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Application of experimental design for quantification and voltammetric studies of sulfapyridine based on a nanostructure electrochemical sensor

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Abstract In the present paper, differential pulse voltammetry (DPV) coupled with experimental design, as a new method, was developed for determination of sulfapyridine (SP). These measurements were carried out in a 0.2 M Britton–Robinson (B–R) buffer solution at the surface of multi-walled carbon nanotubes modified carbon paste electrode (MWCNT/CPE). Operating conditions were improved with central composite rotatable design (CCRD), involving several chemical and instrumental parameters such as pH, MWCNT amount, scan rate, step potential and modulation amplitude. DPV, electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were applied for characterizing of the modified electrode. The modified electrode showed enhanced effect on the oxidation peak current of SP. The electron transfer coefficient ($\alpha = 0.77$), exchanging current density ($j_0 = 1.82 \times 10^{-11} \text{ A cm}^{-2}$) and diffusion coefficient ($D = 2.03 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$) of SP were calculated by linear sweep voltammetry (LSV) and chronoamperometry methods, respectively. Some analytical parameters such as repeatability, linear dynamic range (5.96–161.07 M) and detection limit (DL) (49.55 nM) for SP were also obtained. Finally, the proposed method was successfully applied for determination of SP in plasma samples.

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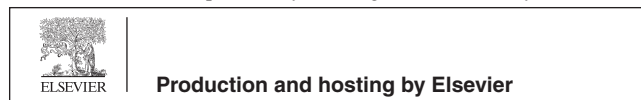
1. Introduction

The classical method based on one factor at a time may be effective in some processes, but fails to consider the combined

effects of several variables involved. When there are various independent variables affecting the responding factors, it is probably that the operational variables interact and influence each other's effects on the response. Thus, it is necessary to apply an optimization strategy that can determine all factors and possible interactions between these independent variables, so that a set of experimental optimal conditions can be determined (Cui et al., 1994). The design of experiments (DOE) fulfills this requirement. The DOE based on the statistical models, started with agricultural experiments in 1920s (Sir Ronald Fisher), followed by chemical experiments in 1950s (George Box) (Patil et al., 2012). In the statistical evaluation and

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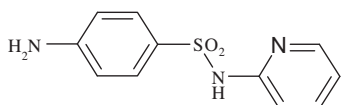


experimental design, response surface methodology (RSM) can be applied for process modeling and analytical optimization (Khuri and Cornell, 1996; Myer and Montgomery, 2002; K rbahti and Rauf, 2008;  lmez, 2009). RSM is a useful modeling tool with a group of statistical and mathematical techniques that can be applied to improve, develop and optimize processes when the responses are influenced by several factors. It characterizes the effect of the independent factors, alone or in combination regarding the studied processes. Furthermore, this experimental methodology develops a mathematical model which is presented in a graphical form (Bas and Boyac, 2007). RSM permits to accomplish optimization study easily as it helps to decrease the number of experimental trials needed as minimum as possible. Thus, it is less laborious and time-consuming than other methods.

Sulfonamides (sulfa drugs) are an important kind of antibiotics widely applied in human and veterinary medicine to treat urinary bronchitis, ear infections, tract infections, skin and soft tissue infections. Important sulfa drugs used for these applications include sulfapyridine (SP) (Scheme 1), sulfamethoxazole, sulfamerazine, sulfamethazine, sulfadiazine and sulfathiazole (Dost et al. 2000; Long et al. 1990; Marek, 1998; Nhat et al. 2012). A variety of methods such as HPLC-UV and HPLC-FLD (Fischer et al., 1983; Teshima et al., 2002), ESI-MS/MS (Pastorini et al., 2008), potentiometric (Nazer et al., 2001), GC (Assassi et al., 2005) and LC-ESI-MS/MS (Guang-Zhi et al. (2011) have been applied to measure sulfonamides. But only a few electrochemical methods have been reported for the detection of sulfonamide drugs (Abdullin et al., 2002; Braga et al., 2010; Preechaworapun et al., 2006). Electrochemical methods have attracted a lot of interest in many cases, and these methods can be fast in detections, low in cost, with merits of high accuracy and low detection limit (Ghoreishi et al., 2012a,b).

Carbon pastes are one of the most convenient materials for the preparation of modified electrodes. By adding mediator materials in carbon paste can improve the electrode sensitivity and selectivity (Ghoreishi et al., 2012a,b). Chemically modified carbon paste electrode is mainly used in the field of voltammetric determination (Ghoreishi et al., 2013; Lin et al., 2008;  svan-cara et al., 2007). Carbon nanotubes (CNTs) are composed of graphitic sheets rolled into closed concentric cylinders with length of micrometers and diameter of the order of nanometers. Since the discovery of CNTs in 1991 (Iijima, 1991), they have been applied more and more in chemical, physical and material science fields due to their unique chemical stability, electrical conductivity and high mechanical strength and modulus. CNTs are able to promote electron transfer in electrochemical reactions when used as the electrode material. Therefore using CNTs, a new manner of electrode surface modification as new electrochemical sensors is obtained (Khoobi et al. 2013; Yao et al., 2006).

In the present work, RSM based on central composite rotatable design (CCRD) was used to design experiments and determine the optimum experimental conditions for



Scheme 1 Structure of sulfapyridine.

desirable responses. The main purpose of this work was investigation of the effect of parameters simultaneously for voltammetric determination of SP in mixed samples and human plasma. This paper is the first report about sensitive determination of SP by the differential pulse voltammetry (DPV) method based on a multi-walled carbon nanotubes modified carbon paste electrode (MWCNT/CPE) in Britton–Robinson (B–R) buffer solution. Additionally, we also determined kinetic parameters of SP with other voltammetric methods.

2. Experimental

2.1. Chemicals and reagents

All solutions were freshly prepared with deionized water. Sulfapyridine was of analytical grade from Sigma–Aldrich. Graphite fine powder (Merck) and paraffin oil (DC 350, density = 0.88 g cm⁻³, Merck) as the binding agent were applied for preparing the pastes. Multi-walled carbon nanotubes with o.d. between 5 and 20 nm, i.d. between 2 and 6 nm, and tube length between 1 and 10 μm were purchased from the Chinese Academy of Science and were purified using nitric acid treatment. All other reagents were of analytical grade from Merck. The buffer solutions of 0.2 M B–R were prepared from orthophosphoric acid, acetic acid and boric acid by adjusting the pH with NaOH solution in the pH range of 2.0–10.0.

