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Electrochemical determination of epinephrine, uric acid and folic acid using a carbon paste electrode modified with novel ferrocene derivative and core–shell magnetic nanoparticles

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Abstract

The purpose of the current research was to construct an intermediate (2-(4-Ferro-cenyl-[1,2,3]triazol-1-yl)-1-(naphthalen-2-yl) ethanone (2FTNE)) and a magnetic core–shell manganese ferrite nanoparticle (CMNP) to synthesis a modified carbon paste electrode (CPE). The electrochemical behavior of epinephrine (EP) was assessed by cyclic voltammetry and measured by square wave voltammetry by exposing to uric acid (UA) and folic acid (FA). High electrocatalytic activity for EP oxidation was seen in the 2FTNECMNP-modified CPE (2FTNEMCNPPE) compared to only CPE- and MCNP-modified CPE. The oxidation peak current of EP was linear within the range of 0.05–60 μM and its correlation coefficient was 0.999. The limit of detection was calculated at 0.016 μM for EP based on three times the standard deviation ($3S_b$) of the blank. According to the results, high selectivity, proper stability and good reproducibility was observed for the 2FTNEMCNPPE that detected EP, UA and FA successfully in an EP ampule, a folic acid tablet and urine samples, introducing 2FTNEMCNPPE with high capacity to develop the electrochemical sensors.

Keywords Epinephrine · Uric acid · Folic acid · Voltammetry · Magnetic core–shell manganese ferrite nanoparticles · Drug analysis

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Introduction

Epinephrine, uric acid and folic acid play a medically interesting and importing role in the metabolism reactions of the human body [1, 2]. Studies have shown that the epinephrine (EP) has functional effects on central nervous, hormonal, and cardiovascular systems. Multiple diseases can be developed following the EP level changes that could be related to many, indicating the importance of any new approach in measuring the EP level to detect the corresponding disorders [3–5].

The abnormal uric acid (UA) level is associated with some disorders, including gout, hyperpiesia and Lesch–Nyhan syndrome [6]; accordingly, it can be claimed that the UA level measured in the body is a good prognosis for such diseases [7].

Given the obvious role of folic acid (FA) and B_{12} in the nucleotide production, cell division and gene expression, any FA deficiency leads to anemia and even can enhance the risk of cardiovascular diseases, as well as can trigger carcinogenesis reportedly [8].

It can summarize that it is really essential to measure precisely the concentration of catecholamines like EP, UA and FA, so that numerous techniques have been developed in this regard, including liquid chromatography [9–12], electro chemiluminescence [13], capillary electrophoresis [14, 15], flow injection analysis [16], spectrophotometry [17, 18], chemiluminescence [19, 20], isotope dilution-liquid chromatography/tandem mass spectrometry [21], and fluorometry [22]. Further attention has been directed towards electrochemical procedures because of their easy to use, low- cost, high sensitivity and miniaturization properties [23–34].

Among these, nanomaterials and novel controlled nanosensors are of great importance [30, 35–39]. Various applicable characteristics like biosensors, prevention of microbial biofilm growth, magnetic drug targeting, controlled drug release, antimicrobial treatment or medical diagnostics have directed the attention of the researches towards the production of core/shell nanomaterials [40, 41].

According to the introduction, we aimed to fabricate a manganese ferrite nanoparticles modified by (3-aminopropyl) trimethoxy silane as a core shell magnetic nanoparticle (CMNP) [42], as well as to produce 2FTNE and to investigate the electrochemical behavior in 2FTNE-modified CMNP paste electrodes (2FTNECMNPPE).

Our review showed that there is no study until now measuring simultaneously the levels of EP, UA and FA by 2FTNECMNPPE. The current research was designed to prepare and apply a novel electrode for electrocatalytic measurement of EP. Moreover, the modified electrode performance was analyzed during quantification of EP in the presence of UA and FA.

Experimental

Devices and chemicals

An Autolab potentiostat/galvanostat (PGSTAT 302N) was used for all electrochemical measurements. The conventional three-electrode cell system was used in a temperature room. A platinum wire as the auxiliary electrode, 2FTNECM-NPPE as the working electrode and an Ag/AgCl/KCl (3.0 M) electrode as reference electrode were used for electrochemical measurements. The pH values were measured using a pH-meter (Metrohm 692 model, Herisau, Switzerland).

EP, UA, and FA and all chemicals had analytical purity (Merck, Darmstadt, Germany). The phosphate buffer solution (PBS) was produced from concentrate phosphoric acid and its salts. As published previously, we synthesized the 2FTNE and magnetic core–shell manganese ferrite NPs in our Lab [42].

