

Full Paper

Voltammetric Determination of Isoproterenol using a Nanostructure based Electrochemical Sensor

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Abstract- 2-Chlorobenzoyl ferrocene (2CBF) was synthesized and used to construct a modified-carbon nanotube paste electrode. The electro-oxidation of isoproterenol at the surface of the modified electrode was studied using cyclic voltammetry (CV), chronoamperometry (CHA) and square wave voltammetry (SWV). Under the optimized conditions, the square wave voltammetric peak currents of isoproterenol increased linearly with isoproterenol concentration in the range of 2.5×10^{-7} to 8.0×10^{-5} M and detection limit of 9.0×10^{-8} M was obtained for isoproterenol. Finally this modified electrode was used for determination of isoproterenol in real samples

Keywords- Isoproterenol, Carbon nanotubes, Chemically modified electrodes, Carbon paste electrode

1. INTRODUCTION

Isoproterenol is a synthetic sympathomimetic amine that is structurally related to epinephrine but acts almost exclusively on beta receptors. Chemically it differs from epinephrine in having an isopropyl group replacing the methyl group attached to the nitrogen atom; rarely used because of the availability of more specific agents [1]. It is a medication used for the treatment of pulmonary hypertension and allergic emergencies, bronchitis, cardiac chock and heart attack. It is also well known that isoproterenol generates free radicals leading to lipid peroxidation, which cause irreversible damage to the myocardium [2,3]. Increase in formation of reactive oxygen species (ROS) during ischemia/reperfusion and the adverse effects of oxygen radicals on myocardium have been well established by both direct and indirect parameters. Thus, the excess of the drug causes loss of functional integrity and necrotic lesion in heart muscle [4,5].

Therefore, from health viewpoints, development of a sensitive analytical method for the determination of isoproterenol is very important. Several methods have been described for the determination of isoproterenol such as spectrofluorimetry, spectrophotometry, liquid and gas chromatographic and electrochemical methods [6-9]. Among them electrochemical methods are practical and attractive because electrochemical instrumentation is usually fast, relatively inexpensive, simple, power efficient, accurate and highly sensitive. Moreover, the development of a rapid electrochemical method for isoproterenol determination that does not require sample pretreatment is possible. It is known that the electrochemical oxidation of isoproterenol at bare electrode surfaces requires high-overpotential [10-17].

Carbon paste is one of the most popular materials to prepare solid-state electrodes, with high applicability in electrochemistry research. The ease and speed of preparation and obtaining a new reproducible surface, low residual current, porous surface and low cost are advantages of carbon paste electrodes (CPEs) over all other solid electrodes [18-20]. Recently, the application of the carbon paste electrodes modified with various materials exhibits considerable improvements in electrochemical behavior of many important compounds [21-23]. In this sense, novel types of CPEs are continuously developed, testing new modifiers and mediators for traditional carbon paste mixtures or using new carbon paste mixtures, in which both graphite and binders are replaced by alternative materials [24,25].

Carbon nanotubes represent one of the best examples of novel nanostructures derived by bottom-up chemical synthesis approaches. Carbon nanotubes, long, thin cylinders of carbon, were discovered in 1990 [26,27]. These are large macromolecules that are unique for their size, shape, and remarkable physical properties. They can be thought of as a sheet of graphite (a hexagonal lattice of carbon) rolled into a cylinder [28].

When the structure of atoms in a carbon nanotube minimizes the collisions between conduction electrons and atoms, a carbon nanotube is highly conductive [29-32]. The strong bonds between carbon atoms also allow carbon nanotubes to withstand higher electric

currents than copper. Electron transport occurs only along the axis of the tube. For the purpose of this paper we are going to focus on the applications related to electrical conductivity [33-39].

