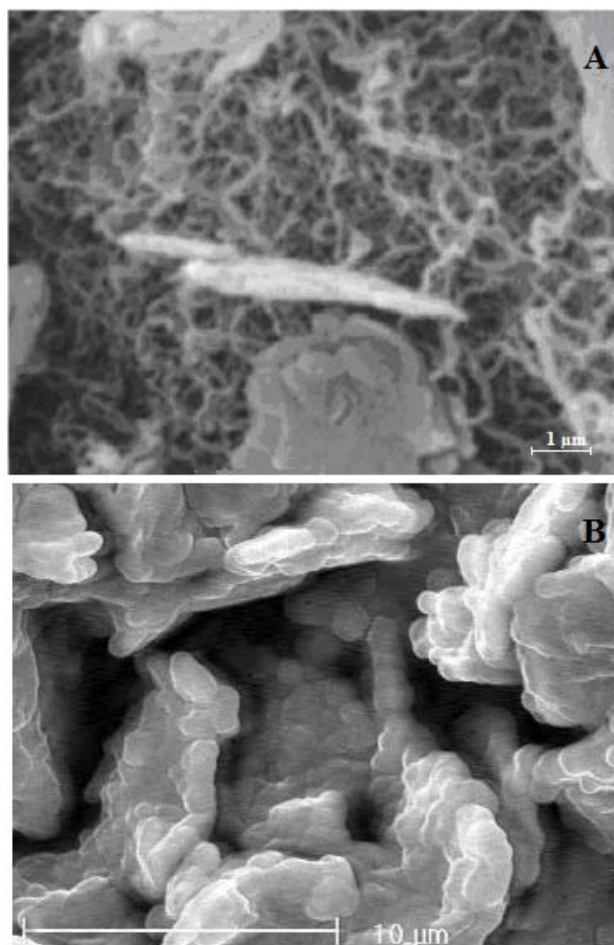


Fig. 1. SEM image of A) CNPE and B) CPE.

3. Results and discussion

3.1. Electrochemical properties of modified DDP-CNPE

As DDP is insoluble in water, therefore, we prepared modified electrodes based on DDP-CNPE and studied their electrochemical properties in a buffered aqueous solution (pH=7.0) using CV. It should be noted that one of the advantages of DDP as an electrode modifier is its insolubility in aqueous media. Experimental results showed reproducible, well-defined, anodic and cathodic peaks with E_{pa} , E_{pc} and $E^{\circ'}$ of 0.235, 0.150 and 0.192 V vs. Ag/AgCl, respectively. For DDP-

CNPE the peak-to-peak separation potential, $\Delta E_p = (E_{pa} - E_{pc})$ of 85 mV, was greater than the value of $59/n$ mV expected for a reversible system, suggesting that the redox couple of DDP in DDP-CNPE has a quasi-reversible behavior in aqueous medium. The effect of the potential scan rate (v) on electrochemical properties of the DDP-CNPE was also studied by CV (Fig. 2). Plots of the both anodic and cathodic peak currents (I_p) were linearly dependent on v in the range of 25 to 1500 mV s^{-1} (Fig. 2A), which showed that the redox reaction of DDP at the modified electrode is diffusionless in nature.

CV technique can be evaluated apparent charge transfer rate constant, k_s , and charge transfer coefficient, α , of a surface-confined redox couple by using the variation of anodic and cathodic peak potentials with logarithm of scan rate, according to the procedure of Laviron [47]. Such plots are shown in Fig. 2B, indicating that the E_p values are proportional to the logarithm of scan rate for v values higher than 15 V s^{-1} (Fig. 2B). The slopes of the plots in Fig. 2B are equal to $-2.303RT/\alpha nF$ and $2.303RT/(1 - \alpha)nF$ for the cathodic and anodic peaks, respectively. The slopes can be used to extract the kinetic parameters α_c and α_a (cathodic and anodic transfer coefficients, respectively). The evaluated value for the α_a is 0.5.