Electrode preparation

A 0.01 g of 2FTNE, 0.89 g graphite powder and 0.1 g of MCNPs was mixed in a mortar, followed by adding 0.7 mL of paraffin and mixing for 15 min. Then, the resulting paste was poured into a glass tube with 3.4 mm i.d. and 10 cm long and was packed. Next, a wire of copper was embedded in the electrode to establish the electrical contact. A novel surface was obtained by pressing additional paste out of the glass tube and using a weighing paper for polishing.

In addition, the same method was used to prepare a 2FTNE modified CPE electrode (2FTNECPE) without CMNP, magnetic core–shell manganese ferrite nanoparticles paste electrode (CMNPPE) without 2FTNE, and bared CPE in the absence of both 2FTNE and CMNP to compare with each other. Figure 1 illustrates a typical SEM of a 2FTNPE, CMNPPE and 2FTNEMCNPPE electrode.

Preparation of real samples

Urine samples were stored in a refrigerator immediately after collection. Ten ml of the samples were centrifuged for 15 min at 2000 rpm. The supernatant was filtered out by using a 0.45 μm filter. Next, different volumes of the solution was transferred into a 25 mL volumetric flask and diluted to the mark with PBS (pH 7.0). The diluted urine samples were spiked with different amounts of epinephrine, uric acid and folic acid. The epinephrine, uric acid and folic acid contents were analysed by the proposed method by using the standard addition method.

Epinephrine injections (Adrenaline Tartrate Injection, Daroupaksk Distribution Company, labelled value epinephrine = 1 mg/injection) and folic acid tablets (Ruzdarou, labelled value folic acid = 5 mg/tablet) were purchased. As many as 10 mL of 0.1 M stock solution of the epinephrine injection was prepared by using deionized water. The required amount of this solution was added to the
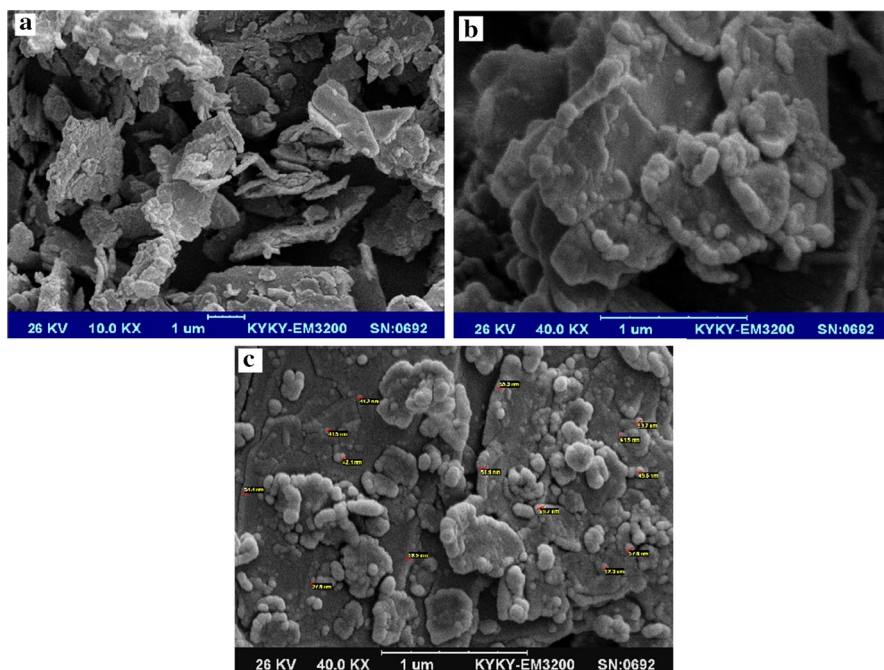
Fig. 1 SEM of 2FTNPE (a), CMNPPE (b) and 2FTNEMCNPPE (c) electrode

electrochemical cell containing 10 mL of 0.1 M PBS (pH 7) for the determination of epinephrine.

The folic acid tablets were completely grounded and homogenized before preparing 10 mL of the 0.1 M stock solution. The solution was sonicated to assure complete dissolution and then filtered. The required amount of clear filtrate was then added to the electrochemical cell containing 10 mL of the 0.1 M PBS (pH 7) to record the SWV voltammogram.