2.2. Apparatus and procedures

The cyclic voltammetry (CV), linear sweep voltammetry (LSV) chronoamperometry and differential pulse voltammetry experiments were carried out using a Sama 500 potentiostat (Isfahan, Iran). Electrochemical impedance spectroscopy (EIS) measurements were carried out by an Autolab potentiostat–galvanostat PGSTAT 35 (Eco chemie Utrecht, Netherlands), equipped with NOVA 1.6 software. Storage and processing of data were carried out by a personal computer (Pentium IV). For preparing buffer solutions with adjusted pH a Metrohm 691 pH/meter was applied. An ultrasound bath (Bandelin Sonorex, Germany) at a constant frequency of 35 kHz was used for dispersing of MWCNT during experiments. A three electrode cell system was applied at 25 ± 1 °C. The MWCNT/CPE, an Ag/AgCl/KCl(sat) and a platinum wire were used as the working, reference and auxiliary electrodes, respectively. A polyethylene tube with a rod (2 mm diameter and 5 mm deep) bored at one end was used as the body of the carbon paste working electrode. For electrical contact a copper wire was placed through the center of the rod. The working electrode was pretreated by pushing paste out of the tube, removing the excess, and mechanically polishing the surface with weighing paper.

2.3. Preparation of the electrodes

For preparation the modified carbon paste electrode, 6.0 mg (optimal amount) of purified MWCNT was added to 5.0 ml deionized water and sonicated for 30 min with an ultrasonic bath to obtain a stable and homogeneous suspension. This suspension was added to graphite powder (500.0 mg) in a small mortar, and allowed to evaporate water at room temperature

in air. Then about 0.2 ml of paraffin oil was added to the above mixture and mixed for 30 min until a uniformly wetted paste was formed. After that, the paste was pressed manually in the cavity of the electrode body, and the surface was smoothed against clean paper. Unless otherwise stated, the paste was carefully removed prior to pressing a new portion into the electrode after every measurement. Unmodified CPE was prepared in the same way without adding MWCNT to the mixture.

2.4. Experimental design and method of analysis

To find the effects of independent factors on the response a central composite rotatable design (CCRD) with the five factors, was performed. CCRD consists of 3 parts: (1) a full or fractional factorial design; (2) an additional design, usually a star design in which experimental points are at a distance α from its center; and (3) a central point. Full uniformly rotatable central composite designs present the following characteristics: (1) require an experiment number according to $N = 2^f + 2f + r$, where f is the number of factors and r is the replicate number of the central point, (2) α -values depend on the number of variables and can be calculated according to $\alpha = 2^{f/4}$. (3) All factors are studied in five levels. In order to reduce the effects of unexplained variability in the real responses due to extraneous parameters, the experiments were done in randomized order. The independent factors and their ranges were selected, based on preliminary experiment results. Then a second-order quadratic equation was fitted to the data by multiple regression procedure (Brereton, 2007). The generalized response surface model for a five-factor system is shown by Eq. (1).

$$Y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{44} x_4^2 + \beta_{55} x_5^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{14} x_1 x_4 + \beta_{15} x_1 x_5 + \beta_{23} x_2 x_3 + \beta_{24} x_2 x_4 + \beta_{25} x_2 x_5 + \beta_{34} x_3 x_4 + \beta_{35} x_3 x_5 + \beta_{45} x_4 x_5 \quad (1)$$

where Y_i is the predicted response, x_i are the independent variables, β_0 is the intercept (constant), $\beta_1, \beta_2, \beta_3, \beta_4, \beta_5$, are linear coefficients, $\beta_{11}, \beta_{22}, \beta_{33}, \beta_{44}, \beta_{55}$, are squared coefficients, $\beta_{12}, \beta_{13}, \beta_{14}, \beta_{15}, \beta_{23}, \beta_{24}, \beta_{25}, \beta_{34}, \beta_{35},$ and β_{45} , are interactions coefficients.

In this work five studied factors are the measured response (DPV current) consist pH (X_1), MWCNT amount (X_2), scan rate (X_3), step potential (X_4) and modulation amplitude (X_5). MINITAB® Release 16, developed by Minitab Inc. (USA), a statistical software package, was used for data processing and studying linear terms, squared terms and interaction between the variables. For each factor five levels and the 95% of significance level were considered.

3. Results and discussion

3.1. Optimization of the procedure using CCRD

A Box–Wilson procedure, that called CCRD, was applied to estimate the correlation of the five independent factors, with five levels for each factor. In this study, $32 (2^5)$ full factorial central composite designs for five independent factors ($f = 5$), with 10 (2×5) axial points and three replicates ($r = 3$) at the center points with $\alpha = 2.38$ were employed to fit a second order polynomial model. Calculations indicated that 45 experiments ($N = 2^5 + (2 \times 5) + 3$) were needed for this procedure. The variables X_1 – X_5 were studied to optimize the DPV current. The name of independent effective factors and their coded levels and scheme of CCRD are shown in Tables 1 and 2, respectively. Thus, we can calculate optimum value of each variable as coded and corresponding uncoded (actual) (Table 3) with MINITAB® Release 16 software for 50.0 M of SP in 0.2 M B–R buffer solutions, and quantified SP by selecting the predicted conditions. Additionally, the optimum response values of each factor can be obtained by the graphical analysis of the surface related to each equation obtained by MINITAB software (Fig. 1).

3.2. Voltammetric behavior of the MWCNT/CPE

For investigation of modifier effect on determination of SP, differential pulse voltammograms of SP in the absence and presence of MWCNT were studied. Fig. 2 shows the differential pulse voltammograms for the oxidation of a solution containing 50.0 M of SP in 0.2 M B–R buffer solution (pH = 7.5) at the surface of bare CPE (curve b) and at the surface of MWCNT/CPE (curve c) (curve a is as c but in the absence of SP). The oxidation peak current for SP at CPE is about 0.50 A (curve b) but at MWCNT/CPE is about 1.16 A (curve c). In other words, at the surface of MWCNT/CPE, SP shows a well-defined peak with a magnification 2.32 times greater than bare CPE. These results clearly remark that the combination of graphite powder and MWCNT improves electron transfer reaction for oxidation of SP.