Also, from eq. 1 it is possible determine the electron transfer rate constant between modifier (DDP) and CNPE:

$$\log k_s = \alpha \log (1-\alpha) + (1-\alpha) \log \alpha - \log (RT/nFv) - \alpha (1-\alpha) nF\Delta E_p / 2.3RT \quad (1)$$

where $(1-\alpha)n_\alpha = 0.5$, vis the sweep rate and other symbols having their conventional meanings. The value of k_s was evaluated to be $39.8 \pm 0.3 \text{ s}^{-1}$ using Eq. (1).

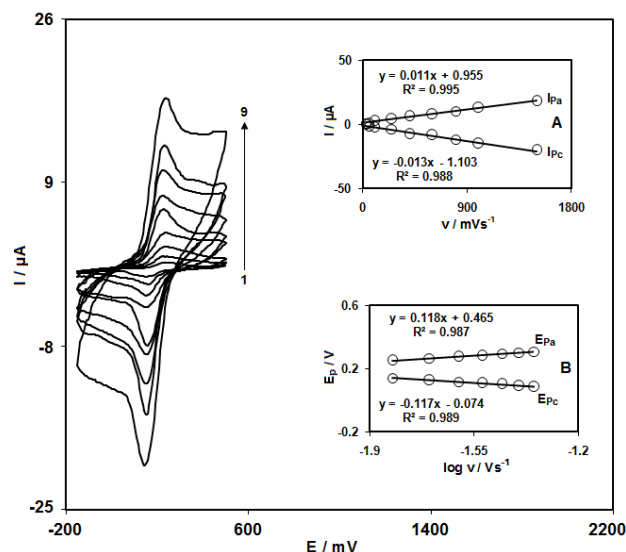


Fig. 2. CVs of DDP-CNPE in 0.1 M phosphate buffer (pH 7.0), at various scan rates, from inner to outer, 25, 50, 100, 250, 400, 600, 800, 1000 and 1500 mV s^{-1} . Insets: variation of (A) I_p vs. scan rate; (B) E_p vs. the logarithm of high scan rates.

3.2. Electrocatalytic oxidation of EP at a DDP-CNPE

The electrochemical oxidation responses of 0.1 mM EP at unmodified CPE (curve b), CNPE (curve d), DDP-CPE (curve e) and DDP-CNPE (curve f) were recorded by cyclic voltammetry. The results are shown in Figure 3. As it is seen, while the anodic peak potential for EP oxidation at the CNPE, and unmodified CPE are 390 and 450 mV, respectively, the corresponding potential at DDP-CNPE and DDP-CPE is ~ 235 mV. These results indicate that the peak potential for EP oxidation at the DDP-CNPE and DDP-CPE electrodes shift by ~ 155 and 215 mV toward negative values compared to CNPE and unmodified CPE, respectively. However, DDP-CNPE shows much higher anodic peak current for the oxidation of EP compared to DDP-CPE. The results demonstrated that the combination of CNTs and the mediator (DDP) had good

properties in electrocatalysing EP oxidation and facilitating electron transfer. In fact, DDP-CNPE in the absence of EP exhibited a well-behaved redox reaction (Fig. 3, curve c) in 0.1 M phosphate buffer (pH 7.0). However, there was a drastic increase in the anodic peak current in the presence of 0.3 mM EP (curve f), which can be related to the strong electrocatalytic effect of the DDP-CNPE towards this compound [48].