Results and discussion

Characterization of 2FTNPE, CMNPPE and 2FTNEMCNPPE

Figure 1 shows the electrode surface morphology of (a) 2FTNPE, (b) CMNPPE and (c) 2FTNEMCNPPE electrode which is characterized by scanning electron microscopy. We can clearly see the existence of modifier (2FTNE) and nanoparticles (CMNPs) on the surface of the 2FTNPE and CMNPPE electrode, respectively. Also, the surface of the 2FTNEMCNPPE electrode presents spheroidal magnetic core–shell manganese ferrite nanoparticles (CMNPs) with well-distributed size, and the particles' average diameter is about 50 nm.
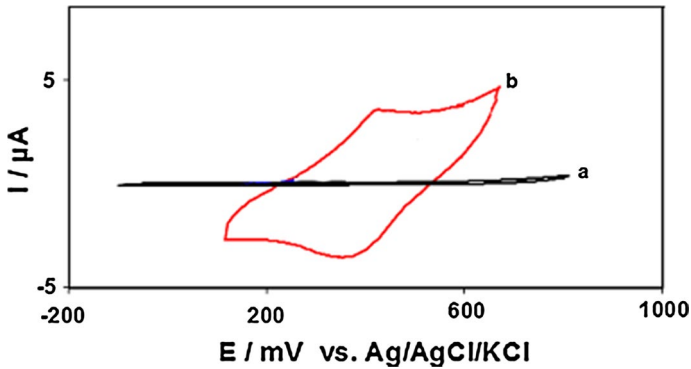
Fig. 2 CVs for CPE (a) and 2FTNEMCNPPE (b) in 0.1 M PBS (pH = 7.0) with the scan rate of 10 mV s^{-1}

Electrochemical behavior of 2FTNECMNPPE

The 2FTNECMNPPE was initially fabricated and then its electrochemical features were investigated in 0.1 M of PBS (pH 7.0) by CV (Fig. 2). Attained test findings indicated respectively the reproducible anodic and cathodic peaks and well-defined with E_{pa}, E_{pc} and $E°'$ of 430, 350 and 390 versus Ag/AgCl/KCl (3.0 M). The calculated peak separation potential, $\Delta E_p = (E_{pa} - E_{pc})$ of 80 mV, was higher than 59/n mV, as expected for a reversible system [43], This shows a quasi-reversible behavior of the redox couple of 2FTNE in 2FTNECMNPPE in aqueous medium.
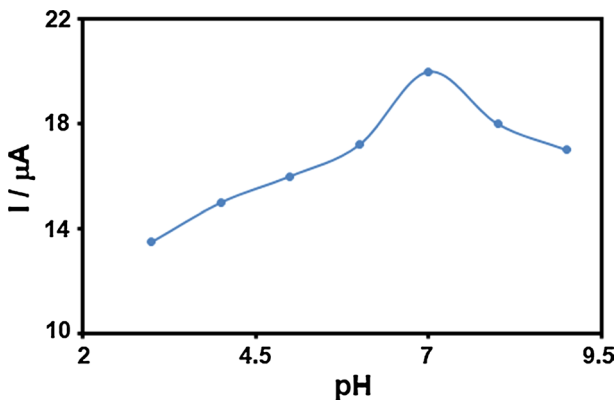

Fig. 3 The current of electro-oxidation of EP at the surface of 2FTNEMCNPPE in the presence of 5.0 μM EP at various buffered pHs (3–9)

Electrocatalytic oxidation of epinephrine at a 2FTNECMNPPE

The electrochemical behavior of epinephrine is dependent on the pH value of the aqueous solution, whereas the electrochemical properties of 2FTNE (Ferocen/Ferocen$^+$ redox couple) is independent of pH. Thus, solution pH optimization appears to be required for the purpose of acquiring the electrocatalytic oxidation of epinephrine. Therefore, the electrochemical activity of epinephrine was examined in 0.1 M PBS with various pH values ($3.0 < pH < 9.0$) at the surface of a 2FTNENFOCPE using CV. It was discovered that the electrocatalytic oxidation of epinephrine at the surface of a 2FTNENFOCPE was more beneficial at neutral circumstances compared to that of an acidic or basic medium (Fig. 3). This seems to be a steady development in the anodic peak current and a concurrent reduction of the cathodic peak current in the cyclic voltammograms of the 2FTNENFOCPE. Hence, pH 7.0 was selected as the optimal pH for the electrocatalysis of epinephrine oxidation at the surface of a 2FTNENFOCPE.