3.3. Electrochemical impedance spectroscopy of the modified electrode

For evidence the additional effect of MWCNT in anodic oxidation of SP, electrochemical impedance spectroscopy (EIS) technique was applied. The EIS is a non-destructive, powerful and informative technique for electrochemical properties about studying of the modified electrodes. Fig. 3 shows the Nyquist plot of a solution containing 5.0×10^{-3} M

Table 1 Levels of independent variables established based on five-level-five-factor CCRD.

Independent variables	Coded levels				
Name	–2.38	–1	0	1	2.38
X_1	pH	5.0	6.0	7.0	8.0
X_2	MWCNT amount (mg)	2.0	4.0	6.0	8.0
X_3	Scan rate ($V s^{-1}$)	0.02	0.06	0.10	0.14
X_4	Step potential (V)	0.001	0.002	0.003	0.004
X_5	Modulation amplitude (V)	0.01	0.03	0.05	0.07
					9.0
					10.0
					0.18
					0.005
					0.09

Table 2 Scheme of CCRD: independent variables (X_i).

Std order	Run order	X1	X2	X3	X4	X5
7	1	-1.00	1.00	1.00	-1.00	-1.00
27	2	-1.00	1.00	-1.00	1.00	1.00
40	3	0.00	0.00	0.00	2.38	0.00
39	4	0.00	0.00	0.00	-2.38	0.00
13	5	-1.00	-1.00	1.00	1.00	-1.00
5	6	-1.00	-1.00	1.00	-1.00	-1.00
31	7	-1.00	1.00	1.00	1.00	1.00
30	8	1.00	-1.00	1.00	1.00	1.00
4	9	1.00	1.00	-1.00	-1.00	-1.00
2	10	1.00	-1.00	-1.00	-1.00	-1.00
9	11	-1.00	-1.00	-1.00	1.00	-1.00
17	12	-1.00	-1.00	-1.00	-1.00	1.00
43	13	0.00	0.00	0.00	0.00	0.00
12	14	1.00	1.00	-1.00	1.00	-1.00
33	15	-2.38	0.00	0.00	0.00	0.00
8	16	1.00	1.00	1.00	-1.00	-1.00
34	17	2.38	0.00	0.00	0.00	0.00
41	18	0.00	0.00	0.00	0.00	-2.38
37	19	0.00	0.00	-2.38	0.00	0.00
26	20	1.00	-1.00	-1.00	1.00	1.00
15	21	-1.00	1.00	1.00	1.00	-1.00
19	22	-1.00	1.00	-1.00	-1.00	1.00
35	23	0.00	-2.38	0.00	0.00	0.00
20	24	1.00	1.00	-1.00	-1.00	1.00
3	25	-1.00	1.00	-1.00	-1.00	-1.00
28	26	1.00	1.00	-1.00	1.00	1.00
36	27	0.00	2.38	0.00	0.00	0.00
25	28	-1.00	-1.00	-1.00	1.00	1.00
38	29	0.00	0.00	2.38	0.00	0.00
44	30	0.00	0.00	0.00	0.00	0.00
32	31	1.00	1.00	1.00	1.00	1.00
29	32	-1.00	-1.00	1.00	1.00	1.00
22	33	1.00	-1.00	1.00	-1.00	1.00
1	34	-1.00	-1.00	-1.00	-1.00	-1.00
6	35	1.00	-1.00	1.00	-1.00	-1.00
23	36	-1.00	1.00	1.00	-1.00	1.00
21	37	-1.00	-1.00	1.00	-1.00	1.00
16	38	1.00	1.00	1.00	1.00	-1.00
18	39	1.00	-1.00	-1.00	-1.00	1.00
11	40	-1.00	1.00	-1.00	1.00	-1.00
10	41	1.00	-1.00	-1.00	1.00	-1.00
45	42	0.00	0.00	0.00	0.00	0.00
42	43	0.00	0.00	0.00	0.00	2.38
24	44	1.00	1.00	1.00	-1.00	1.00
14	45	1.00	-1.00	1.00	1.00	-1.00

$[\text{Fe}(\text{CN})_6]^{3-/4-}$ in 0.2 M B-R buffer solution (pH = 7.5) at the surface of CPE and MWCNT/CPE. It can be observed that the impedance spectrum is composed from two regions: (1) a semicircle in high frequencies, related to the charge-transfer

process and (2) a 45° line indicating a region of semi-infinite diffusion of species in the electrode. We focused at the high frequency region. The value of the electron transfer resistance (R_{ct} , semicircle diameter) depends on the dielectric and insulating features at the electrode/electrolyte interface (Luo et al., 2005). An extensive difference in R_{ct} was observed upon the stepwise formation of the modified electrode. The R_{ct} value for the bare CPE was 5.58 k. After the MWCNT was assembled on the CPE, the R_{ct} value was found to be 1.91 k. As compared with bare CPE, the R_{ct} value of MWCNT/CPE reduced obviously. This might be due to the presence of MWCNT, which played an important role in accelerating the transfer of the electrons.

3.4. Kinetic investigations of MWCNT/CPE

For evaluation kinetic parameters of the modified electrode such as the surface concentration of mediator, charge transfer coefficient, α , and apparent charge transfer rate constant, k_s , the CV method was used. Fig. 4 shows CV responses of $\text{Fe}^{2+}/\text{Fe}^{3+}$ (probes solution) in 0.2 M B-R buffer solution (pH = 7.5) and various scan rates, at the surface of MWCNT/CPE. The surface coverage of the electrode can be calculated using the Sharp et al. method (Eq. (2)) (Sharp, 1979).

$$I_p = n^2 F^2 A \Gamma v / 4RT \quad (2)$$

Where n represents the number of electrons involved in the reaction (n is 1 for $\text{Fe}^{2+}/\text{Fe}^{3+}$), A is the surface area of the MWCNT/CPE ($3.14 \times 10^{-2} \text{ cm}^2$), Γ (mol cm^{-2}) is the surface coverage of modifier and other symbols have their usual meanings. Using the slope of anodic peak currents versus scan rate (Fig. 4A) the surface coverage of MWCNT/CPE, Γ , is obtained $8.23 \times 10^{-6} \text{ mol cm}^{-2}$.