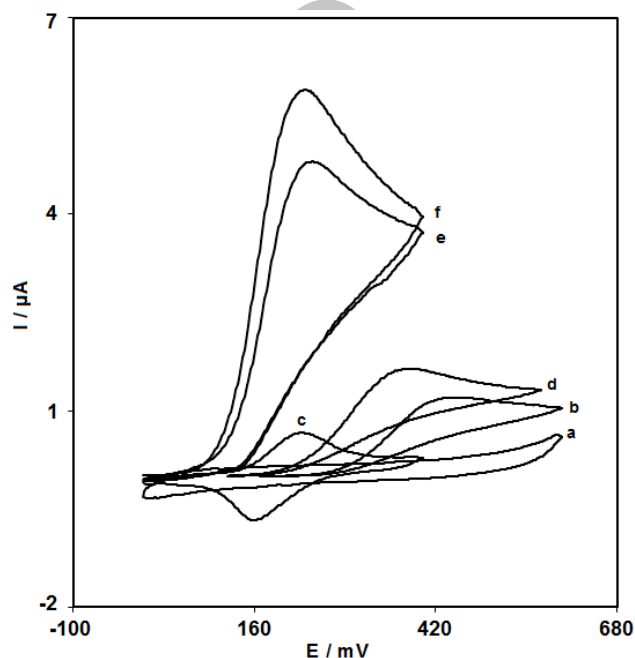


Fig. 3. CVs of (a) unmodified CPE in 0.1 M phosphate buffer solution (pH 7.0) at scan rate of 10 mV s^{-1} ; (b) as (a) + 0.1 mM EP; (c) as (a) at the surface of DDP-CNPE; (d) as (b) at the surface of CNPE; (e) as (b) at the surface of DDP-CPE; (f) as (b) at the surface of DDP-CNPE.

The cyclic voltammogram of DDP-CNPE at various scan rates in the presence of EP was studied (Fig. 4). It showed that the oxidation peak potential shifted to more positive potentials with increasing scan rate, confirming the kinetic limitation in the electrochemical reaction. The oxidation current (I_p) increased linearly vs. the square root of scan rate ($v^{1/2}$), suggesting that, at

sufficient overpotential, the reaction is mass transfer controlled (Fig. 4A) [48].

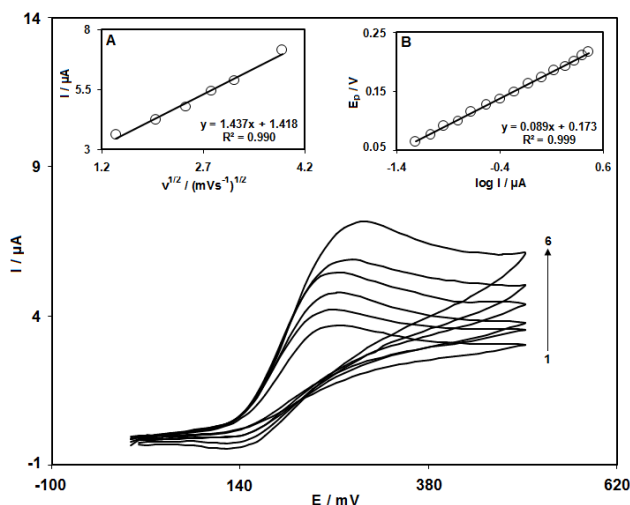


Fig. 4. CVs of DDP-CNPE in 0.1 M phosphate buffer solution (pH 7.0) containing 100.0 μM EP at various scan rates; The numbers 1-6 correspond to scan rates of 2, 4, 6, 8, 10 and 15 mV s^{-1} , respectively. Insets: variation of (A) anodic peak current vs. $v^{1/2}$; (B) Tafel plot derived from the rising part of the voltammograms recorded at the scan rates of 10 mV s^{-1} .

3.3 Chronoamperometric measurements

The chronoamperometry as well as the other electroanalytical methods was used for the investigation of electrode reaction at chemically modified electrodes. Fig. 5 shows chronoamperometric measurements of EP at DDP-CNPE. Chronoamperometric experiments were carried out at the working electrode potential of 300 mV for various concentrations of EP (Fig. 5). For an electroactive material (EP 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 [48]. Experimental plots of I vs. $t^{-1/2}$ were used, with the optimum fits for different concentrations of EP (Fig. 5A). The slopes of the resulting straight lines were then plotted vs. EP

concentration (Fig. 5B). From the resulting slope and Cottrell equation the mean value of the D was found to be $7.3 \times 10^{-6} \text{ cm}^2/\text{s}$.