You can see the CV responses for the electrochemical oxidation of 5.0 μM EP at the bare CPE (curve *b*), CMNPPE (curve *d*), 2FTNECPE (curve *e*) and
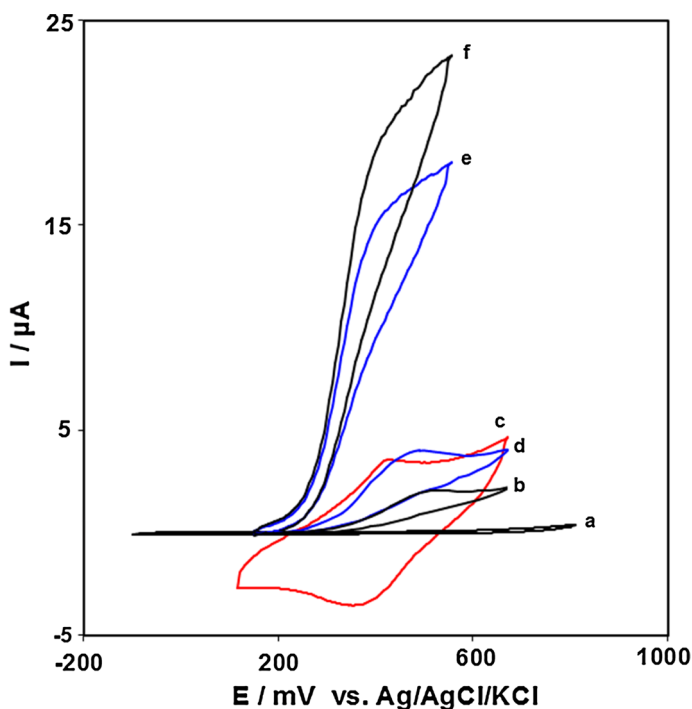

Fig. 4 CVs for (a) Bare CPE in 0.1 M PBS (pH = 7.0), (b) Bare CPE in 5.0 μM EP, (c) 2FTNEMCNPPE in PBS (pH = 7.0), (d) MCNPPE in 5.0 μM EP, (e) 2FTNEPE in 5.0 μM EP, and (f) 2FTNEMCNPPE in 5.0 μM EP, with the scan rate of 10 mV s^{-1}

2FTNECMNPPE (curve *f*) in Fig. 4. Further, curve *a* reveals the bare CPE in 0.1 M PBS (pH = 7.0).

As can be seen, the anodic peak potentials for EP oxidation are 490 and 530 mV, respectively, at the CMNPPE and the bare CPE, but approximately 430 mV at the 2FTNECMNPPE and the 2FTNEPE, indicating the ability of 2FTNE as an appropriate mediator. In comparison with CMNPPE and bare CPE, the peak potential for the oxidations of EP at the 2FTNECMNPPE and 2FTNEPE is shifted respectively by about 60 and 100 mV toward negative values. However, in comparison with 2FTNEPE, peak current of EP at 2FTNECMNPPE is much higher and confirms that the combined CMNP and 2FTNE could improve significantly the electrode performance toward EP oxidation. Finally, 2FTNECMNPPE without EP and in 0.1 M PBS (pH = 7.0) showed a well-behaved redox reaction (Fig. 3, curve *c*). However, a dramatic increase in the anodic peak current in the 5.0 μM EP solution (curve *f*) was observed as a result of potent electrocatalytic impact of the 2FTNECMNPPE over the EP [43].
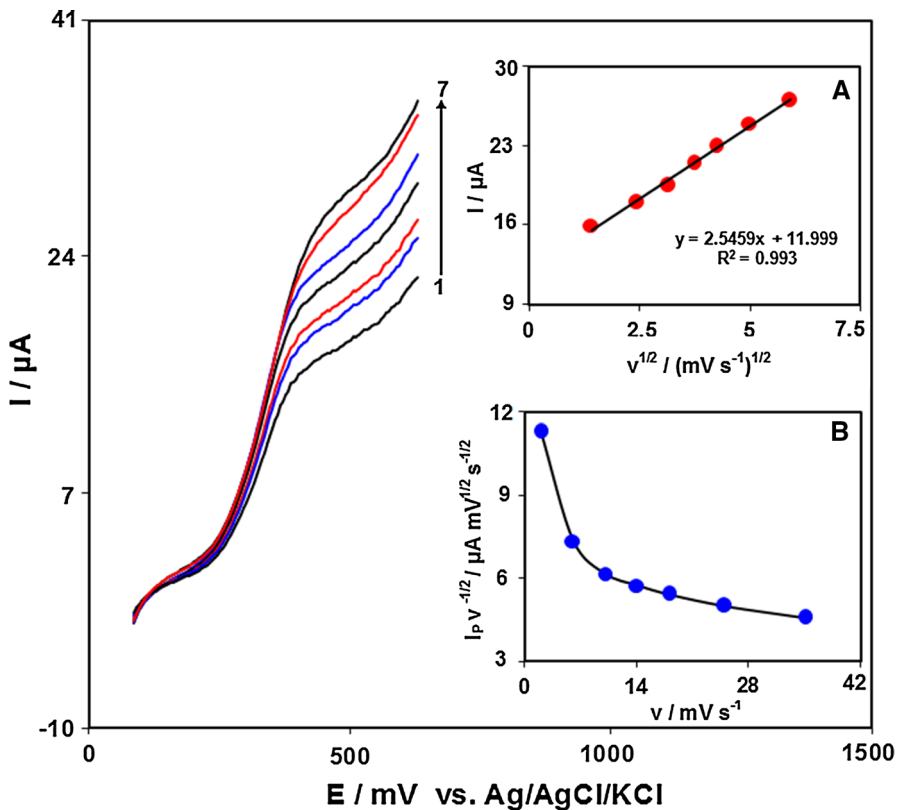

