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Using multiwalled carbon nanotubes/ferrocene nanocomposite paste electrode for sensitive electrocatalytic determination of isoprenaline

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ABSTRACT

A novel voltammetric sensor for the determination of isoprenaline (ISP) was fabricated based on a ferrocene-multiwalled carbon nanotubes (Fc-MWCNTs) composite paste electrode. Then the electrochemical behavior of this sensor was investigated in detail by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The kinetic parameters such as electron transfer coefficient, α, and apparent rate constant for the redox reaction between ISP and the Fc-MWCNTs paste electrode were also determined using electrochemical approaches. The sensor displayed highly sensitive electrocatalytic activity towards the oxidation of ISP, and under the optimized conditions, its electrocatalytic oxidation peak current showed two linear concentration range from 5.0 to 50.0 μmol L^{-1} and 500.0 to 2000.0 μmol L^{-1} with a detection limit (S/N=3) of 0.1 μmol L^{-1} ISP using the differential pulse voltammetric (DPV) method. The proposed method was applied for the sensitive and selective determination of ISP in the pharmaceutical formulations and human urine samples with satisfactory results.

KEYWORDS

Carbon nanotube paste electrode;
Ferrocene;
Isoprenaline;
Electrocatalysis;
Electrochemical impedance spectroscopy.

INTRODUCTION

Isoprenaline (ISP) or isoproterenol 4-[1-hydroxy-2-[(1-methylethyl)-amino] ethyl]-1,2-benzenediol is a catecholamine drug and a sympathominetic agent which acts almost exchustively on beta-adrcnergic receptors. Catecholamines play a major role as neurotransmitters in the function of brain and nerve signal transduction and are widely used in the treatment of bronchial asthma, hypertension, in cardiac surgery etc[1]. ISP stimulates the central nervous system, and it has a powerful stimulating action on the heart and increases cardiac output, excitability, and rate. The cardiovascular effects of isoprenaline are compared with the epinephrine, adrenaline and noradrenaline, which can relax almost every kind of the smooth musculature that receives adrenergic nervous, but this effect is pronounced in the musculature of bronchus and also in the gastrointestinal tract. Isoprenaline is better absorbed when dispensed by inhalation. It is used for the symptomatic relief of bronchial asthma, in the treatment of bradycardia in patients with heart block[1, 2].

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The therapeutic importance of ISP required the development of sensitive and rapid method for industrial quality control and clinical monitoring. Various analytical methods have been reported for the separation and determination of isoprenaline based on spectrophotometry[3-5], spectrofluorimetry[6-8], chemiluminescence[9, 10], capillary electrophoresis[11-13], high-performance liquid chromatography[14-16] and electrochemical methods[17, 18]. HPLC has been the most widely used technique for determination of this group of drugs, but presents some disadvantages such as necessity to the large amount of high purity organic solvents and long equilibration time. Electrochemical methods have been very useful for the determination of electroactive species in pharmaceuticals and body fluids due to their simplicity and low cost. However, in electrochemical detection of ISP, the reported voltammetric methods may suffer from low sensitivity and selectivity that leads to an inactive overpotential due to the irreversibility of voltammetric behavior of catecholamines. However, compared to other techniques, modified electrodes provide certain advantages such as long term stability and suitable sensitivity, selectivity and response repeatability.

Carbon nanotubes (CNTs), due to their high electrical conductivity, strong adsorptive ability, good mechanical strength and excellent biocompatibility, have received enormous attention in biological applications, particularly for constructing electrochemical biosensors because of utilizing the high electron transfer ability between biomolecules and CNTs modified electrode surface acting as nanowires[19, 20]. However, the insolubility of CNTs in most solvents and their hydrophobicity limit their application in the design of CNT-based biosensing devices[21]. The challenge of solubility of CNTs has been addressed by chemical oxidation and functionalization with carboxylate or sulfonate groups, which facilitates covalent binding to specific target molecules[22-24]. However, the covalent functionalization can often destroy the sp^2 structures thereby diminishing their pristine mechanical and electronic properties[25, 26]. Thus, non-covalent functional strategies, such as hydrophobic or van der Waals interactions, seem to be a more moderate and effective alternative for this purpose through non-covalent interactions[27-30]. Since the CNTs exhibit a special sidewall curvature and possess a π–conjugative structure with highly hydrophobic surface, which allow them to interact with aromatic rings such as porphyrin, pyrene due to effective stacking and van der Waals interactions[31-35]. The high ratio with large surface area of MWCNTs makes them possible to immobilize tethering molecules mediators with high density either via covalently bonding or via van der Waals interaction and show enhanced electrochemical detection capability[36-38].

Ferrocene (Fc) and its derivatives are well-known mediators[39, 40] due to their various very desirable properties, e.g., relatively low molecular mass, reversibility, regeneration at low potential, and generation of stable redox forms[41]. In this study, the carboxilated multiwalled carbon nanotubes (MWCNTs) were simply mixed with ferrocene electrocatalyst and this composite was fabricated as a highly sensitive electrochemical sensor for the detection of ISP.