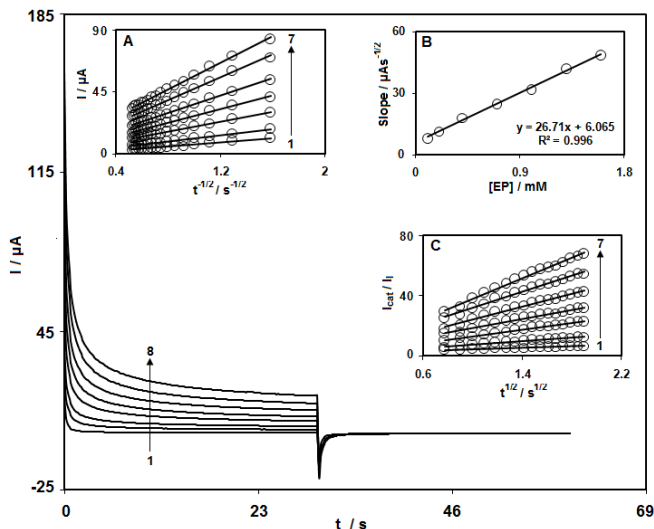


Fig. 5. Chronoamperograms obtained at DDP-CPE in 0.1 M phosphate buffer solution (pH 7.0) for different concentrations of EP. The numbers 1–8 correspond to 0.0, 0.1, 0.2, 0.4, 0.7, 1.0, 1.3 and 1.6 mM of EP. Insets: (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 2–8. (B) Plot of the slope of the straight lines against EP concentration. (C) Dependence of I_{cat}/I_1 on $t^{1/2}$ derived from the data of chronoamperograms 1–8.

3.4 Calibration plot and limit of detection

DPV method was used to determine the concentration of EP. The plot of peak current vs. EP concentration consisted of two linear segments with slopes of 0.152 and 0.019 $\mu\text{A } \mu\text{M}^{-1}$ in the concentration ranges of 0.1 to 35.0 μM and 35.0 to 750.0 μM , respectively. The decrease in sensitivity (slope) of the second linear segment is likely due to kinetic limitation. The detection limit (3σ) of EP was found to be 70 nM.

3.5. Simultaneous determination of EP, UA and FA

To our knowledge, there is no report on the simultaneous determination of EP, UA and FA using modified CNT-paste electrodes. The influences of UA and FA on the determination of EP were investigated by cyclic voltammetry. This was performed by simultaneously changing the concentrations of EP, UA and FA, and recording the DPVs. Fig.6 showed well-defined anodic peaks at potentials of 200, 400 and 730 mV, corresponding to the oxidation of EP, UA and FA, respectively, indicating that UA and FA had no influence on the determination of EP. Therefore simultaneous determination of these compounds is feasible at the DDP-CNPE.

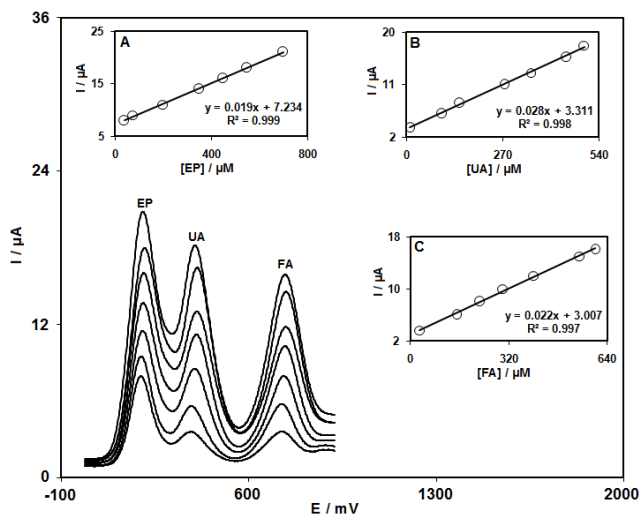


Fig. 6. DPVs of DDP-CNPE in 0.1 M phosphate buffer solution (pH 7.0) containing different concentrations of EP+UA+FA in μM, from inner to outer: 40.0+10.0+30.0, 75.0+100.0+150.0, 200.0+150.0+225.0, 350.0+275.0+300.0, 450.0+350.0+400.0, 550.0+450.0+550.0 and 700.0+500.0+600.0, respectively. Insets (A) (B) and (C) are plots of I_p vs. EP, UA and FA concentrations, respectively.