Fig. 5 LSVs for 2FTNEMCNPPE in PBS containing 5.0 μM EP at different scan rates; numbers 1–7: (1) 2, (2) 6, (3) 10, (4) 14, (5) 18, (6) 25 and (7) 35 mV s^{-1}. Insets: **a** Ip versus $v^{1/2}$ and **b** normalized current ($I_p/v^{1/2}$) versus v

The LSV was used to evaluate the scan rate effect on the electrocatalytic oxidation of EP at the 2FTNECMNPPE (Fig. 5). The results indicated that elevating the scan rate shifted the oxidation peak potential towards higher positive potentials. This shows the kinetic restriction in the electrochemical reaction. A linear plot of peak current (I_p) versus the square root of scan rate ($\nu^{1/2}$) was in the range of 2–35 mV s^{-1} with the linear regression equations $y = 2.5459\,x + 11.999$ and the correlation coefficient of 0.993. This means that the process is diffusion controlled [43].

Chronoamperometric measurements

For EP chronoamperometry at 2FTNEMCNPPE, the working electrode potential was set at 0.75 V versus Ag/AgCl/KCl (3.0 M) at different concentrations of EP in 0.1 M PBS (pH 7.0) (Fig. 6). The Cottrell equation can be used for determination of the diffusion coefficient (1) [43].

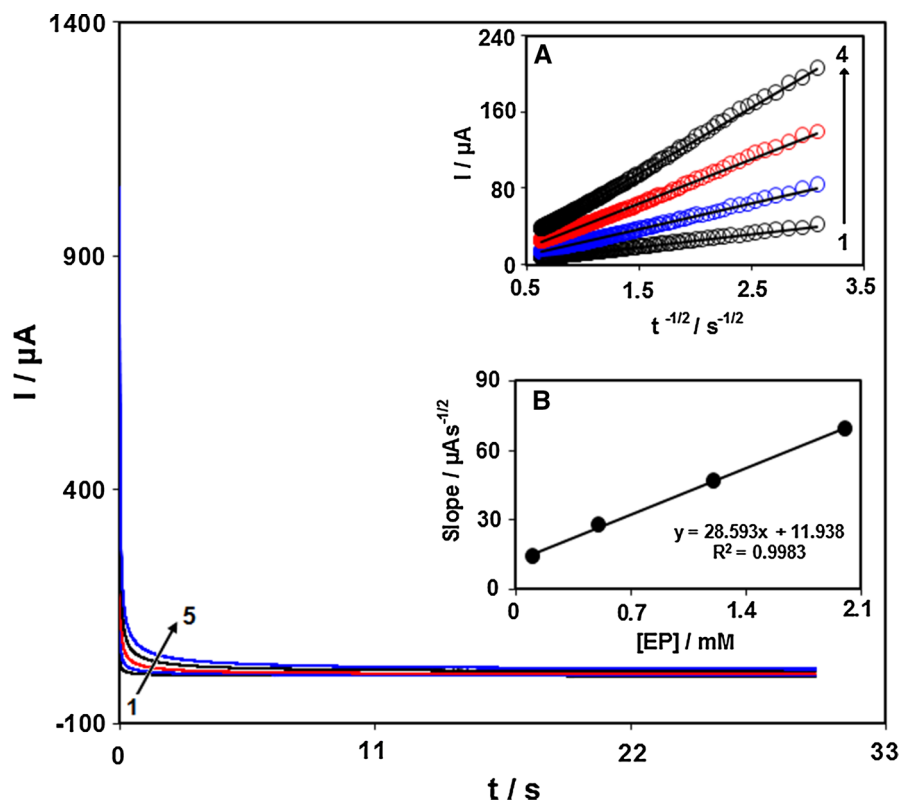$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \tag{1}$$