In the present work, following the idea of searching new methods for isoproterenol detection, we describe the preparation of a new electrode composed of 2-chlorobenzoyl ferrocene modified carbon nanotube paste electrode (2CBFCNPE). We described initially the preparation and suitability of a 2CBFCNPE as a new electrode in the electrocatalysis and determination of isoproterenol in an aqueous buffer solution. Finally this new constructed electrochemical sensor was used for determination of isoproterenol in the real samples.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and 2CBFCNPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was used for pH measurements.

Isoproterenol and all of the other reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-9.0. Multi-walled 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 & Amorphous Materials, Inc. 2CBF was synthesized in our laboratory as reported previously [21].

2.2. Preparation of the electrode

The 2CBFCNPEs were prepared by hand mixing 0.01 g of 2CBF with 0.89 g graphite powder and 0.1 g carbon nanotubes 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, 2CBF modified CPE electrode (2CBFCPE) without carbon nanotubes, carbon nanotubes paste electrode (CNPE) without 2CBF, and unmodified CPE in the absence of both 2CBF and carbon nanotubes were also prepared in the same way.

3. RESULT AND DISCUSSION

3.1. Electrochemical Behavior of 2CBFCNPE

2CBFCNPE was constructed and its electrochemical properties were studied in a 0.1 M PBS (pH 4.0) using CV (Fig. 1). The experimental results show well-defined and reproducible anodic and cathodic peaks with E_{pa} , E_{pc} and $E^{\circ'}$ of 655, 540 and 597 vs. Ag/AgCl/KCl (3.0 M) respectively. The observed peak separation potential, $\Delta E_p=(E_{pa}-E_{pc})$ of 115 mV, was greater than the value of $59/n$ mV expected for a reversible system [40] suggesting that the redox couple of 2CBF in 2CBFCNPE has a quasi-reversible behavior in aqueous medium.

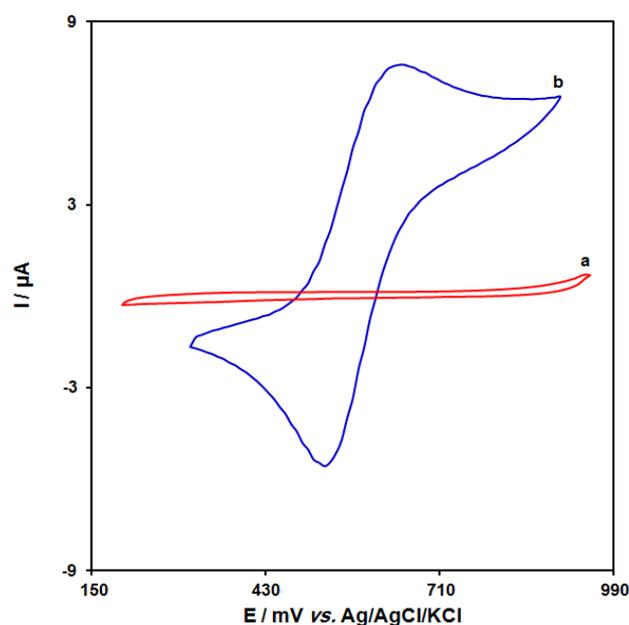


Fig. 1. CVs of CPE (a) and 2CBFCNPE (b) in 0.1 M PBS (pH 4.0). In all cases, the scan rate is 100 mVs^{-1} .

3.2. Electrocatalytic oxidation of isoproterenol at a 2CBFCNPE

Fig. 2 depicts the CV responses for the electrochemical oxidation of $40.0 \mu\text{M}$ isoproterenol at unmodified CPE (curve a), CNPE (curve b), 2CBFCPE (curve c) and 2CBFCNPE (curve d). As can be seen, while the peak potential for isoproterenol oxidation at the CNPE, and unmodified CPE are 690 and 720 mV, respectively, the corresponding potential at 2CBFCNPE and 2CBFCPE is ~ 655 mV. These results indicate that the peak potential for isoproterenol oxidation at 2CBFCNPE and 2CBFCPE shifts by ~ 35 and 65 mV toward negative values compared to CNPE and unmodified CPE, respectively. However, 2CBFCNPE shows a much higher anodic peak current for the oxidation of isoproterenol compared to 2CBFCPE, indicating that the combination of carbon nanotubes and the