Determination of the charge transfer coefficient, α , and apparent charge transfer rate constant, k_s , of a surface-confined redox couple can be obtained by the variation of anodic peak potentials with logarithm of scan rate, according to the procedure of Laviron (Bard and Faulkner, 2001). Fig. 4 inset B demonstrates the variations of peak potentials (E_p) as a function of logarithm of the potential scan rate (v). Fig. 4 inset C, indicating that for scan rate values higher than 50.0 mV s^{-1} the peak potential values are proportional to the logarithm of scan rate. Under these conditions the slope of the plot (Fig. 4 inset C) can be used to calculate transfer coefficient (α) and using Eq. (3) we can obtain electron transfer rate constant (k_s , s^{-1}) between probes and MWCNT/CPE.

$$\log k_s = \alpha \log(1 - \alpha) + (1 - \alpha) \log \alpha - \log(RT/nFv) - \alpha(1 - \alpha)n_x F \Delta E_p / 2.3RT \quad (3)$$

Table 3 Optimized parameters to the SP determination by DPV.

Variables	Name of variables	Coded optimized values	Actual optimized values
X_1	pH	0.4565	7.39
X_2	MWCNT Amount (mg)	-0.0240	6.0
X_3	Scan Rate (V s^{-1})	0.6976	0.124
X_4	Step potential (V)	-0.1682	0.0028
X_5	Modulation amplitude (V)	-0.8409	0.0355

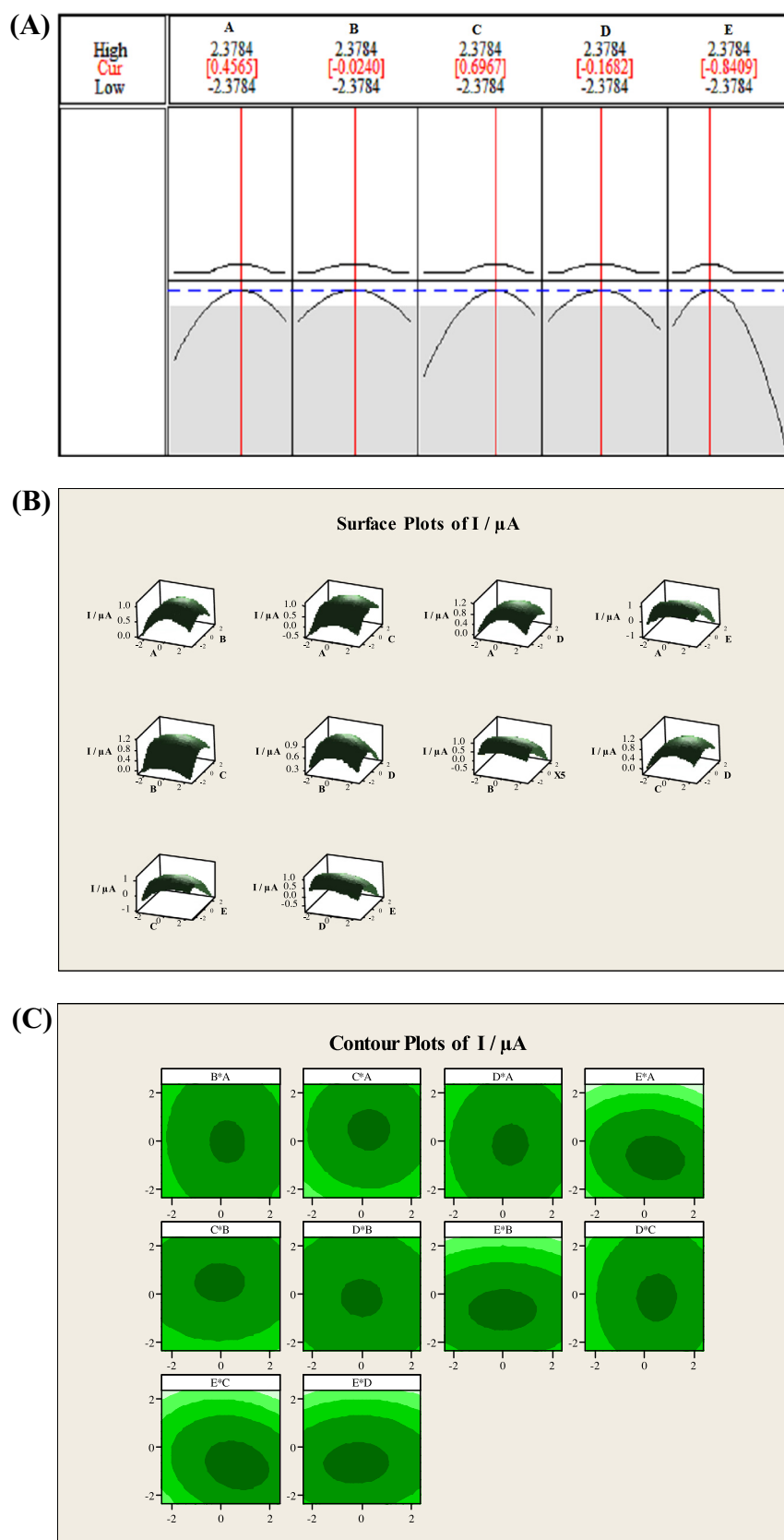


Figure 1 (A) Three-dimensional (3-D) response obtained from the regression equation (coded values of variables). A–D and E variables correspond to pH, MWCNT content, scan rate, step potential and modulation amplitude. (B) and (C) response surface and contour plot of the combined effect of A–D and E variables on DPV current of the SP, respectively.