Fig. 6 **a** Chronoamperograms of 2FTNEMCNPPE in PBS for various concentrations of EP; the numbers 1–5 show to 0.0, 0.1, 0.5, 1.2 and 2.0 mM of EP. Insets: **a** I versus $t^{-1/2}$ **b** slope of straight lines versus EP concentration

According to the Cottrell equation, at different concentrations of EP, diagrams of I versus $t^{-1/2}$ were drawn (Fig. 6a). Then, the slopes of the resulting lines were plotted versus EP concentration (Fig. 6b). With using this slope and Cottrell equation, D was obtained as 8.5×10^{-6} cm^2/s.

Stability and reproducibility of the modified electrode

The reproducibility of the 2FTNEMCNPPE for measurement of EP was assessed using 20 µM EP ($n = 10$). Based on the findings, proper reproducibility was obtained with a relative standard deviation (RSD) of 2.7%. After each measurement, the modified electrode was polished. Then, the 2FTNEMCNPPE was maintained in air for 2 weeks. According to the results, the electrochemical activity of the 2FTNEMC-NPPE over the measurement of EP remained almost the same, which confirmed the good reproducibility and stability of 2FTNEMCNPPE.
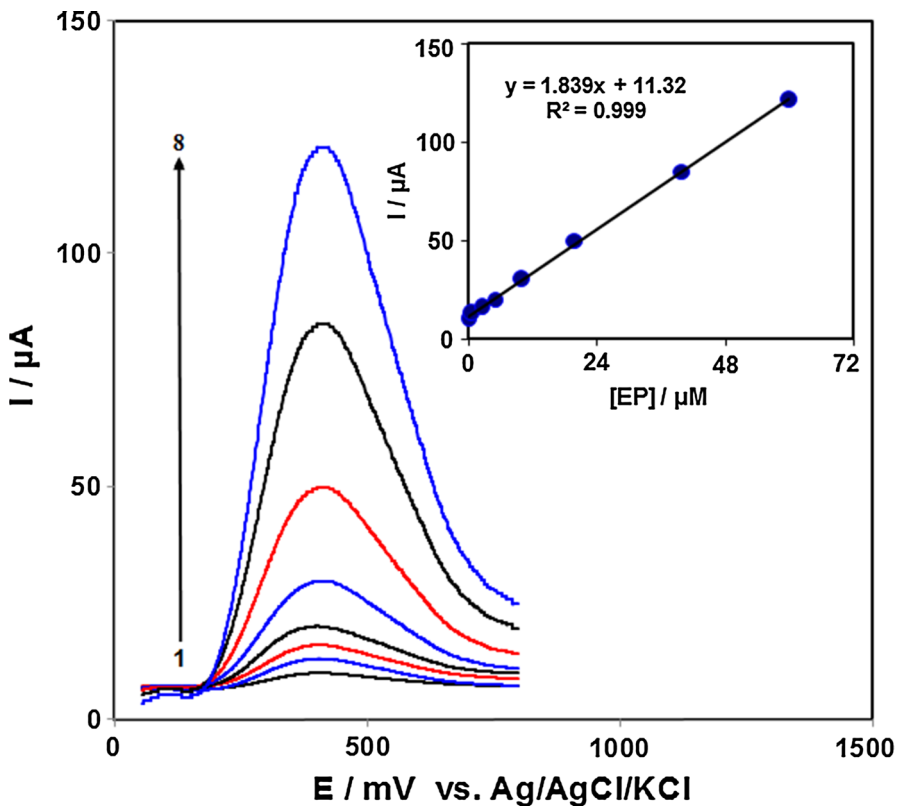

Fig. 7 SWVs for 2FTNEMCNPPE in PBS with various EP concentrations. Numbers 1–8: (1) 0.05, (2) 0.075, (3) 2.5, (4) 5.0, (5) 10.0, (6) 20.0, (7) 40.0 and (8) 60.0 µM. Insets: plots of I_p versus EP concentrations

Table 1 Comparison of the efficiency of some modified electrodes used in the electro-oxidation of epinephrine