mediator (2CBF) has significantly improved the performance of the electrode toward isoproterenol oxidation. 2CBFCNPE, in 0.1 M PBS (pH 4.0) and without isoproterenol in solution (Fig. 1 curve b), exhibited a well-behaved redox reaction and with addition of 40.0 μM isoproterenol, increased the anodic peak current (Fig. 2 curve d), indicating a strong electrocatalytic effect [40].

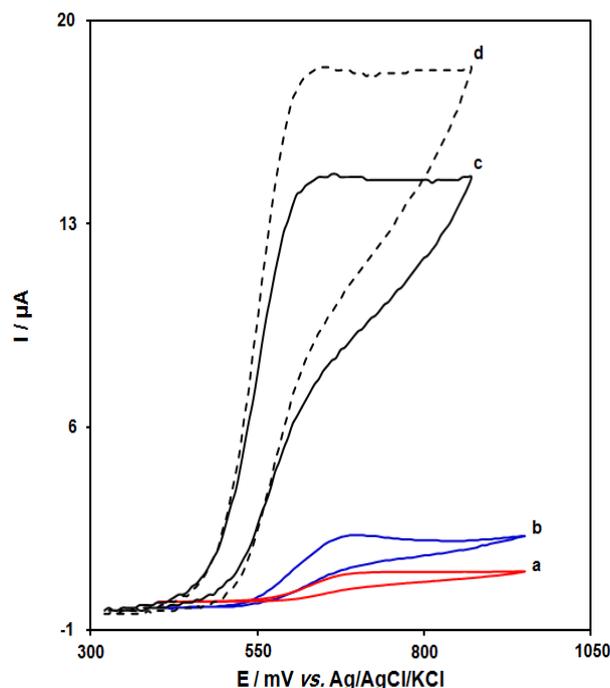


Fig. 2. CVs of CPE (a), CNPE (b), 2CBFCPE (c) and 2CBFCNPE (d) in 0.1 M PBS (pH 4.0) containing 40.0 μM isoproterenol. In all cases, the scan rate is 10 mV s^{-1}

The effect of scan rate on the electrocatalytic oxidation of isoproterenol at the 2CBFCNPE was investigated by LSV (Fig. 3). As can be observed in Fig. 3, 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 ($v^{1/2}$) was found to be linear in the range of 4-35 mV s^{-1} , suggesting that, at sufficient overpotential, the process is diffusion rather than surface controlled [40].

A plot of the scan rate-normalized current ($I_p/v^{1/2}$) vs. scan rate (Fig. 3B) exhibits the characteristic shape typical of an EC' process [40].

Fig. 4 shows the LSV of 2CBFCNPE obtained in 0.1 M PBS (pH 4.0) containing 2.5 μM isoproterenol, with a sweep rate of 4 mV s^{-1} . The points show the rising part of the voltammograms (known as the Tafel region), which is affected by the electron transfer kinetics between isoproterenol and 2CBFCNPE. If deprotonation of isoproterenol is a sufficiently fast step, the number of electrons involved in the rate determining step can be estimated from the slope of the Tafel plot. The inset of Fig. 4 shows a Tafel plot that was

drawn from points of the Tafel region of the LSV. The Tafel slope of 0.1329 V obtained in this case agrees well with the involvement of one electron in the rate determining step of the electrode process, assuming a charge transfer coefficient of $\alpha=0.56$.