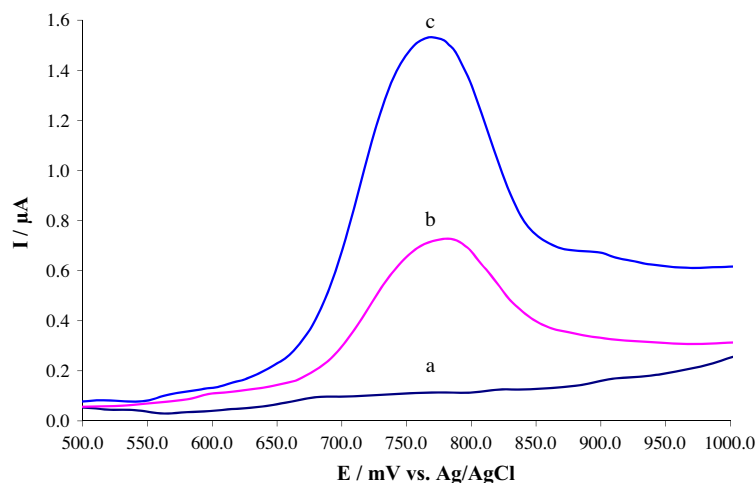


Figure 2 Differential pulse voltammograms (a): MWCNT/CPE in B-R buffer solution (pH = 7.5), (b) CPE in the presence of 50.0 M SP, (c) as (a) + 50.0 M SP.

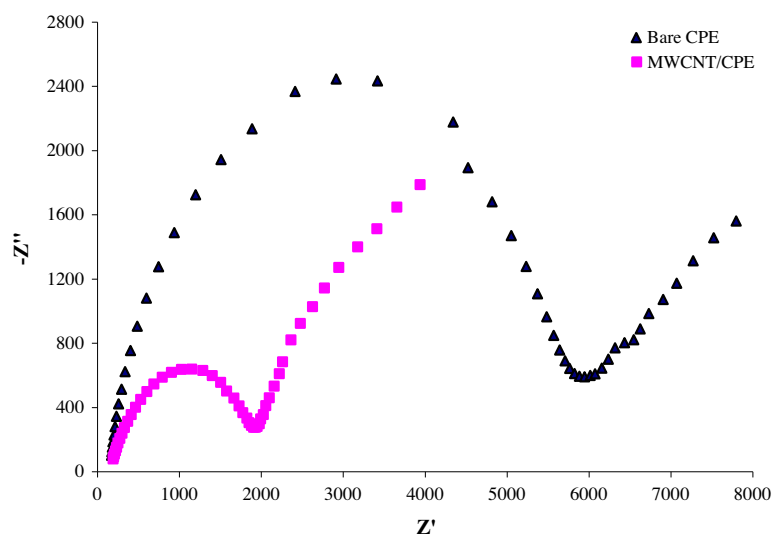


Figure 3 The Nyquist plot of the bare CPE and MWCNT/CPE in 0.2 M B-R buffer solution (pH = 7.5), containing 5.0 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$.

where $n = 1$, $\Delta E_p = E_{pa} - E_{pc}$, v (V s^{-1}) is the scan rate and all other symbols have their conventional meanings. From the values of ΔE_p corresponding to different scan rates, an average value of k_s and α was evaluated to be 11.68 s^{-1} and 0.49, respectively.

3.5. Kinetic investigations of SP using LSV

Linear sweep voltammetry is a useful technique for electrochemical studies of electroactive substances. The linear sweep voltammograms of solution containing different concentrations of SP with a sweep rate of 100 mV s^{-1} are shown in Fig. 5 A. The points show the rising region of the voltammograms (known as the Tafel region), which is affected by the electron transfer kinetics between SP and MWCNT/CPE. If deprotonation of SP is a sufficiently fast step, with assuming the number of electrons involved in the rate determining step

of $n_\alpha = 1$, charge transfer coefficient (α) and ionic exchanging current density (j) can be calculated from the average of the slopes and intercepts of the Tafel plot, respectively by the Tafel equation (Bard and Faulkner, 2001). Fig. 5 B shows the relationship between logarithm i (A) and peak potential in different concentrations of SP in 0.2 M B-R buffer solution (pH = 7.5) at the surface of MWCNT/CPE which is linear (Tafel region). With the average of these slopes and intercepts and assuming that $n_\alpha = 1$, the values of α and j were found to be 0.77 and $1.82 \times 10^{-11} \text{ } \mu\text{A cm}^{-2}$, respectively.

3.6. Effect of scan rate

The electrochemical behavior of SP on the modified electrode was studied by the CV method. These studies showed an irreversible chemical behavior for SP at the surface of MWCNT/CPE (Fig. 6A). Additionally the effect of scan rate

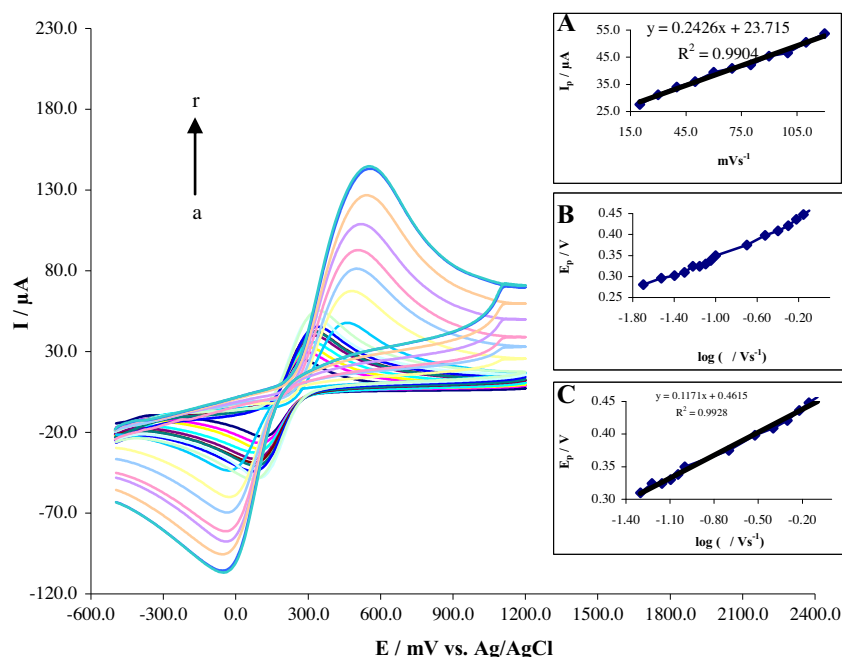


Figure 4 CV of MWCNT/CPE in $\text{Fe}^{2+}/\text{Fe}^{3+}$ probe solution in 0.2 M B–R buffer (pH = 7.5), at various scan rates, a to r correspond to 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0, 100.0, 110.0, 120.0, 200.0, 300.0, 400.0, 500.0, 600.0, 700.0 and 800.0 mV s^{-1} scan rates, respectively. (A) Variations of I_p versus scan rates, (B) variation of E_p versus logarithm of the scan rate and (C) magnification of the same plot for high scan rates.