The objective of the current work is to develop a simple, selective and sensitive method for the determination of isoprenaline, based on the unusual properties of carbon nanotubes such as strong adsorptive ability, huge specific area, subtle electronic properties as well as excellent electrocatalytic activity, and its synergetic effects with ferrocene electrocatalyst. Thus a simple mixing method was used for the preparation of the homogeneous composite of carboxylated MWCNTs and ferrocene. Then, the electrochemical behavior of ISP on the Fc-MWCNTs paste electrode was investigated by voltammetry and electrochemical impedance spectroscopy techniques to determine the interaction between isoprenaline and the modified electrode. The results showed that this modified electrode strongly enhanced the electron transfer rate of ISP oxidation and the determination sensitivity of the analyte was significantly improved. Consequently, an ultrasensitive differential pulse voltammetric method based on the Fc-MWCNTs electrode was developed for the quantification of ISP in the pharmaceutical and human urine samples. This newly proposed method possesses some advantages such as high sensitivity, rapid response, low cost and simplicity.

EXPERIMENTAL

Chemicals

All chemicals, unless mentioned otherwise, were of

analytical grade and were used as received. The MWCNTs were bought from Iran's Research Institute of Petroleum Industry and synthesized by chemical vapor deposition (CVD) with a diameter of 8–15 nm, a length 50 μmol L^{-1} and the purity of 95%. Also, High viscosity paraffin oil (density = 0.88 g cm^{-3}) from Fluka were used for preparation of carbon nanotube paste electrode, and isoprenaline was purchased from Fluka. All aqueous solutions were made with demineralized water, which was further purified with a Milli-Q system (Millipore) with the resistivity not less than 18.0 MΩ at 25 ºC. Universal buffer (boric acid, phosphoric acid, acetic acid and sodium hydroxide, 0.1 mol L^{-1}) solutions with different pH values were used for the study of the pH influence.

Apparatus

Electrochemical measurements were carried out in a conventional three-electrode cell, powered by an electrochemical system comprising the Autolab system with PGSTAT 12 and FRA2 boards (Eco Chemie B. V., Utrecht, The Netherlands). The system was run on a PC using GPES and FRA 4.9 software. For impedance measurements, a frequency range of 100.0 kHz to 10.0 mHz was employed. The AC voltage amplitude used was 5 mV, and the equilibrium time was 20 minutes. The Fc-MWCNTs paste electrode, a graphite electrode and a saturated Ag/AgCl reference electrode was employed as a working, auxiliary and reference electrode, respectively. The Fc-MWCNTs composites were characterized by scanning electron microscopy (SEM) with a Philips XL Model 30 microscope.

Preparation of the Fc-MWCNTs paste Electrode

To eliminate metal oxide catalysts within the nanotubes, multi-walled carbon nanotubes were refluxed in the 2.0 mol L^{-1} HNO_3 for 15 hours, and then washed with twice-distilled water and dried at room temperature. Ferrocene (0.05 g) was dissolved in 20 mL acetone and homogenized with ultrasonic agitation in the presence of 0.950 g of carbon nanotube, then it was hand mixed by a mortar and pestle, until the solvent was evaporated. Using a syringe, 0.1 g paraffin was added to the mixture and mixed well for 30.0 min until a uniformly-wetted paste was obtained. The paste was then packed into a glass tube (geometric surface area; 0.0434 cm^2). Electrical contact was made by pushing a copper wire down the glass tube into the back of the mixture. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing it on a weighing paper. The unmodified carbon nanotube paste electrode (CNPE) was prepared in the same way without adding ferrocene to the mixture to be used for comparison purposes.

Preparation and analysis of Real samples

The fresh human urine samples were collected in dark glass containers, filtered through 0.22 μm pore size membrane, stored in refrigerator, and analyzed within 8 h after collection. Without any pretreatment, samples were diluted with ultrapure water to the working range of the determination of ISP, and then used for analysis. Liquid formulations of ISP, labeled with amount of 1.0 mg mL^{-1} per ampule, were appropriately diluted to 10 mL with 0.1 mol L^{-1} universal buffer solution with pH 6.0. No other treatment of the samples was required. The standard addition method was used for the determination of isoprenaline in real samples.

The cyclic voltammograms were recorded by cycling the potential between -0.1 and +0.5 V (versus Ag/AgCl) at a scan rate of 50 mVs^{-1}. Differential pulse voltammetric analysis of real samples were performed in the universal buffer solution with pH 6.0, by scanning the potential between 0.0 and 0.5 V at 10 mV s^{-1} and a 50 mV pulse amplitude. The percent content of ISP in these samples was determined by the standard addition method.

RESULTS AND DISCUSSION

Characterization and Electrochemical Behavior of Fc-MWCNTs electrode

The dispersing state and homogeneity of the carboxylated MWCNTs and their composites with ferrocene were examined by scanning electron microscopy. As shown in Figure 1, SEM micrographs showed that ferrocene agglomerates and particles have uniformly adsorbed on the nanotube structures.

Cyclic voltammetry was employed for investigating the electrochemical properties of Fc-MWCNTs in the pure buffered aqueous solutions (pH 2.0 to 10.0). A

typical cyclic voltammogram (see Figure 2) in pH 6.0 and the potential scan rate 50 mV s^{-1}, exhibits an anodic and corresponding cathodic peaks with E_{pa}=0.33 V and E_{pc}=0.15 V vs. saturated Ag/AgCl reference electrode. The experimental results show well-defined and reproducible anodic and cathodic peaks related to Fc/Fc^+ redox couple with quasi-reversible behavior. Because the peak separation potential, $\Delta E_p = (E_{pa} - E_{pc})$, is greater than that 59/*n* mV expected for a reversible system. Also, the obtained result from cyclic voltammetry of this modified electrode in various buffered solutions does not show any shift in the anodic and cathodic peak potentials. Therefore, the electrochemical behavior of the redox process of Fc/Fc^+ in Fc-MWCNTs is independent on the pH of aqueous solution.