Electrode	Modifier	Method	LOD (µM)	LDR (µM)	Refs.
Glassy carbon	SnO$_2$/graphene nanocomposite	Voltammetry	0.01	0.5–200.0	[44]
Au	Au nanorod	Voltammetry	2.8	10.0–150.0	[45]
Screen printed	Multi-walled carbon nanotubes	Voltammetry	0.1	2.0–2500.0	[46]
Glassy carbon	Poly (fuchsine acid)	Voltammetry	0.075	0.1–300.0	[47]
Carbon paste	2FTNECMNP	Voltammetry	0.016	0.05–60	This work

Calibration plot and limit of detection

The linearity of EP oxidation peak current on the 2FTNEMCNPPE was assessed using a SWV experiment with different concentrations of EP (Fig. 7). The obtained results indicated linear peak current of the EP oxidation within the range 0.5×10^{-7} to 6.0×10^{-5} M. The detected limit on the basis of three times the standard deviation (3σ) of the blank was 1.6×10^{-8} M. The obtained values correspond to values recorded by other researchers regarding EP oxidation at chemically adjusted electrode surfaces. These results are presented in Table 1.

Simultaneous determination of EP, UA and FA at 2FTNEMCNPPE

The main objective of this study was the concurrent measurements of EP, UA, and FA. For this purpose, the concentrations of three analyte were simultaneously changed. Consequently, three well-distinguished anodic peaks at potentials of 430, 730, and 930 mV, corresponding to the oxidation of EP, UA, and FA, respectively, were obtained, and confirmed that the concurrent measurements of EP, UA, and FA at the 2FTNEMCNPPE is possible (Fig. 8). However, with use of the bare CPE, an overlapping voltammogram was obtained for the analytes. The sensitivity of the 2FTNEMCNPPE toward EP was 1.813 µA µmol^{-1} L, but the sensitivity toward EP without UA and FA was found to be 1.839 µA µmol^{-1} L. Therefore, it is possible to have the independent or the concurrent measurements of EP, UA, and FA.

Interference study

We investigated the effect of different species on measuring the 20 µM EP, 100 µM UA and 20 µM FA. The tolerance limit was adjusted for the concentration of species with $\pm 5\%$ error in the determination. Based on the obtained results, the tolerance limit for Na$^+$, Cl$^-$ and K$^+$ was 0.1 M; for Mg^{2+} and Ca^{2+} was 0.05 M; for glycine, L-lysine, glutamic acid, glucose, acetaminophen, l-asparagines, and NADH was 0.004 M.
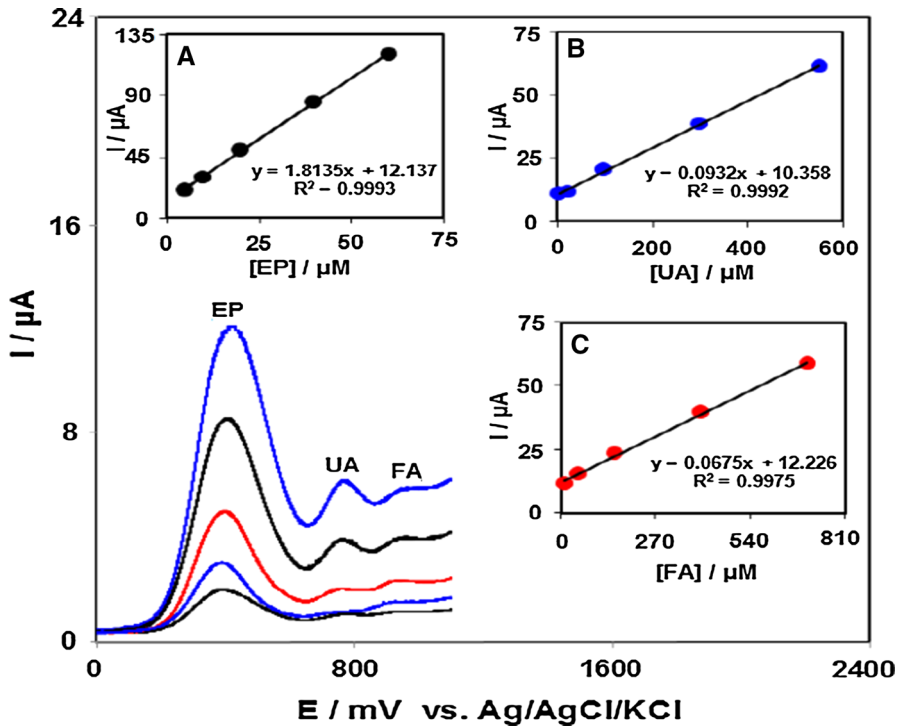
Fig. 8 a SWVs of 2FTNEMCNPPE in 0.1 M PBS (pH = 7.0) with various concentrations (µmol/L) of EP + UA + FA mixed solutions: (1) 5.0 + 5.0 + 10.0, (2) 50.0 + 25.0 + 10.0, (3) 150.0 + 100.0 + 20.0, (4) 400.0 + 300.0 + 40.0, (5) 700.0 + 550.0 + 60.0, **a–c** are plots of peak currents as a function of EP + UA + FA concentration, respectively

