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ORIGINAL PAPER

Electrochemical behavior of isoproterenol in the presence of uric acid and folic acid at a carbon paste electrode modified with 2,7-bis(ferrocenyl ethyl)fluoren-9-one and carbon nanotubes

Hadi Beitollahi · Jahan-Bakhsh Raoof · Hassan Karimi-Maleh · Rahman Hosseinzadeh

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Abstract This paper reports the selective determination of isoproterenol (IP) in the presence of uric acid (UA) and folic acid (FA) using 2,7-bis(ferrocenyl ethyl)fluoren-9-one modified carbon nanotube paste electrode (2,7-BFCNPE) in 0.1 M phosphate buffer solution (PBS) (pH 7.0). The bare carbon paste electrode does not separate the voltammetric signals of IP, UA, and FA. However, 2,7-BFCNPE not only resolved the voltammetric signals of IP, UA, and FA with potential differences of 150, 325, and 475 mV between IP–UA, UA–FA, and IP–FA, respectively, but also dramatically enhanced the oxidation peak currents of them when compared to bare carbon paste electrode. In PBS of pH 7.0, the oxidation current increased linearly with two concentration intervals of IP, one is 0.08 to 17.5 μM and the other is 17.50 to 700.0 μM. The detection limit (3σ) obtained by DPV was 26.0±2 nM. The practical application of the modified electrode was demonstrated by determining IP in IP injection, urine, and human blood serum.

Keywords Isoproterenol · Uric acid · Folic acid · Modified electrode · Electrocatalysis · Carbon nanotubes

H. Beitollahi (✉)
Environment Department, Research Institute of Environmental Sciences, International Center for Science, High Technology & Environmental Sciences, Kerman, Iran
e-mail: h.beitollahi@yahoo.com

J.-B. Raoof
Electroanalytical Chemistry Research Laboratory, Department of Analytical Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran

H. Karimi-Maleh
Department of Chemistry, Majlesi Branch, Islamic Azad University, Isfahan, Iran

R. Hosseinzadeh
Department of Organic Chemistry, Faculty of Chemistry, University of Mazandaran, Babolsar, Iran

Introduction

Isoproterenol (IP) is a catecholamine drug that is widely used in the treatment of allergic emergencies such as styptic, bronchial asthma, status asthmaticus, cardiac arrest, glaucoma, and ventricular bradycardia [1]. The cardiovascular effects of IP compare with epinephrine and norepinephrine, which can relax almost every kind of smooth musculature that contains adrenergic nerves, but this effect is pronounced in the musculature of bronchus and also in the gastrointestinal tract [2]. IP has positive inotropic and chronotropic effects on the heart. In skeletal muscle arterioles, it produces vasodilatation. Its inotropic and chronotropic effects elevate systolic blood pressure, while its vasodilatory effects tend to lower diastolic blood pressure. The adverse effects of IP are also related to the drug's cardiovascular effects. IP can produce an elevated heart rate (tachycardia), which predisposes patients to cardiac dysrhythmias. Also, IP should not be administered to patients with myocardial ischemia.

Several methods have been described in the literature for the determination of IP such as flow injection spectrophotometry [3], ultra-performance liquid chromatography/time-of-flight mass spectrometry [4], and capillary electrophoresis [5]. Due to the advantages of relatively low cost, fast

response, simple instrumentation, high sensitivity, facile miniaturization, and low power requirement, numerous voltammetric methods have been developed for the determination of IP [2, 6–8].

Uric acid (UA) is the final product of purine metabolism in the human body [9]. It is one of the major parameters monitored in urine and in blood. UA concentration changes are associated with the altered metabolism of purines that are related to numerous illnesses and physiological disorders [10].

Therefore, its determination in physiological fluids is necessary in the diagnosis and treatment of diseases such as gout, hyperuricemia, heavy hepatitis, and Lesch–Nyhan syndrome [11]. UA is also a marker for renal failure as well as toxicity. Most analytical methods applied in routine clinical analysis, including UA assays, use an optical detection [12]. Therefore, the determination of UA with a simple method is essential because it serves as a marker for the detection of the above diseases. Comparing with other technologies, electrochemical method is more desirable because of its convenience and low cost [13–17].