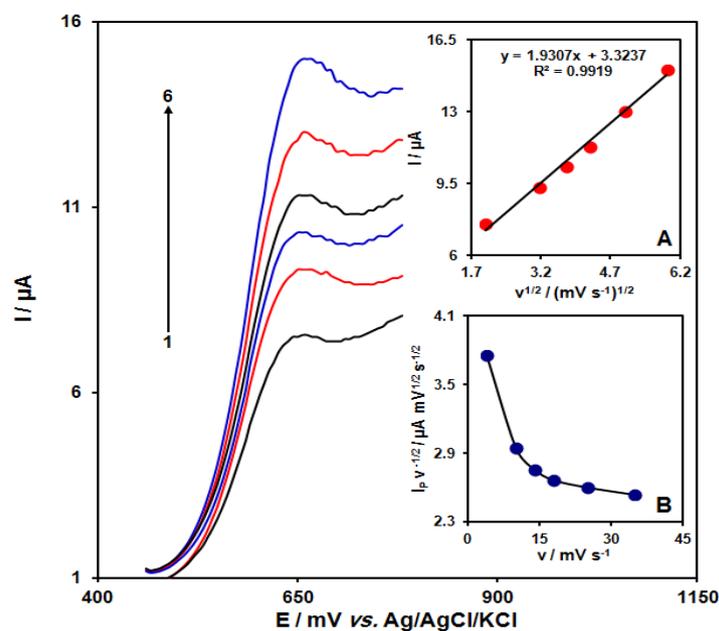


Fig. 3. LSVs of 2CBFCNPE in 0.1 M PBS (pH 4.0) containing 2.5 μM isoproterenol at various scan rates; numbers 1-6 correspond to 4, 10, 14, 18, 25 and 35 mV s^{-1} , respectively. Insets: Variation of (A) anodic peak current vs. $v^{1/2}$ and (B) normalized current ($I_p/v^{1/2}$) vs. v

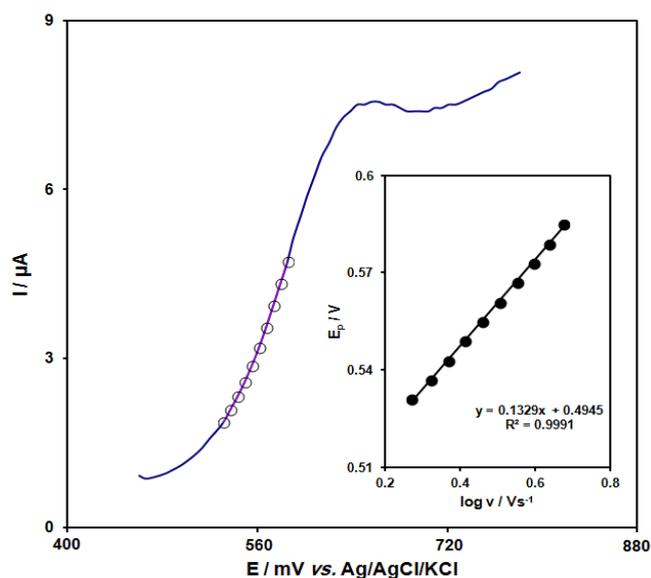


Fig. 4. LSV (at 4 mVs^{-1}) of a 2CBFCNPE in 0.1 M PBS (pH 4.0) containing 2.5 μM isoproterenol. The points are the data used in the Tafel plot. The inset shows the Tafel plot derived from the LSV

3.3. Chronoamperometric measurements

Chronoamperometric measurements of isoproterenol at 2CBFCNPE were carried out by setting the working electrode potential at 0.75 V (at the first potential step) and at 0.4 V (at second potential step) *vs.* Ag/AgCl/KCl (3.0 M) for the various concentrations of isoproterenol in 0.1 M PBS (pH 4.0) (Fig. 5). For an electroactive material (isoproterenol 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 [40].

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \quad (1)$$

Where D and C_b are the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I *vs.* $t^{-1/2}$ were employed, with the best fits for different concentrations of isoproterenol (Fig. 5A). The slopes of the resulting straight lines were then plotted *vs.* isoproterenol concentration (Fig. 5B). From the resulting slope and Cottrell equation the mean value of the D was found to be $1.43 \times 10^{-6} \text{ cm}^2/\text{s}$.