Real sample analysis

Finally, 2FTNEMCNPPE was applied for measurement of EP, UA, and FA in an EP ampoule, a FA tablet, and urine samples. For this purpose, the measurement of EP, UA, and FA in the real samples was carried out (Table 2). Also, the recovery of EP, UA, and FA of the samples spiked with known amounts of EP, UA, and FA was assessed. The results showed quantitatively recovery of the added analytes from the real samples. These results demonstrate the applicability of the 2FTNEMCNPPE for measurement of EP, UA, and FA in the real samples. Also, the mean RSD indicated the reproducibility of the method.

The amounts of EP and FA in ampules and tablets were found to be 0.98 mg/mL and 1.01 mg/tablet, respectively. The results exhibited no significant difference between the results of the 2FTNEMCNPPE and the nominal value on the ampoule label and tablet label (1.00 mg/mL and 1.00 mg/tablet, respectively). The *t* test used for setting the results revealed no significant difference at the 95%.

Table 2 The use of 2FTNEMCNPPE in simultaneous measurement of EP, UA and FA in an EP ampule, a FA tablet and urine samples ($n = 5$)

Sample	Spiked (μM)			Found (μM)			Recovery (%)			R.S.D. (%)		
	EP	UA	FA	EP	UA	FA	EP	UA	FA	EP	UA	FA
EP ampoule	0	0	0	9.0	–	–	–	–	–	2.7	–	–
	2.5	15.0	17.5	11.4	15.5	17.1	99.1	103.3	97.7	3.2	1.9	2.8
	5.0	25.0	27.5	14.3	24.8	27.9	102.1	99.2	101.4	3.1	2.3	2.7
	7.5	35.0	37.5	17.1	35.1	37.3	103.6	100.3	99.5	1.9	3.3	2.4
	10.0	450	47.5	18.5	45.6	48.8	97.3	101.3	102.7	2.2	1.8	3.1
FA tablet	0	0	0	–	–	17.0	–	–	–	–	3.4	–
	5.0	17.5	2.5	5.1	17.1	19.7	102.0	97.7	101.0	2.3	1.9	3.2
	10.0	22.5	5.0	9.8	22.9	21.9	98.0	101.8	99.5	3.1	2.3	1.9
	15.0	27.5	7.5	15.1	27.1	24.3	100.7	98.5	99.2	1.7	2.8	2.7
	20.0	32.5	10.0	19.8	33.5	27.5	99.0	103.1	101.8	2.8	3.1	1.8
Urine	0	0	0	–	10.0	–	–	–	–	–	1.7	–
	7.5	10.0	30.0	7.4	20.2	30.9	98.7	101.0	103.0	2.9	3.2	1.6
	12.5	20.0	40.0	12.7	29.5	39.1	101.6	98.3	97.7	3.4	2.7	2.6
	17.5	30.0	50.0	18.1	41.2	49.5	103.4	103.0	99.0	1.6	2.6	3.1
	22.5	40.0	60.0	22.4	49.8	61.5	99.5	99.6	102.5	2.2	1.8	2.9

Conclusion

The 2FTNE and MCNPs were synthesized in our laboratory and a modified electrode fabricated for electrocatalytic measurement of EP. The EP electro-oxidation at the 2FTNEMCNPPE surface occurred at 430 mV. In the present study, the use of the 2FTNEMCNPPE for the measurement of EP, UA, and FA simultaneously was demonstrated. The potential differences in 400, 600, and 200 mV between EP–UA, EP–FA, and UA–FA, respectively, were large enough to determine EP, UA, and FA simultaneously and individually. Finally, the measurement of EP, UA, and FA was successfully carried in the real samples, including an EP ampoule, a FA tablet, and urine samples. The potential sensing applications of 2FTNEMCNPPE was confirmed due to low detection limit, high selectivity, and high sensitivity in detecting the EP.

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