The effect of varying the scan rate on the performance of the electrode was also studied. With increasing the scan rate, the CV peak currents of the Fc-MWCNTs modified electrode increased in the scan rate range of 10.0-100.0 mV s^{-1}, and ΔE_p was slightly dependent on the scan rate, indicating that all of Fc mediators were not efficiently connected on the MWCNTs surfaces. The cathodic and anodic peak currents increased linearly with the increase of the square root of scan, and the following equation obtained: I / μA= 14.3940 × $v^{1/2}$ / (V s^{-1}) – 56.0380, R^2=0.9929, suggesting that the electrochemical reaction of MWCNTs-Fc modified electrode is a diffusion-controlled process.

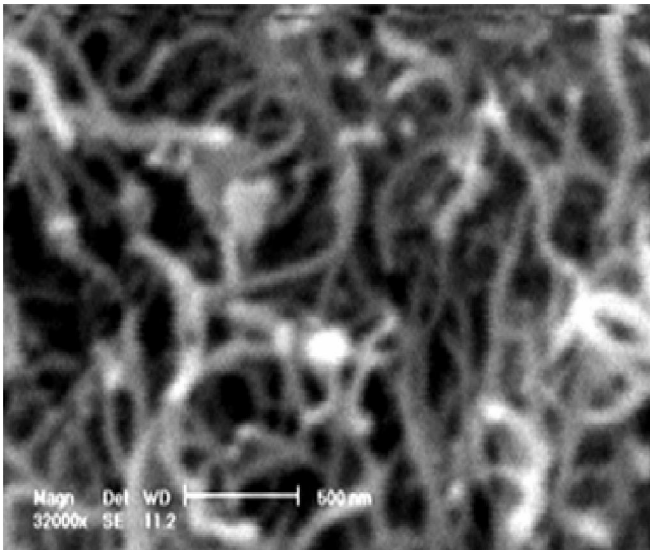

Figure 1 : Typical SEM images of ferrocene-MWCNTs composite.

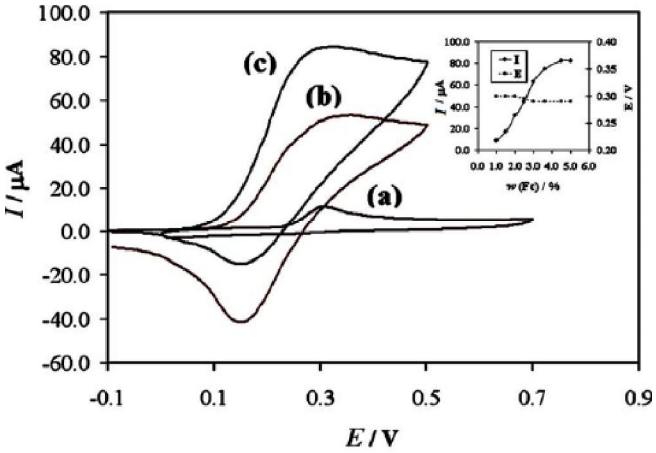

Figure 2 : The cyclic voltammograms of (a) the bare MWCNTs paste electrode in the presence of ISP, and Fc-WCNTs paste electrode in the absence (b) and presence of ISP (c). Inset shows the effect of modifier fraction, *w*/%, on the oxidation peak current of ISP. Other conditions; pH 6.0, 1.0 mmol L^{-1} ISP and the scan rate 50.0 mV s^{-1}.

Electrocatalytic oxidation of isoprenaline on the modified electrode

Figure 2 shows the typical cyclic voltammograms of ISP in a pH 6.0 buffer solution at a bare CNPE and a Fc-MWCNTs (*w*(Fc) = 4.5 %) paste electrodes between -0.1 to 0.5 V at the scan rate 50.0 mV s^{-1} for 1.0 mmol L^{-1} ISP solution. This Figure illustrates the cyclic voltammetric responses of a bare carbon nanotube paste electrode in the presence of ISP (curves a) and Fc-modified MWCNTs (curves b and c) without and with ISP analyte, respectively. At the surface of the unmodified electrode, the direct oxidation of ISP produces an anodic peak at the potential of 310.0 mV and a depressed cathodic peak at near to 100.0 mV. Under the identical conditions, the ferrocene modified MWCNTs gives increased peak currents for ISP. Thus, by using Fc as an electron mediator in the matrix of the modified electrode, the overpotential for the anodic oxidation of isoprenaline becomes considerably lower and the rate of the heterogeneous electron transfer is increased. This suggests an efficient electrocatalytic reaction of ISP on the Fc-MWCNTs paste electrode.

The amount of ferrocene in the Fc-MWCNTs paste electrode has a significant influence on the voltammetric response of the modified electrode. As shown in the inset of Figure 2, which is a plot of peak current and peak potential vs. the modifier mass fraction, *w*(Fc), the oxidation current for 1.0 mmol L^{-1} ISP increases

gradually with modifier, and at *w*(Fc) =4.5% the oxidation current achieves a maximum and then become constant with a further increase of the modifier mass fraction. Also, the oxidation peak potential of ISP reduces slightly with adding modifier. This occurs may be, due to increasing of interactions between the analyte molecules and electrocatalyst. Therefore, the role of modifier is to enhance the peak current and also to decrease the overpotential for the oxidation of ISP. It was found that the best carbon nanotube paste composition for an electrode is with *w*(Fc) = 4.5%, *w*(MWCNTs) = 86.41% and *w*(paraffin oil) = 9.1%.