Chronoamperometry can also be employed to evaluate the catalytic rate constant, k , for the reaction between isoproterenol and the 2CBFCNPE according to the method described by Galus [41].

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

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

$$I_C / I_L = \pi^{1/2} \gamma^{1/2} = \pi^{1/2} (kC_b t)^{1/2} \quad (3)$$

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 isoproterenol concentration. From the values of the slopes (Fig. 5C), the average value of k was found to be $2.3 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$.

3.4. Calibration plot and limit of detection

The electrocatalytic peak current of isoproterenol oxidation at the surface of the 2CBFCNPE can be used for determination of isoproterenol in solution. Therefore, square wave voltammetry (SWV) experiments were performed using modified electrode in 0.1 M PBS (pH 4.0) containing various concentration of isoproterenol (Fig. 6).

The plot of peak current *vs.* isoproterenol concentration consisted of two linear segments with slopes of 1.0159 and 0.0915 $\mu\text{A}/\mu\text{M}$ in the concentration ranges of 2.5×10^{-7} - 1.0×10^{-5} M

and 1.0×10^{-5} - 8.0×10^{-5} M respectively. The detection limit (3σ) of isoproterenol was found to be 9.0×10^{-8} M.

3.5. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of isoproterenol in isoproterenol injection and urine samples. The results are listed in Table 1. Satisfactory recovery of the experimental results was found for isoproterenol. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

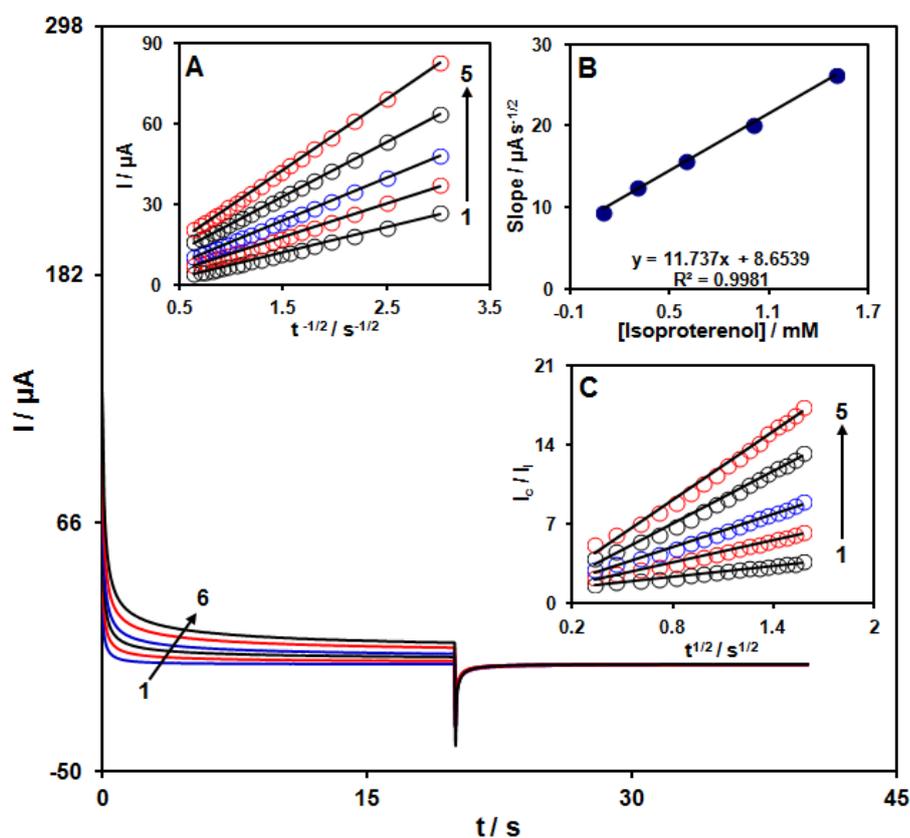


Fig. 5. Chronoamperograms obtained at 2CBFCNPE in 0.1 M PBS (pH 4.0) for different concentrations of isoproterenol. The numbers 1–6 correspond to 0.0, 0.1, 0.3, 0.6, 1.0 and 1.5 mM of isoproterenol. Insets: (A) Plots of I vs. $t^{1/2}$ obtained from chronoamperograms 2–6; (B) Plot of the slope of the straight lines against isoproterenol concentration; (C) Dependence of I_c/I_i on $t^{1/2}$ derived from the data of chronoamperograms 1-6