Folic acid (FA) is chosen as the analyte for this investigation because it is an electroactive component of considerable biological importance [1]. It has long been recognized as part of the vitamin B complex found in some enriched foods and vitamin pills. It is usually employed in the treatment or prevention of megaloblastic anemia during pregnancy, childhood, and other clinical situations often associated with alcoholism and liver diseases [18]. A lack of FA gives rise to gigantocytic anemia, associated with leukopaenia, devolution of mentality, psychosis, etc. Simple and sensitive methods are required for its determination in pharmaceutical, clinical, and food samples. Published methods for the determination of FA include high-performance liquid chromatography [19], spectrophotometry [20], flow injection chemiluminescence [21], and electrochemistry. Among these methods, electrochemical methods maybe the most widely applied because of high sensitivity, simplicity, and reproducibility of this approach. Many electrochemical techniques such as square wave voltammetry [22], amperometry [23], and differential pulse voltammetry [24, 25] have been used for the determination of FA.

Electrode surface modification is a field of paramount importance in the modern electrochemistry especially due to the various application possibilities of modified electrodes. In recent years, chemically modified carbon paste electrodes have received increasing attention due to their potential applications in various analysis and also due to its relative ease of electrode preparation and regeneration [26–29]. Carbon nanotubes (CNTs) are one of the most important nanomaterials due to their high chemical stability, high surface area, high mechanical properties, unique electrical conductivity, metallic structural characteristics, and mechanical strength and elasticity [30]. Carbon-based electrodes are currently in widespread use in electroanalytical chemistry because of their broad potential window, low cost, rich surface chemistry, low background current, and chemical inertness [31–33].

In the present work, we describe the preparation of a new electrode composed of CNT paste electrode (CNPE) modified with 2,7-bis(ferrocenyl ethyl)fluoren-9-one (2,7-BFCNPE) and investigate its performance for the electrocatalytic determination of IP in aqueous solutions. We also evaluate the analytical performance of the modified electrode for quantification of IP in the presence of UA and FA.

Experimental

Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT-302 N, Eco Chemie, The Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System software. A conventional three-electrode cell was used at 25±1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and the 2,7-BFCNPE were used as the reference, auxiliary, and working electrodes, respectively. A Metrohm 691 pH/ion meter was used for pH measurements.

All solutions were freshly prepared with double distilled water. IP, UA, FA, and all other reagents were of analytical grade from Merck (Darmstadt, Germany). Graphite powder (particle diameter=0.1 mm) and paraffin oil (DC 350, density=0.88 gcm^{-3}) as the binding agent (both from Merck) were used for preparing the pastes. Multiwalled carbon nanotubes (purity more than 95%) with o.d. between 10 and 20 nm, i.d. between 5 and 10 nm, and tube length from 10 to 30 μm were prepared from Nanostructured and Amorphous Materials, Inc. The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0–11.0. 2,7-BF was synthesized in our laboratory as reported previously [26].

Preparation of the electrode

The 2,7-BFCNPEs were prepared by hand mixing 0.01 g of 2,7-BF with 0.89 g graphite powder and 0.1 g CNTs with a mortar and pestle. Then, ~0.7 mL of paraffin oil 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, 2,7-BF-modified CPE electrode (2,7-BFCPE) without CNTs, CNPE without 2,7-BF, and unmodified CPE in the absence of both 2,7-BF and CNT were also prepared in the same way.

Results and discussion

Electrochemical behavior of 2,7-BFCNPE

We have previously shown that a carbon paste electrode spiked with 2,7-BF can be constructed by the incorporation of 2,7-BF in a graphite powder–paraffin oil matrix [26]. The experimental results show well-defined and reproducible anodic and cathodic peaks related to Fc/Fc^+ redox system, which show a quasireversible behavior in an aqueous medium [34]. The surface reproducibility of the modified electrode was examined by cyclic voltammetric data obtained in optimum solution pH 7.0 from five separately prepared 2,7-BFCNPEs (Table 1).