in the oxidation of SP at the surface of the modified electrode is shown (Fig. 6A). For totally irreversible diffusion controlled process according to Eq. (4) (Antoniadou et al., 1989).

$$I_p = 3.01 \times 10^5 n [(1 - \alpha) n_\alpha]^{1/2} A D^{1/2} C v^{1/2} \quad (4)$$

where n , α , n_α , A , D , C and v are the number of electrons involved in the thorough oxidation process, transfer coefficient, the number of electrons involved in the rate determining step, surface area, diffusion coefficient, concentration of reagent and scan rate, respectively.

Fig. 6 A shows cyclic voltammograms of the solution containing 50.0 M of SP in 0.2 M B–R buffer (pH = 7.5) at the surface of MWCNT/CPE. As can be seen, in the range of 50–170 mV s^{-1} for scan rate (v), there is a linear relationship between the peak current and square root of scan rate (Fig. 6B). According Eq. 4 this result indicates that oxidation peak current of SP is a diffusion-controlled process on the modified electrode.

3.7. Chronoamperometric measurements of SP

Considering that the oxidation peak of SP is a diffusion-controlled process, we used chronoamperometry technique to determine the diffusion coefficient (D) of SP of the modified electrode. Fig. 7A shows chronoamperograms of solutions containing various concentrations of SP ranging from 8.0 to 38.0 M in B–R buffer (pH = 7.5) at the surface of MWCNT/CPE, using a potential step of 1000 mV. For an electroactive substance (such as SP) with a diffusion coefficient of D , the current for the electrochemical reaction (at a mass transport limited rate) is described by the Cottrell equation (Eq. (5)) (Bard and Faulkner, 2001).

$$I = nFA D^{1/2} C_b \pi^{-1/2} t^{-1/2} \quad (5)$$

where D ($\text{cm}^2 \text{s}^{-1}$) is the diffusion coefficient and C_b (mol cm^{-3}) is the bulk concentration. According to this equation, under diffusion limited transport, a plot of I versus $t^{-1/2}$ ($\text{s}^{-1/2}$) is linear, and therefore the slope of the plot can be obtained for the value of D . The fitted experimental plots for different concentrations of SP are shown in Fig. 7B and C. The slopes of the resulting straight lines were plotted versus the SP concentration, and the average value of D was obtained $2.03 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$.

3.8. Influence of pH

The effect of pH on SP oxidation at the surface of MWCNT/CPE was studied using DPV technique. As shown in Fig. 8A, DPV was conducted on solutions containing 50.0 M SP in 0.2 M B–R buffer with pH values ranging from 5.0–9.0 in optimal conditions i.e. a scan rate of 124 mV s^{-1} , a step potential of 2.8 mV and modulation amplitude of 35.4 mV. As can be seen in Fig. 8B, in pH = 7.5 the maximum anodic current for SP is observed. This result shows a good agreement with the optimum value obtained from experimental design data. Additionally, according to the Nernst equation (Eq. (6)), where n and m represent the number of electrons and protons involved in reaction, a and b represented the coefficient reagents in the reaction equation, a slope of $-0.0598 \text{ V pH}^{-1}$ indicates that the proportion of the electron and proton involved in the reaction is same (Fig. 8C). Because for similar sulfonamides the number of electron was reported equals 2 (Arvand et al., 2011), therefore we suggest that for SP the proportion of the electron and proton involved in the reaction is 2:2. The dependence of E_{pa} on the pH indicates that the electrochemical reaction involves proton transfer. Oxidation process of SP probably starts with an electron oxidation to form a cation radical at the nitrogen. Subsequently, a rapid loss of

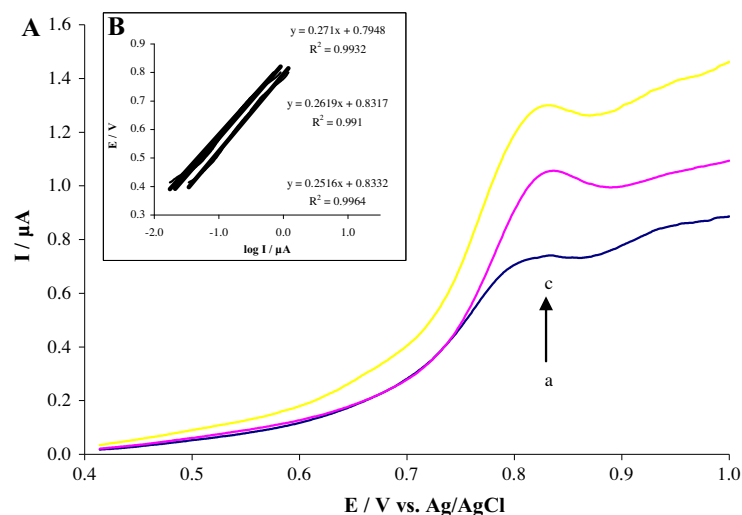


Figure 5 (A): Linear sweep voltammograms of MWCNT/CPE in B-R buffer (pH = 7.5) containing 23.0, 31.0 and 38.0 M (a–c) SP at a sweep rate of 100.0 mV s⁻¹, (B): Tafel plot derived from linear sweep voltammograms.