Kinetic studies by chronoamperometry confirm the electron transfer rate in the presence and absence of 1.0 mmol L^{-1} ISP. At the intermediate times, the catalytic current (I_C) is dominated by the rate of electrocatalyzed oxidation of isoprenaline. So the rate constant for the reaction between ISP and Fc-MWCNTs is determined according to the method described in the literature[42]:

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

where I_C and I_L show current in the presence and absence of ISP, respectively. $\gamma = kC_0t$ (k is the catalytic rate constant ((mol L^{-1})$^{-1}$ s^{-1}), t the time elapsed (s), and C_0 is the concentration of isoprenaline) is the argument of error function. Where γ exceed 2, the error function is almost equal to 1 and the above equation can be considered as follows:

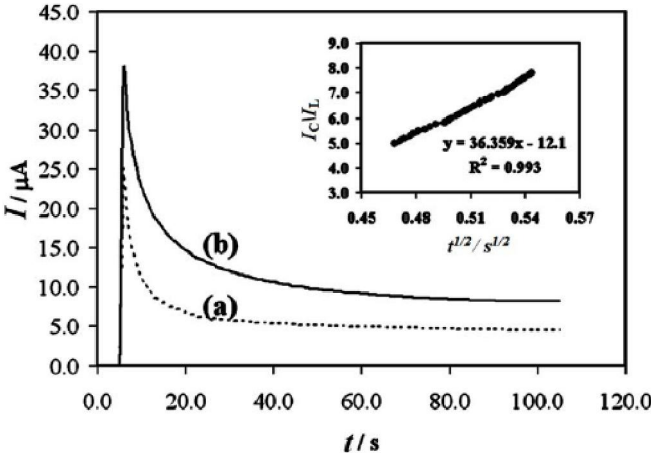

Figure 3 : Chronoamperograms obtained at the potential step of +300 mV on the Fc-MWCNTs paste electrode and in the absence (a) and presence of 1.0 mmol L^{-1} ISP (b). Inset shows the related plot of I_C/I_L vs. $t^{1/2}$ driven from the chronoamperogram data.

$$\frac{I_C}{I_L} = \gamma^{1/2}\pi^{1/2} = \pi^{1/2}(kC_0t)^{1/2} \quad (2)$$

According to the Figure 3, the slopes of the I_C/I_L vs. $t^{1/2}$ for 1.0 mmol L^{-1} ISP was determined and k was calculated to be (4.21±0.11) ×10^4 M^{-1} s^{-1}.

In this study, we used the above process on the Fc-MWCNTs paste electrode for the determination of isoprenaline in pharmaceutical and human urine samples and optimized the various parameters that can affect on the amount of that electrocatalytic oxidation signal.

Effect of pH

The peak current closely depends on the pH of buffer solution. Experimental results of 1.0 mmol L^{-1} ISP in 0.1 mol L^{-1} buffer solution at different pHs from 2.0 to 11.0 are shown in Figure 4. It can be seen that the oxidation peak current enhances as increasing pH, and decreases after attaining a maximum. Also, the potential peaks gradually decrease in the pHs from 2.0 to 10.0. The calibration curve of E versus pH has a slope of -6.2 mV pH^{-1}. This slope approximately indicates the relatively slight participation of hydrogen ions in the oxidation reaction of ISP. The electrocatalytic oxidation of isoprenaline at the surface of modified electrode is more favoured under pH 6.0 condition than in highly acidic or basic mediums. This appears as a gradual growth in the anodic peak current and a simultaneous decrease in the cathodic peak current of cyclic voltammograms. Therefore, pH 6.0 was chosen as the optimum condition for the electrocatalytic process.

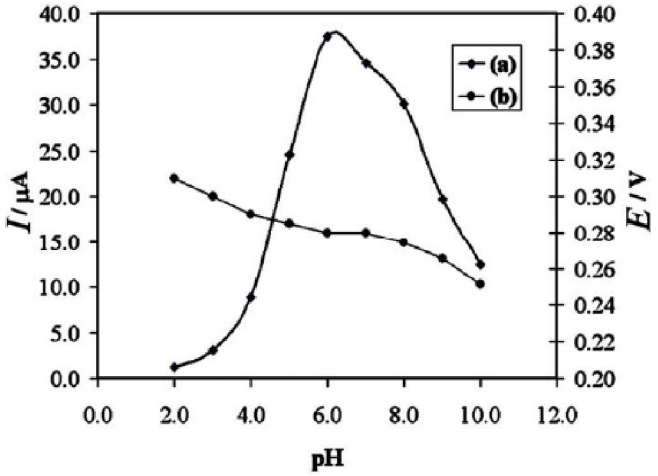

Figure 4 : Effect of pH on the peak current (a) and peak potential (b) of 1.0 mmol L^{-1} ISP on the modified electrode, at the scan rate of 50.0 mV s^{-1} and pH 6.0.