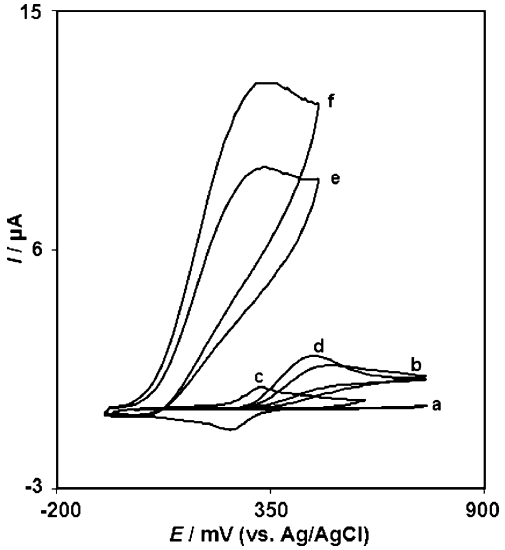

Fig. 1 CVs of *a* unmodified CPE in 0.1 M PBS (pH 7.0); *b* unmodified CPE in 0.1 M PBS (pH 7.0) and 0.1 mM IP; *c* 2,7-BFCNPE in 0.1 M PBS (pH 7.0); *d* CNPE in 0.1 M PBS (pH 7.0) and 0.1 mM IP; *e* 2,7-BFCPE in 0.1 M PBS (pH 7.0) and 0.1 mM IP, and *f* 2,7-BFCNPE in 0.1 M PBS (pH 7.0) and 0.1 mM IP. In all cases, the scan rate is 10 mV s^{-1}

Electrocatalytic oxidation of IP at a 2,7-BFCNPE

Figure 1 depicts the cyclic voltammetry (CV) responses for the electrochemical oxidation of 0.1 mM IP at unmodified CPE (curve b), CNPE (curve d), 2,7-BFCPE (curve e), and 2,7-BFCNPE (curve f). As it is seen, while the anodic peak potential for IP oxidation at the CNPE and unmodified CPE are 460 and 500 mV, respectively, the corresponding potential at 2,7-BFCNPE and 2,7-BFCPE is ~320 mV. These results indicate that the peak potential for IP oxidation at the 2,7-BFCNPE and 2,7-BFCPE electrodes shift by ~140 and 180 mV toward negative values compared to CNPE and unmodified CPE, respectively. However, 2,7-BFCNPE shows higher anodic peak current for the oxidation of IP compared to 2,7-BFCPE, indicating that the combination of CNTs and the mediator (2,7-BF) has significantly improved the performance of the electrode toward IP oxidation. In fact, 2,7-BFCNPE in the absence of IP exhibited a well-behaved redox reaction (Fig. 1, 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 0.1 mM IP (curve f), which can be related to the strong electrocatalytic effect of the 2,7-BFCNPE towards this compound [34].

The electrochemical behavior of IP is dependent on the pH value of the aqueous solution, whereas the electrochemical properties of Fc/Fc^+ redox couple are independent on pH. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of IP. Thus, the electrochemical behavior of IP was studied in 0.1 M PBS in different pH values ($2.0<pH<11.0$) at the surface of 2,7-BFMCNPE by cyclic voltammetry. It was found that the electrocatalytic oxidation of IP at the surface of 2,7-BFMCNPE was more favored under neutral conditions than in acidic or basic medium.

This appears as a gradual growth in the anodic peak current and a simultaneous decrease in the cathodic peak current in the cyclic voltammograms of 2,7-BFCNPE. The variation of I_{pa} vs. the variation of pH was studied. The results showed that the anodic peak current value for electrooxidation of IP are high at a biological pH. Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of IP oxidation at the surface of 2,7-BFMCNPE.

Table 1 Cyclic voltammetric data obtained for constructed 2,7-BFCNPE in 0.1 M PBS (pH 7.0) at 10 mV s^{-1}

E_{pa} (V)[a]	E_{pc}(V)	$E_{1/2}$ (V)	E_p (V)Δ	I_{pa} (μA)	I_{pc} (μA)
0.320	0.255	0.287	0.065	0.79	0.77
(3.52×10^{-3})[b]	(3. 44×10^{-3})	(2.87×10^{-3})	(7.15×10^{-4})	(1.42×10^{-3})	(1.46×10^{-3})

[a] Versus Ag/AgCl/KCl (3.0 M) as reference electrode

[b] The values in parentheses indicate the calculated standard deviations

The effect of scan rate on the electrocatalytic oxidation of IP at the 2,7-BFCNPE was investigated by linear sweep voltammetry (Fig. 2). As can be observed in Fig. 2, 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 5–100 mV s^{-1} (Fig. 2a), suggesting that, at sufficient overpotential, the process is diffusion rather than surface controlled. A plot of the scan rate-normalized current ($I_p/\nu^{1/2}$) vs. scan rate (Fig. 2b) exhibits the characteristic shape typical of an EC′ process [34].