3.6. Interference study

The influences of various foreign substances such as some amino acids, glucose, NADH, L-penicillamine, levodopa, carbidopa, methionine and phenylalanine on the determination of EP were also investigated under the optimal condition. The tolerance limit was taken as the maximum concentration of the foreign substances which caused an approximately $\pm 5\%$ relative error in the determination. The results showed that the substances such as L-lysine, glucose, NADH, L-asparagine, glutamic acid, glycine, L-cystine, L-cysteine, penicillamine, levodopa, carbidopa, methionine and phenylalanine had no obvious influence on the results of the determination of EP.

3.7. Real sample analysis

To verify our results, one millilitre of an EP ampoule was diluted to 10 mL with phosphate buffer solution (0.1 M, pH 7.0); then, different capacity of the diluted solution was transferred into each of a series of 10 mL volumetric flasks and diluted to the mark with phosphate buffer. Each sample solution was transferred into the electrochemical cell and DPV was recorded between 0.0 and 0.5 V at a scan rate of 10 mV s^{-1} . The I_{pa} was measured at the oxidation potential of EP and the concentration of this compound was obtained from the calibration plot. This procedure was repeated five times for each sample, and the average amount of EP in the injection was found to be 0.989 mg, a value in well agreement with the value on the ampoule label (1.0 mg).

Also, to a series of 10 mL volumetric flasks, different capacity of the diluted EP injection solution together with standard UA and FA solutions were added and diluted to the mark with

phosphate buffer. The DPVs were recorded and the anodic peak currents for each of EP, UA and FA were measured at their own oxidation potentials. The recoveries were between 98.4-102.8 for the determinations of EP, UA and FA. Results were obtained with high reproducibility, which indicates that the sensor can be applied for the analysis of these compounds with no significant influence from each other.

4. Conclusion

In the present study, carbon-paste electrode modified was developed based on DDP and CNTs for the determination of EP in the presence of UA and FA. The ability of the electrode for analyzing of EP was demonstrated by CV and DPV. The detected potential differences of 200 mV, 530 mV and 330 mV between EP-UA, EP-FA and UA-FA, respectively, are large enough to allow simultaneous determination of EP, UA and FA in mixtures without significant interferences. In addition, the electrode enjoys some advantages as high sensitivity, selectivity and reproducibility of the voltammetric responses, and very low detection limit (70 nM), together with the ease of preparation and surface regeneration. The proposed nanostructure electrode is very useful for accurate determination of EP in real samples.

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Determination of epinephrine in the presence of uric acid and folic acid using nanostructure-based electrochemical sensor

M. Mazloum-Ardakani^{*,a}, H. Beitollahi, B.B.F. Mirjalili, A. Akbari

Department of Chemistry, Faculty of Science, Yazd University, Yazd, I. R. Iran

اندازه گیری اپی نفرین در حضور اوریک اسید و فولیک اسید با استفاده از سنسور
الکتروشیمیایی بر پایه ی نانو ساختارها

چکیده:

در این کار توصیف الکتروشیمیایی یک سنسور برای اندازه گیری اپی نفرین، اوریک اسید و فولیک اسید مورد بحث قرار گرفته است. سنسور با استفاده از الکتروود کربنی (CPE) با ۴۳- دی هیدروکسی بنز آلدهید-۲ و ۴- دی نیترو فنیل هیدرازون و نانوتیوب های کربنی که الکتروود اصلاح شده با حساسیت بالا را می سازد برای شناسایی الکتروشیمیایی این ترکیبات تهیه شده است. به منظور بررسی این الکتروود اصلاح شده جدید، جواب های الکتروشیمیایی برای ولتامتری سیکلی انجام دهنده، ولتامتری پالس تفاضلی و آمپرومتری گرانو ارزیابی شده اند. تحت pH بهینه ۷ اکسید شدن اپی نفرین در پتانسیل حدود ۲۱۵ میلی ولت کمتر از CPE اصلاح نشده اتفاق می افتد. ولتامتری پالس تفاضلی اپی نفرین در الکتروود اصلاح شده دو رنج دینامیکی خطی با یک حد شناسایی ۷۰ nM نشان می دهد. ولتامتری پالس تفاضلی برای اندازه گیری همزمان اپی نفرین، اوریک اسید و فولیک اسید در الکتروود اصلاح شده و تغییر خاصیت اپی نفرین در نمونه های واقعی به روش افزایش استاندارد مورد استفاده قرار گرفته است..