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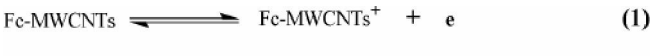
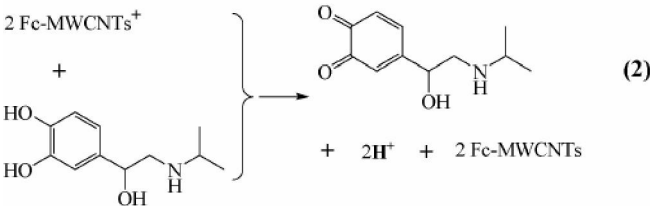
Effect of scan rate

The effect of the potential scan rate on the electrocatalytic property of Fc-MWCNTs electrode toward ISP oxidation was studied by cyclic voltammetry in the presence of 1.0 mmol L^{-1} analyte at pH 6.0 buffer solution. The results showed that with increasing the scan rate, the peak potential for the electrooxidation of isoprenaline shifts to more positive potentials, suggesting a kinetic limitation in the reaction between the redox sites of ferrocene and ISP. In addition, the cathodic current would increase with increasing the scan rate, because in short time-scale experiments, there is no enough time for the catalytic reaction to take place completely. However, the oxidation current of ISP increased linearly with the square root of the scan rate of potentials, and the following equation obtained: I / μA= 17.2310 × $\upsilon^{1/2}$ / (V s^{-1}) + 39.1620, R^2=0.9955, suggesting that at sufficient overpotentials the process is diffusion rather than surface controlled. In order to get the information on the rate determining step, the values of αn_α (where α is the transfer coefficient and n_α is the number of electrons involved in the rate determining step) were calculated for the oxidation of ISP at pH 6.0 at both modified and unmodified MWCNTs according to the following equation[42]:

$$\alpha n_\alpha = 0.048/(E_P - E_{P/2}) \quad (3)$$



(1)



(2)

Scheme 1 : The electrocatalytic reaction for the oxidation of ISP on the Fc-MWCNTs electrode

Where, $E_{P/2}$ is the potential corresponding to $I_{P/2}$. The values for αn_α were found to be 0.44 and 0.36 for the oxidation of ISP at the surface of the Fc-MWCNTs and unmodified CNPE, respectively. These values clearly show that not only the overpotential for ISP oxidation is reduced at the surface of Fc-MWCNTs, but also the rate of the electron transfer process is greatly enhanced, this phenomenon is thus confirmed by the larger I_{pa} values recorded during cyclic voltammetry on the modified electrode.

The overall oxidative reaction process, similar to other reports[17], can be attributed to the contribution of two electrons and two protons. Finally, the electrocatalytic process that is compatible with the observed behavior is given in Scheme 1.

Chronoamperometric studies

The apparent diffusion coefficient of ISP on the Fc-MWCNTs was also studied by chronoamperometry. Chronoamperograms obtained at a potential step of +300 mV are depicted in Figure 5. For an electroactive material with a diffusion coefficient of D, the current for the electrochemical reaction (at a mass transport limited rate) is described by the Cottrell equation[42]:

$$I = \frac{nFAD_O^{1/2}C^*}{\pi^{1/2}t^{1/2}} \quad (4)$$

where D_O and C^* are the diffusion coefficient (cm^2 s^{-1}) and the bulk concentration (mol cm^{-3}), respectively. Under diffusion control, a plot of I versus $t^{-1/2}$ will be linear, and from its slope, the value of D can be obtained. We have carried out such studies at various ISP concentrations. Inset (a) of Figure 5 shows the experimental plots with the best fits for different concentration of ISO. The slopes of the resulting straight lines were then plotted versus the ISP concentration (Figure 5, inset b), from whose slope we calculated an apparent diffusion coefficient of 4.06×10^{-6} cm^2 s^{-1}.

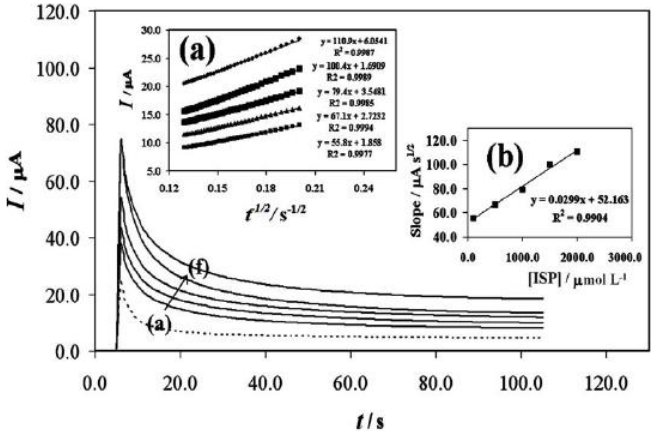


Figure 5 : Chronoamperometric response for different concentration of ISP in 0.1 mol L^{-1} buffer solution (pH 6.0) at potential step of +300 mV. The numbers of a-f correspond to 0.0, 100.0, 500.0, 1000.0, 1500.0, 2000.0 and 2500.0 μmol L^{-1} ISO. Insets: (a) Plots of I vs. $t^{-1/2}$ obtained from the chronoamperograms, and (b) plot of the slopes of the straight lines against the ISP concentration.

Electrochemical impedance spectroscopy studies

Electrochemical impedance spectroscopy was also employed to investigate the oxidation of isoprenaline on both bare CNPE and Fc-MWCNTs electrodes. Figure 6 shows the Nyquist plots of the impedance (Ω cm^2) the mentioned electrodes recorded at 0.1 V as dc-offset for 1.0 mmol L^{-1} ISP in pH 6.0.