Figure 2, inset C, shows Tafel plots that was drawn from the data of the rising part of the current voltage curves recorded at scan rates of 10 mV s^{-1}. This part of voltammogram, known as Tafel region, is affected by electron transfer kinetics between the substrate (IP) and the modified electrode [34]. In this condition, transfer coefficient (α) can be estimated from the slope of Tafel plot. The Tafel slope was found to be 83.5 mV (Fig. 2, inset C), which indicates that a one-electron transfer process is the rate-limiting step assuming a transfer coefficient (α) is about 0.29.

Chronoamperometric measurements

Chronoamperometric measurements of IP at 2,7-BFCNPE were carried out by setting the working electrode potential at 0.4 V vs. Ag/AgCl/KCl (3.0 M) for the various concentrations of IP in PBS (pH 7.0) at 25±1 °C. For an electroactive material (IP 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 [34]. Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of IP. The slopes of the resulting straight lines were then plotted vs. IP concentration. From the resulting slope and Cottrell equation, the mean value of the D was found to be (9.3± 0.2)×10^{-6} cm^2/s.

Chronoamperometry can also be employed to evaluate the catalytic rate constant, k, for the reaction between IP and the 2,7-BFCNPE according to the method of Galus [35]:

$$I_C/I_L = \gamma^{1/2}\left[\pi^{1/2}\mathrm{erf}\left(\gamma^{1/2}\right) + \exp(-\gamma)/\gamma^{1/2}\right] \quad (1)$$

where I_C is the catalytic current of IP at the 2,7-BFCNPE, I_L is the limited current in the absence of IP, and $\gamma = kC_b t$ is the argument of the error function (C_b is the bulk concentration of IP). In cases where γ exceeds the value of 2, the error function is almost equal to 1, and therefore, the above equation can be reduced to:

$$I_C/I_L = \pi^{1/2}\gamma^{1/2} = \pi^{1/2}(kC_b t)^{1/2} \quad (2)$$

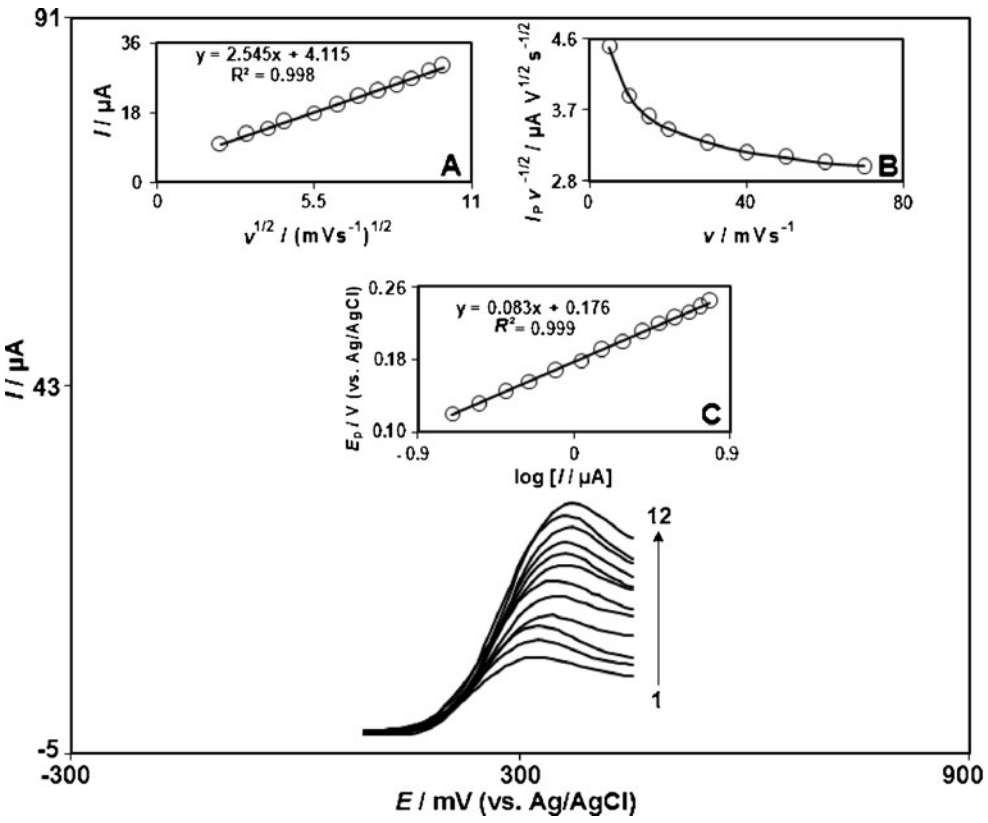

Fig. 2 Linear sweep voltammograms of 2,7-BFCNPE in 0.1 M PBS (pH 7.0) containing 100.0 μM IP at various scan rates; numbers 1–12 correspond to 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mV s^{-1}, respectively. *Insets*—variation of *A* anodic peak current vs. $\nu^{1/2}$; *B* normalized current ($I_p/\nu^{1/2}$) vs. ν; *C* Tafel plot derived from the rising part of voltammogram recorded at a scan rate 10 mV s^{-1}

where t is the time elapsed. The above equation can be used to calculate the rate constant, k, of the catalytic process from the slope of I_C/I_L vs. $t^{1/2}$ at a given IP concentration. From the values of the slopes, the average value of k was found to be 4.7×10^4 $M^{-1}s^{-1}$ which is significantly higher than those previously reported for other modifiers (4.21×10^2 $M^{-1}s^{-1}$ [7], 4.85×10^3 $M^{-1}s^{-1}$ [8], and 1.72×10^2 $M^{-1}s^{-1}$ [9]).

Calibration plot and limit of detection

Differential pulse voltammetry (DPV) method was used to determine the concentration of IP. The plot of peak current vs. IP concentration consisted of two linear segments with slopes of 0.5206 and 0.0255 μA μM^{-1} in the concentration ranges of 0.08 to 17.5 μM and 17.5 to 700.0 μM, respectively. The decrease in sensitivity (slope) of the second linear segment is likely due to kinetic limitation. The detection limit (3σ) of IP was found to be 26.0±2 nM. These values are compared with values reported by other research groups for electrocatalytic oxidation of IP at the surface of chemically modified electrodes by other mediators (Table 2).

Simultaneous determination of IP, UA, and FA

To our knowledge, there is no report on the simultaneous determination of IP, UA, and FA using 2,7-BFCNPE. Therefore, the main objective of this study was to develop a modified electrode with the capability of separating the electrochemical responses of IP, UA, and FA. Therefore, DPV was used for the simultaneous determination of IP, UA, and FA. Using 2,7-BFCNPE as the working electrode, the analytical experiments were carried out by varying the concentration of UA or FA in the presence of constant concentrations of IP in 0.1 M PBS (pH 7.0). Figure 3 shows DPVs obtained in 250.0 μM IP containing increasing concentrations of UA and FA. It can be clearly seen that the response of the 2,7-BFCNPE to IP is independent of both UA and FA. The utilization of the 2,7-BFCNPE for the simultaneous determination of IP, UA, and FA was demonstrated by simultaneously changing the concentrations of IP, UA, and FA. The voltammetric results showed well-defined anodic peaks at potentials of 295, 445, and 770 mV corresponding to the oxidation of IP, UA, and FA respectively indicating that simultaneous determination of these compounds is feasible at the 2,7-BFCNPE as shown in Fig. 4.

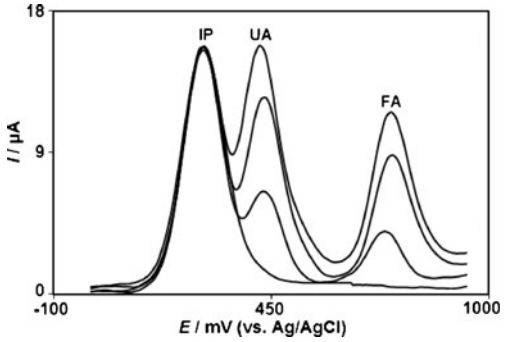

Fig. 3 DPVs of 2,7-BFCNPE in 0.1 M PBS (pH 7.0) containing 250.0 μM IP and different concentrations of UA + FA in micromolar, from inner to outer, 0.0+0.0, 175.0+150.0, 400.0+350.0, and 525.0+500.0, respectively

The sensitivity of the modified electrode towards the oxidation of IP was found to be 0.5250 μA μM^{-1}. This is very close to the value obtained in the absence of UA and FA (0.5206 μA μM^{-1}), indicating that the oxidation processes of these compounds at the 2,7-BFCNPE are independent, and therefore, simultaneous determination of their mixtures is possible without significant interferences.