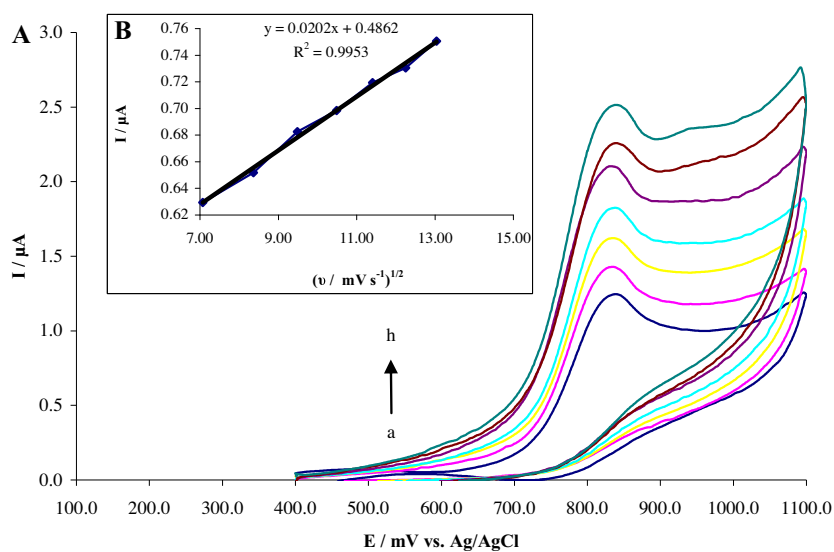


Figure 6 Cyclic voltammograms of MWCNT/CPE in a B-R buffer solution (pH = 7.5) for 50.0 μM SP, using various scan rates: 50.0, 70.0, 90.0, 110.0, 130.0, 150.0, 160.0 and 170.0 mVs⁻¹ (from inner to outer), (A): Peak current vs. E (mV), (B): Intensity, I (μA) vs. v^{1/2}.

a second electron and proton occurred to obtain an iminium ion to which the water is subsequently added (Braga 2010).

$$E_p = E^\circ + (0.0591/n)\log[(Ox)^a/(R)^b] - (0.0591m/n) \text{pH} \quad (6)$$

$$E_{pa}(\text{V vs. Ag/AgCl}) = 1.214 - 0.0598\text{pH}, R^2 = 0.9972$$

3.9. Differential pulse voltammetry investigations for the measurement of SP

Since DPV has a better resolution with a negligible charging current contribution to the background current than CV, it was applied to estimate the detection limit of SP. Fig. 9 shows the DPV data obtained for the oxidation of different concentrations of SP on the MWCNT/CPE in 0.2 M B-R buffer solutions (pH = 7.5) at the optimum conditions established

in Section 3.1. The dependencies of the peak currents on the SP concentrations are shown in inset A of Fig. 9. In Fig. 9B it is shown that the peak current increases linearly with the SP concentration with a linear dynamic range from 5.96 up to 161.07 M. The respective calibration equation for this concentration range is:

$$I_p(\mu\text{A}) = 0.0259C(\mu\text{M}) - 0.1605, R^2 = 0.9977$$

Using the slope of the calibration curve (Fig. 9 B) the detection limit (3σ) of SP was obtained 49.55 nM at the surface of MWCNT/CPE. The relative standard deviation (RSD) of 3.3% in the oxidation peak current and 0.55% in the peak potential for five repeated detections of 50.0 M SP suggests that there is excellent reproducibility of results using our modified electrode.

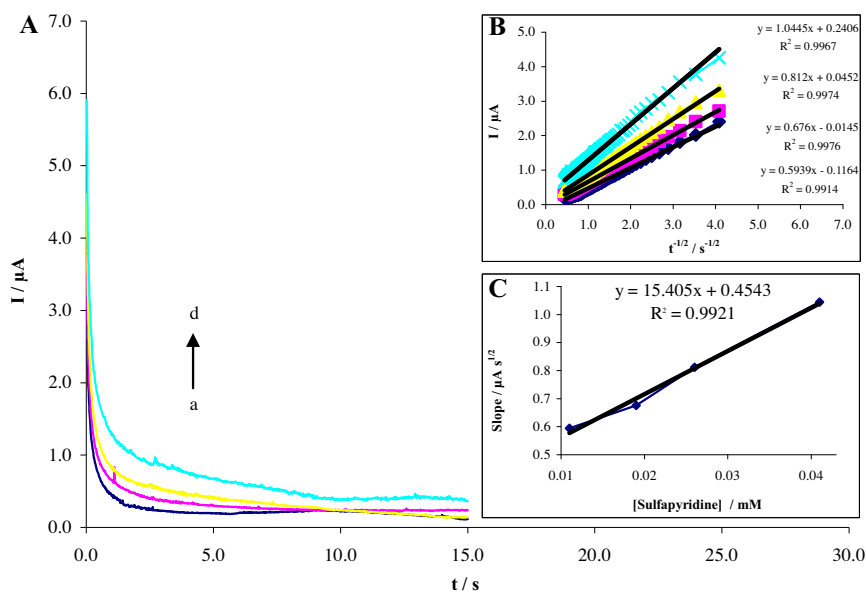


Figure 7 (A): Chronoamperograms obtained at MWCNT/CPE in 0.2 M B-R buffer solution (pH = 7.5) for various concentrations of SP, with a potential step at 1000.0 mV. a–d correspond to 8.0, 16.0, 23.0 and 38.0 μM of SP, respectively (B): Plots of I vs. $t^{-1/2}$ ($\text{s}^{-1/2}$) obtained from chronoamperograms a–d, (C): A plot of the slope of the straight lines versus the concentration of SP.