The equivalent circuit compatible with the Nyquist diagram recorded in the absence and presence of ISP is depicted in Scheme 2. In this circuit, R_s, *CPE*, and R_{ct} represent solution resistance, a constant phase element corresponding to the double-layer capacitance, and the charge transfer resistance associated with the oxidation of ISP species. *W* is a finite-length Warburg short-circuit term coupled to R_{ct}, which accounts for the Nernstian diffusion. In the presence of ISO, the diameter of the semicircle decreases, confirming the electrocatalytic capability of the mentioned electrocatalyst for the oxidation of isoprenaline. This is due to the instant chemical reaction of ISP with the high-valence ferrocene species. The catalytic reaction of oxidation of ISP that occurred via the participation of MWCNTs and Fc electrocatalysts virtually caused an increase in the surface concentration of low valence species of Fc, and the charge transfer resistance declined, depending on the concentration of ISP in the solution. This behavior is consistent with the result of cyclic voltammetry and chronoamperometry (See Figures 2 and 5). Impedance of *CPE* and *W* elements can be expressed as[43, 44]:

$$\mathbf{Z_{CPE} = Y_0^{-1}(j\omega)^{-n}} \quad (5)$$

$$\mathbf{Z_W = Y_0^{-1}(j\omega)^{-1/2}} \quad (6)$$

where Y_0 (the admittance parameter, S cm^{-2} s^{-n}) and *n* (dimensionless exponent) are two parameters independent of frequency; $j = (-1)^{1/2}$ and ω = angular frequency = $2\pi f$. "*n*" reflect the roughness of the electrode surface or any inhomogeneity in the reaction rates and it is related to α (phase angle) by $\alpha = (1\text{-}n)\ 90^\circ$. Smooth electrode surfaces and homogenous reaction rates show a perfect capacitor with $n = 1$ and $\alpha = 0$, and increasing of microscopic inhomogeneities in the charge transfer processes lead to the reduction of "*n*". When $n = 0$, CPE is reduced to a resistor. When n = 0.5, it is equal to a Warburg impedance.

In the mentioned circuits, the charge-transfer resistance of the electrode reaction is the only circuit element that has a simple physical meaning describing how fast the rate of charge transfer during electro-oxidation of isoprenaline changes with the electrode potential or analyte concentration. The most widely accepted explanation for the presence of constant phase elements and the appearance of depressed semicircles in the Nyquist plots is the microscopic roughness on the electrode surface[45].

TABLE 1 shows the values of the equivalent circuit elements obtained by fitting the experimental results. The goodness of the fit can be judged by the estimated relative errors presented in the parentheses. According to the values of the electrical equivalent elements reported in this TABLE, upon increasing the concentration of ISP on the Fc-MWCNTs electrode, the charge transfer resistances (R_{ct}) decreased due to the facile occurrence of the faradic process related to the electro-oxidation process. Moreover, the concentration of isoprenaline shows relatively little effect on *CPE*, related to the double-layer capacitance. The electron transfer resistance, R_{ct}, for the 1.0 mmol L^{-1} ISP on the unmodified nanotube paste electrode and Fc-MWCNTs electrode, equals to 4790.7 and 462.3 Ω cm^2, respectively, indicating the very faster charge transfer rate for oxidation of isoprenaline on the modified electrode surface, due to the electrocatalytic effect of ferrocene and nanotubes on the electro-oxidation process. The apparent electron transfer rate constant k_{app} can be obtained from the following conventional equation[42]:

$$\mathbf{k_{app} = \frac{RT}{n^2F^2AR_{ct}C}} \quad (7)$$

where *n* is the number of electron transferred (*n*=1), *A* is the microscopic area of the electrodes, *C* is the concentration of the electroactive species (in mol cm^{-3}), *R*, *T* and *F* have their usual meanings. Thus, the value of k_{app} for the Fc-MWCNTs is about 10.36 times higher than that of the unmodified electrode, demonstrating enhancement of charge transfer reaction kinetics.

The dependence of drug concentration on R_{ct} and *CPE* elements has been illustrated in Figure 7 and TABLE 1. It is interesting that the concentration of isoprenaline is related to R_{ct} of Nyquist diagrams. The equation (7) may explain the relation between bulk concentra-