Real sample analysis

Determination of IP in IP injection

One milliliter of an IP ampoule was diluted to 10 mL with 0.1 M PBS (pH 7.0); then, different volume of the diluted solution was transferred into each of a series of 10 mL volumetric flasks and diluted to the mark with PBS. Each sample solution was transferred into the electrochemical

Table 2 Comparison of the efficiency of some modified electrodes used in the electrocatalysis of IP

Electrode	Modifier	Method	pH	Peak potential shift (mV)	Scan rate (mV/s)	Limit of detection (M)	Dynamic range (M)	References
Glassy carbon	Poly(1-methylpyrrole)-DNA	Cyclic voltammetry	4.0	334	50	1.6×10^{-7}	2.0×10^{-6}–6.0×10^{-5}	[6]
Carbon nanotube paste	p-chloranil	Differential pulse voltammetry	10.5	250	10	9.0×10^{-9}	1.5×10^{-8}–1.0×10^{-4}	[7]
Carbon nanotube paste	Ferrocenemonocarboxylic acid	Differential pulse voltammetry	5.0	90	20	2.0×10^{-7}	5.0×10^{-7}–5.0×10^{-5}	[8]
Carbon nanotube paste	2,7-BF	Differential pulse voltammetry	7.0	180	10	2.6×10^{-8}	8.0×10^{-8}–7.0×10^{-4}	This work

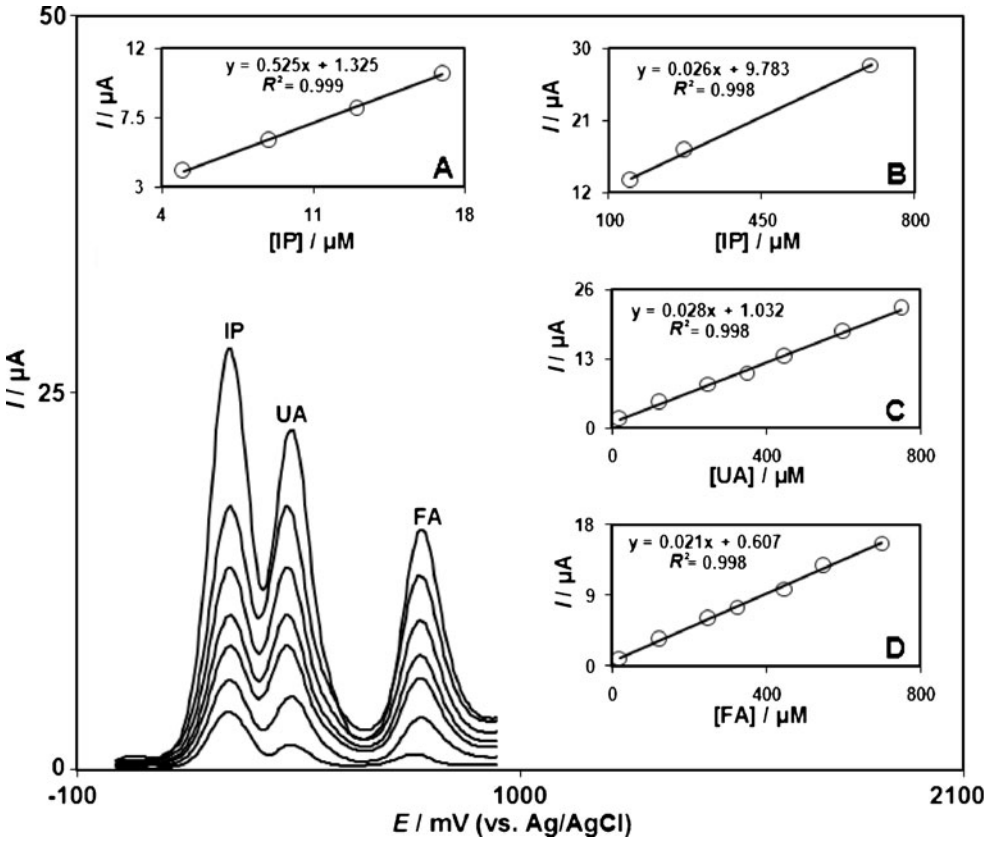

Fig. 4 DPVs of 2,7-BFCNPE in 0.1 M PBS (pH 7.0) containing different concentrations of IP + UA + FA in micromolar, from inner to outer, 5.0+20.0+20.0, 9.0+125.0+125.0, 13.0+250.0+250.0, 17.0+350.0+325.0, 150.0+450.0+450.0, 275.0+600.0+550.0, and 700.0+750.0+700.0, respectively. *Insets A*, *B*, *C*, and *D* are plots of I_p vs. IP, UA, and FA concentrations, respectively