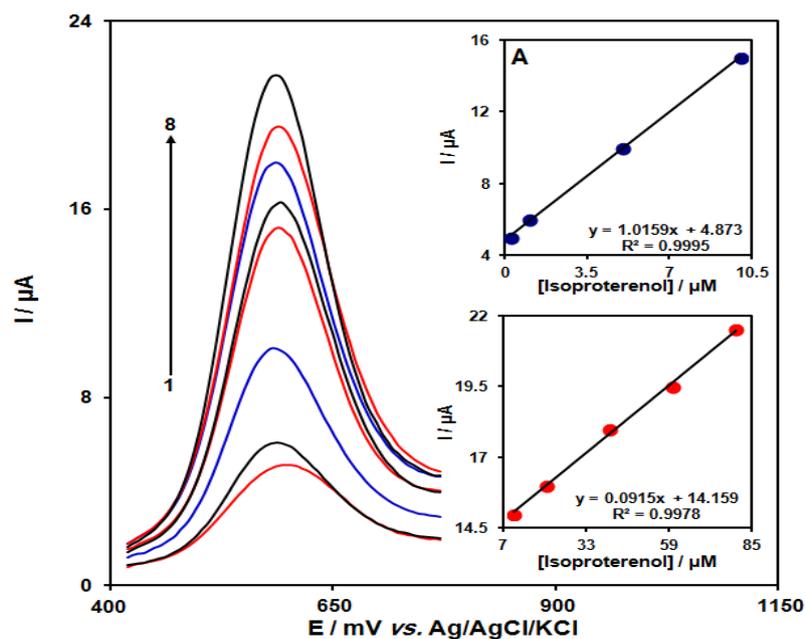


Fig. 6. SWVs of 2CBFCNPE in 0.1 M PBS (pH 4.0) containing different concentrations of isoproterenol. Numbers 1-8 correspond to: 0.25, 1.0, 5.0, 10.0, 20.0, 40.0, 60.0 and 80.0 μM . Insets: plots of I_p vs. isoproterenol concentrations in the range of 0.25-10.0 μM (A) and 10.0-80.0 μM (B)

Table 1 The application of 2CBFCNPE for determination of isoproterenol in isoproterenol injection and urine samples (n=5)

Sample	Spiked (μM)	Found (μM)	Recovery (%)	R.S.D. (%)
Isoproterenol injection	0	9.0	-	3.1
	2.5	11.4	99.1	1.8
	7.5	16.9	102.4	2.5
	12.5	21.8	101.4	2.3
	17.5	25.9	97.7	3.2
Urine	0	ND ^a	-	-
	5.0	5.1	102.0	2.8
	10.0	9.9	99.0	2.1
	20.0	20.1	100.5	3.3
	30.0	29.1	97.0	1.7

^a ND: Not detected

4. CONCLUSION

The 2CBFCNPE was prepared and used for the investigation of the electrochemical behavior of isoproterenol. Two pairs of well-defined redox peaks were obtained at the

2CBFCNPE. The 2CBFCNPE showed excellent electrocatalytic activity for the isoproterenol. The SWV currents of isoproterenol at 2CBFCNPE increased linearly with the isoproterenol concentration in the range from 2.5×10^{-7} to 8.0×10^{-5} M with a detection limit of 9.0×10^{-8} M. Finally, this method was used for the determination of isoproterenol in some real samples.

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