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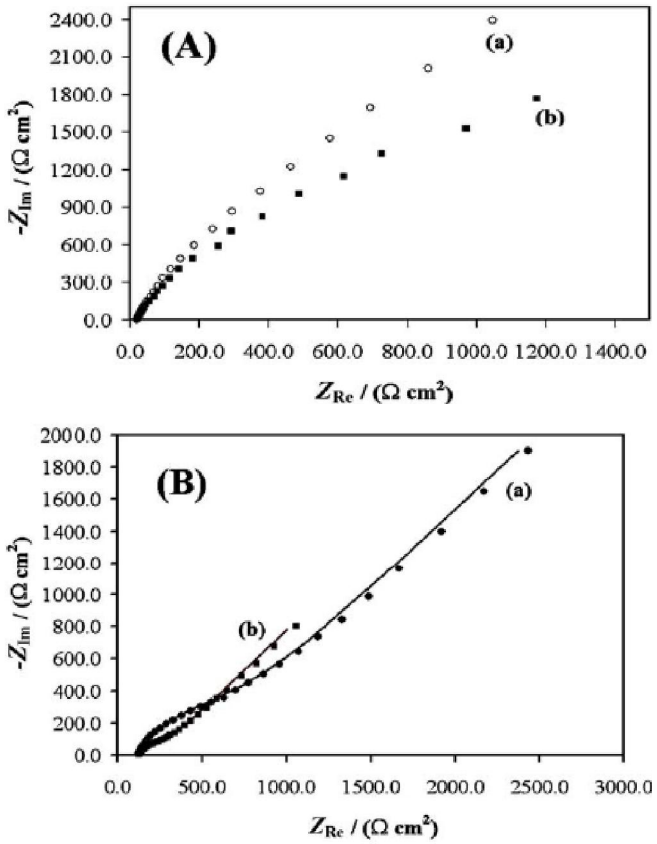
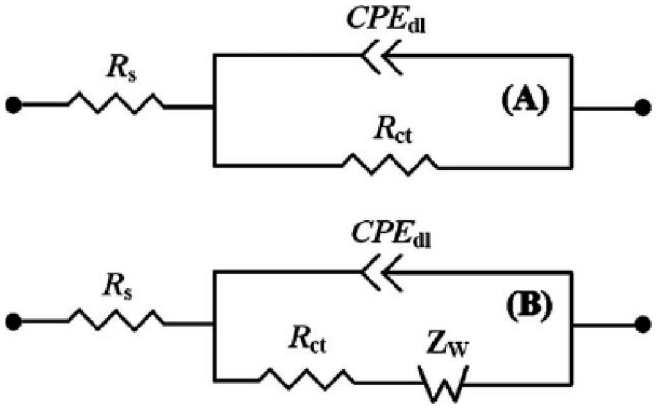


Figure 6 : The Nyquist diagrams of the impedance (Z_{Im} vs. Z_{Re}) for the A) CNPE and B) Fc-MWCNTs paste electrodes in the absence (a) and presence of 0.1 mmol L^{-1} ISP (b) by applying a bias of 0.1 V and ac voltage with 5 mV amplitude in a frequency range from 10.0 Hz to 100.0 kHz in pH 6.0.



Scheme 2 : The equivalent circuits compatible with the Nyquist diagrams represented in Figure 6.

tion of the redox probe and charge transfer resistance. We can replace C with k_1[ISP], where k_1 is a constant. Because all the other parameters are also constant, simply a linear relation of the form $1/R_{ct} = k$[ISP] is obtained, in which k includes all constants. Results show that the values of the charge transfer resistances linearly

TABLE 1 : The values of the elements in equivalent circuit and the corresponding relative errors for the oxidation of ISP on the CNTPE and Fc-MWCNTs electrodes.

Electrode	C_{ISP} (μmol L^{-1})	R_{ct} (Ω cm^2)	*CPE*	
			$Y_0 \times 10^{6}$ (S cm^{-2} s^n)	*n*
CNPE	0.0	6945.6 (3.3%)	0.5210 (3.7%)	0.8166 (2.8%)
CNPE	1000.0	4790.7 (3.2%)	0.4631 (1.9%)	0.8023 (3.3%)
Fc-MWCNTs	0.0	1236.8 (3.5%)	0.1623 (3.1%)	0.7432 (2.6%)
Fc-MWCNTs	100.0	970.4 (3.2%)	0.1981 (2.9%)	0.8741 (2.1%)
Fc-MWCNTs	500.0	650.1 (3.5%)	0.2890 (1.7%)	0.8346 (1.4%)
Fc-MWCNTs	1000.0	462.3 (2.7%)	0.3380 (3.2%)	0.8192 (2.2%)
Fc-MWCNTs	1500.0	318.7 (3.4%)	0.3550 (3.7%)	0.8231 (2.7%)

decrease upon increasing of ISP concentration (see Figure 7 and TABLE 1). The extent of the decrease in R_{ct} depends on the electrocatalytic activity of the working electrode and the magnitude of the applied DC potential, provided the AC potential is small and the diffusion layer produced by the DC potential is not perturbed by the AC potential.

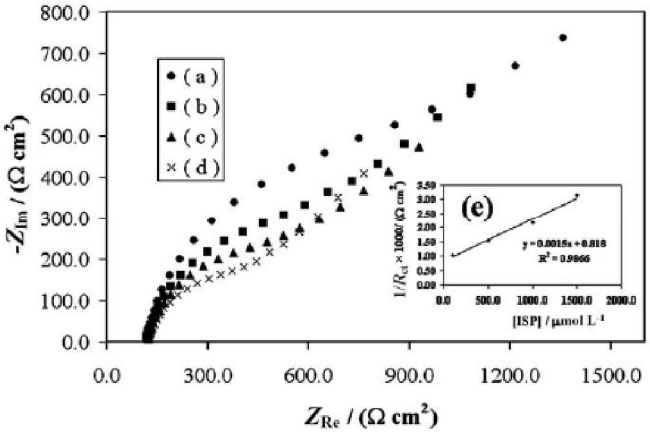


Figure 7 : Complex plane plots obtained on the modified electrode for different concentrations of ISP: a) 100.0 b) 500.0 c) 1000.0 and d) 1500.0 μmol L^{-1} ISP. The inset shows calibration curve obtained using $1/R_{ct}$ as a function of ISP concentration. Other conditions are similar to Figure 6.