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 IP and the concentration of this compound was obtained using standard addition method. This procedure was repeated three times for each sample, and the average amount of IP in the injection was found to be 0.195± 0.005 mg, a value in well agreement with the value on the ampoule label (0.20 mg). Also, to a series of 10-mL volumetric flasks, different capacity of the diluted IP injection solution together with standard UA and FA solutions were added and diluted to the mark with PBS. The DPVs were recorded and the anodic peak currents for

Table 3 The application of 2,7-BFCNPE for simultaneous determination of IP, UA, and FA in IP injection, urine, and human blood serum

Sample	Spiked (μM)			Found (μM)			Recovery (%)			R.S.D. (%)		
	IP	UA	FA	IP	UA	FA	IP	UA	FA	IP	UA	FA
IP injection	0	0	0	7.1±1.1	ND	ND	–	–	–	2.4	–	–
	2.5	15.0	20.0	9.7±2.2	15.5±1.9	19.7±2.3	101.0	103.3	98.5	3.1	1.9	2.4
	5.0	17.5	22.5	11.9±2.4	17.2±3.2	23.2±1.9	98.3	98.3	103.1	2.9	1.6	3.3
	7.5	20.0	25.0	15.1±1.8	19.8±2.7	25.3±2.9	103.4	99.0	101.2	2.8	2.3	2.5
Urine	0	0	0	ND	25.0±3.1	ND	–	–	–	–	1.4	–
	5.0	15.0	20.0	5.1±1.9	39.1±2.6	20.6±1.7	102.0	97.7	103.0	1.8	2.3	3.2
	7.5	17.5	22.5	7.3±2.8	43.2±2.4	21.9±3.1	97.3	101.6	97.3	2.3	1.7	2.2
	10.0	20.0	25.0	9.9±2.6	46.7±3.5	24.8±2.9	99.0	103.8	99.2	2.1	3.2	2.3
Human blood serum	0	0	0	ND	15.0	ND	–	–	–	–	2.9	–
	5.0	15.0	20.0	4.9±2.3	31.1±2.2	20.3±3.5	98.0	103.7	101.5	2.7	2.9	1.6
	7.5	17.5	22.5	7.7±2.6	31.9±1.9	23.2±2.9	102.7	98.1	103.1	2.2	1.9	3.2
	10.0	20.0	25.0	10.1±1.7	34.5±2.8	24.5±2.3	101.0	98.6	98.0	1.7	3.1	2.6

All concentrations are in micromolar. All "±" values are R.S.D% (n=3)

ND not detected, *IP* isoproterenol, *UA* uric acid, *FA* folic acid, *R.S.D.* relative standard deviation

each of IP, UA, and FA were measured at their own oxidation potentials. According to the results listed in Table 3, very good recoveries for the determinations of IP, UA, and FA 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.

Determination of IP, UA, and FA in urine and human blood serum

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of IP, UA, and FA in urine and human blood serum. Urine samples were stored in a refrigerator immediately after collection. Ten milliliters of the sample was centrifuged for 15 min at 2,000 rpm. The supernatant was filtered out using a 0.45-μm filter. Then, different volume of the solution was transferred into a 10-mL volumetric flask and diluted to the mark with PBS (pH 7.0). The diluted urine sample was spiked with different amounts of IP, UA, and FA.

The serum sample was centrifuged and then after filtering, diluted with PBS (pH 7.0) without any further treatment. The diluted serum sample was spiked with different amounts of IP, UA, and FA.

The results are given in Table 3. Satisfactory recovery of the experimental results was found for IP, UA, and FA. The reproducibility of the method was demonstrated by the mean relative standard deviation.

Conclusions

In the present study, a carbon paste electrode modified with carbon nanotubes and 2,7-bis(ferrocenyl ethyl)fluoren-9-one was used for the determination of IP. The CV and DPV investigations showed effective electrocatalytic activity in lowering the anodic overpotential for IP. The high sensitivity and very low detection limit (26.0±2), together with the ease of preparation and surface regeneration of the modified electrode, are the advantages of the studied modified electrode. The modified electrode displays high selectivity in the voltammetric measurements of IP, UA, and FA in their mixture solutions.

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