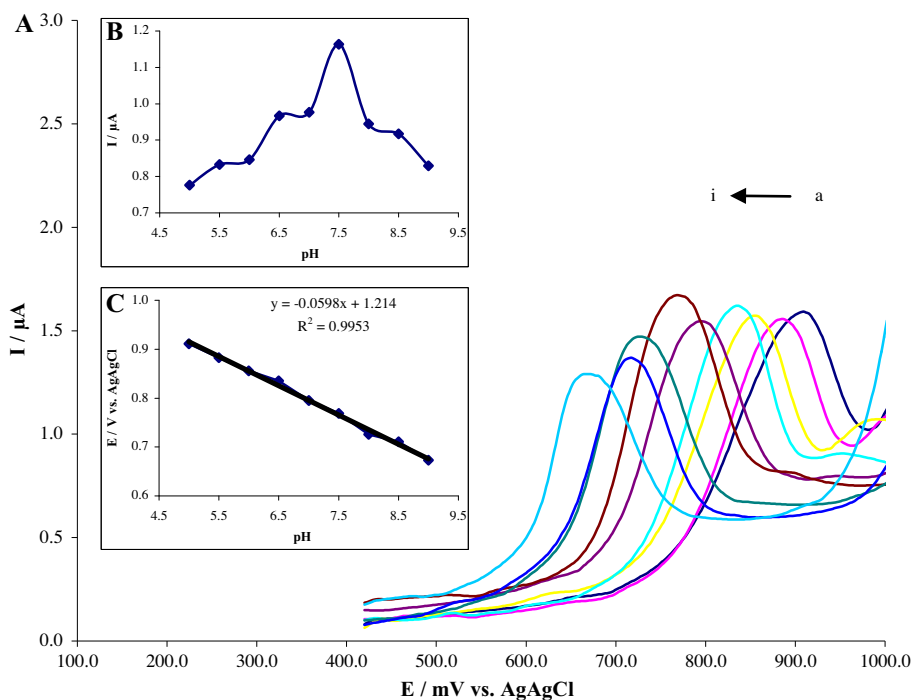


Figure 8 (A) DPV of a solution of 50.0 μM SP at various buffered pHs, a–i correspond to pH values of 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0, respectively. (B): Intensity, I (μA) vs. pH and (C) electrochemical potential, E_{pa} (V) vs. pH. DPV conditions: scan rate, 124.0 mV s^{-1} ; step potential, 2.8 mV; modulation amplitude, 35.5 mV.

3.10. Interferences

The influence of common interfering species was investigated in the presence of 55.0 M SP. The results showed that Na^+ ,

K^+ , NH_4^+ , Cl^- , CO_3^{2-} , L-phenyl alanine and glycin have not significantly influenced the height of the peak current of SP (with concentration ratio up to 200 times rather than of SP). The tolerance limit was defined as the concentrations

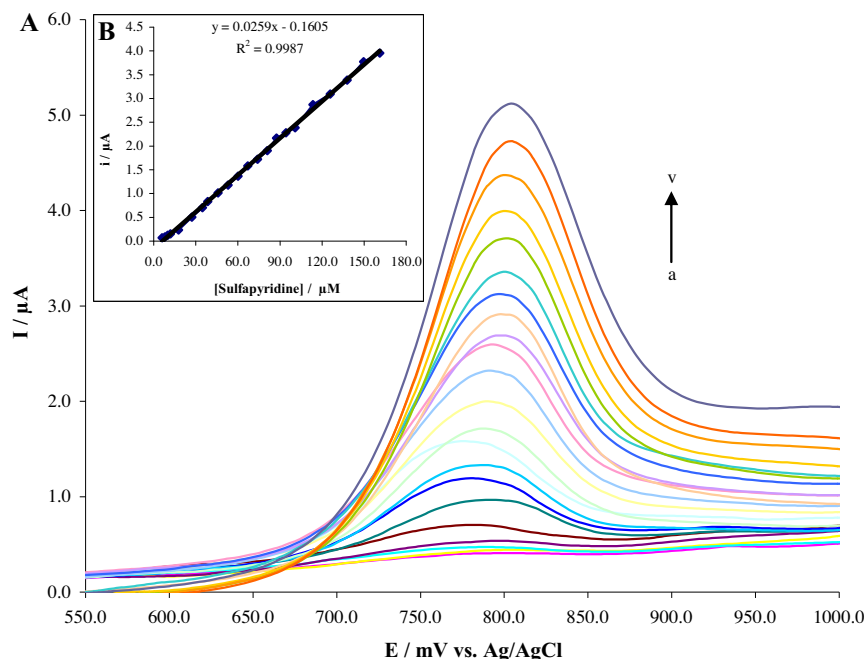


Figure 9 (A) DPV of MWCNT/CPE in a 0.2 M B–R buffer solution (pH = 7.5), containing different concentrations of SP. The letters a to v correspond to: 5.96, 7.94, 9.90, 11.86, 17.68, 27.24, 34.74, 38.46, 45.80, 53.03, 60.15, 67.16, 74.07, 80.88, 87.59, 94.20, 100.72, 113.47, 125.87, 137.93, 149.66 and 161.07 μM of SP, (B): Plot of the electrocatalytic peak current as a function of SP concentration.

Table 4 Determination of SP in human blood serum samples.

Sample	Added (μM)	Found (μM)	Recovery (%)
Human blood serum	–	–	–
	2.00	1.95	95.54
	5.00	5.18	103.64

which give an error of $< 5.0\%$ in the determination of SP. These results indicate that the proposed method has good selectivity for the determination of SP.

3.11. Determination of SP in human blood plasma samples

The application of the proposed method in real sample analysis was investigated by direct analysis of SP in human blood plasma samples. Prior to measurement, all plasma samples were diluted 100 times with B–R buffer solution (pH = 7.5). The standard addition method was used for testing recovery. The recovery rates of the spiked samples ranged from 95.54% and 103.64%. (Table 4), indicating the plasma sample matrix does not interfere with the detection procedures.

4. Conclusions

This paper describes a novel and selective method for optimization of voltammetric parameters and determination of SP using combination of CCRD and DPV. CCRD allowed analyzing the interaction between the variables with a minimum

number of experiments. By using the modified electrode an increase in the current response is observed relative to the electrochemical response of a bare CPE. Also the modified electrode was characterized by EIS technique. Kinetic parameters of MWCNT/CPE such as the surface concentration of mediator, Γ , charge transfer coefficient, α , and apparent charge transfer rate constant, k_s , are calculated using CV technique. Kinetic constants of SP such as charge transfer coefficient (α), ionic exchanging current density (j) and diffusion coefficient (D) were determined by the LSV and chronoamperometry methods. Then by using the DPV method the detection limit for SP (with Signal to Noise > 3) was found to be 49.55 nM with a wide linear dynamic range (5.96–161.07 M). Finally the proposed method was applied for the analysis of SP in real samples. Our results indicate that the MWCNT/CPE is highly sensitive, with great potential as a sensor for electrochemical applications.

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