Performance of the system for the determination of isoprenaline

Because differential pulse voltammetry (DPV) has a much higher current sensitivity than cyclic voltammetry, it was used for the determination of isoprenaline in real

samples. The calibration plot of ISP determination is linear in two concentration region of 5.0-50.0 and 500.0–2000.0 μmol L^{-1} with detection limit of 0.1 μmol L^{-1} (see Figure 8). Using DPV under the optimum conditions were selected as: pH 6.0 (universal buffer) by scanning the potential between 0.0 and 0.5 V at 10 mV s^{-1} and a 50 mV pulse amplitude. Equations of linear least square calibration curves over this ranges are: I / μA = 0.28 × C_{ISP} + 1.50 (R^2 = 0.9983) and I / μA = 0.012 × C_{ISP} + 18.92 (R^2 = 0.9987). The detection limit, linear dynamic range and sensitivity of this method are significantly better than other electrochemical methods reported by Aslanoglu[17] and Fatibello-Filho[18] and provide useful route for the detection and determination of ISP in the human urine and pharmaceutical samples.

To prove the precision and practicability of the proposed method, the reproducibility and storage stability of the sensor were examined. The relative standard deviation (RSD) of the modified electrode response to 1.0 mmol L^{-1} ISP was 2.6% (n = 5). The RSD for five different electrodes using the same conditions response to isoprenaline was 3.6%. These results showed that such kinds of electrodes exhibited good reproducibility. Furthermore, the long term stability of the Fc-MWCNTs was tested over a three-week period. The cyclic voltammetry of ISP at the surface of Fc-MWCNTs after the modified electrode was stored in an atmosphere at room temperature showed that the oxidation peak potential of ISP was unchanged and the anodic peak current was only decreased less than 2.5 % of the initial oxidation peak current.

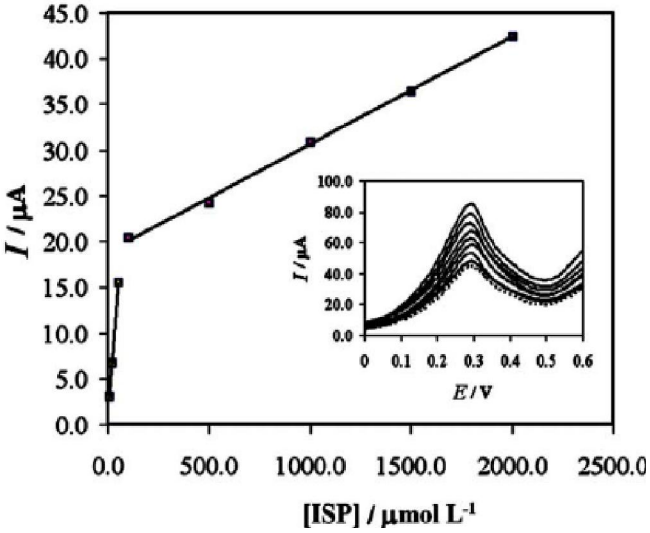


Figure 8 : Calibration curve for the determination of ISP by DP`V on the Fc-MWCNTs paste electrode in pH 6.0 buffer solution. Inset shows the related differential pulse voltammograms containing different concentrations of ISP.

Interference studies

Under optimized experimental conditions described above, the effects of some foreign species on the determination of ISP at 1.0 mmol L^{-1} level were evaluated in detail. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than 3% for determination of ISO. 800-fold of Na^+, K^+, Ca^{2+}, Mg^{2+}, NH_4^+, stearate, Cl^-, SO_4^{2-}, CO_3^{2-}, NO_3 and urea; 250-fold of glucose, sucrose, lactose and fructose and 5-fold of Fe^{3+} have almost no influence on the current response of ISO. All these indicate that the peak current of isoprenaline is not affected by all conventional cations, anions, and organic substances, presenting in the mentioned real samples, but other electrochemically reducible materials such as iron cations can be interfered.

Application

To evaluate the applicability of proposed method, the recovery of ISP was determined in the ampule and urine samples by adding the standard value of ISP to them. The standard addition method was used for the analysis of prepared samples. The data given in TABLE 2 show the satisfactory results for analytical determination of isoprenaline in the real samples.

TABLE 2 : Determination of ISP in pharmaceutical formulations and urine samples by DPV technique in pH 6.0 buffer solution.

No.	Sample	Added (μmol L^{-1})	Found[a] (μmol L^{-1})	Recovery (%)	RSD (%)
	Urine				
1		100	98.0 ± 0.1	98.0	0.3
2		500	480.8 ± 0.5	96.1	1.8
3		1000	1020.4 ± 1.0	102.0	2.6
	Ampule				
4		100	101.6 ± 1.7	101.6	2.6
5		200	196.5 ± 2.9	98.25	0.8
6		300	294.6 ± 3.8	98.2	0.4

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CONCLUSION

In conclusion, we have demonstrated that the Fc-MWCNTs nanocomposite can be effectively employed for the fabrication of isoprenaline electrochemical sensor in the pharmaceutical and human urine samples. The synergetic effects between the MWCNTs and Fc in the nanocomposite enhance the electrocatalytic action of Fc to the oxidation of ISP, resulting in a sensitive voltammetric sensor for this drug. The proposed sensor exhibits wide linear detection range, acceptable reproducibility, and high sensitivity and selectivity for the detection of ISP in the clinical and pharmaceutical samples.

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