

Development of electrochemical sensor based on multiwall carbon nanotube for determination of anticancer drug idarubicin in biological samples

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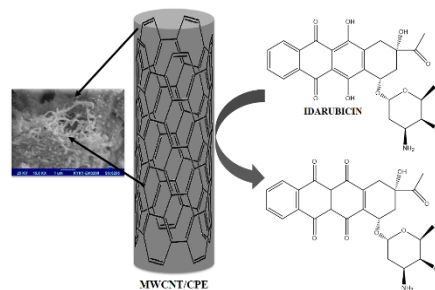
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Abstract

A graphite paste electrode modified with Multiwall Carbon Nanotube (MWCNT) was prepared and used for low level determination of anticancer drug idarubicin (IDA) in biological fluids. The measurement was made using differential pulse voltammetry (DPV). The influence of some experimental variables such as composition of carbon paste, pH of solution, scan rate and possible interferences were studied. The charge transfer property of the fabricated composite electrode was characterized by electrochemical impedance spectroscopy (EIS). Furthermore, the morphology of the electrode was characterized by scanning electron microscopy (SEM). Under optimum conditions, the proposed sensor provides two linear DPV responses toward IDA over the concentration ranges of 1–100 nM and 100–1000 nM for with a detection limit of 0.2 nM. Finally, the constructed sensor was successfully applied for determination of IDA in human urine and serum samples.



Keywords: Idarubicin (IDA), Multiwall carbon nanotube (MWCNT), Differential pulse voltammetry (DPV), Modified graphite paste electrode.

Introduction

Despite the extensive efforts in cancer treatment, the best therapeutic strategy is not found yet. Chemotherapy remains the first line treatment regimens of cancer.¹ Anthracyclines (or anthracycline antibiotics) are a class of drugs used in cancer chemotherapy derived from *Streptomyces* bacterium *Streptomyces peucetius* var. *caesius*. Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Valrubicin and Mitoxantrone are examples of anthracyclines.² One of the most potent chemotherapeutic anthracyclines is idarubicin (IDA, see Figure 1), approved for use against a wide spectrum of tumors namely acute and chronic myelogenous leukemia, acute lymphoblastic leukemia and myelodysplastic syndrome.³ The absence of a methoxy group at position 4 of the anthracycline structure gives the high lipophilic character, and is therefore used orally.⁴ IDA also displays anti-tumor activity in melanoma, sarcoma, lung, ovarian and breast cancers.⁵ IDA has a similar anticancer mechanism of action to other anthracycline drugs, by interfering with DNA synthesis.⁶ This drug acts as topoisomerase II poisons, inducing DNA damage by disrupting the cleavage-religation equilibrium and increasing the concentration of DNA topoisomerase II covalent complexes leading to apoptosis.⁷ IDA is reported to be 5-10 times more potent but less cardiotoxic than daunorubicin. Both IDA and its lipophilic metabolite daunorubicinol are considered unique BBB-crossing anthracyclines.⁸

Very few methods appear in the literature for the determination of IDA and its metabolites using high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) in bulk solutions, plasma, saliva, and urine with amperometric,⁹ UV,⁹ fluorescence,¹¹⁻¹³ and mass spectrometry (MS)^{14,15} detectors. Compared with the above methods, the electrochemical assay is simple, reliable and practical with low detection limit and wide dynamic range. The electrochemical detection has been found more attractive technique for the determination of electroactive compounds because of its sensitivity, fast operation, reproducibility, accuracy, low-cost, negligible sample and solvent consumption and recently it has been developed as a potentially useful technique for pharmaceutical applications.¹⁶ The electrochemical detection of electroactive compounds has been shown to be facilitated by modification of accessible and conventional electrodes.¹⁷⁻²⁴ However, up to now only voltammetric technique was used for electrochemical determination of IDA, where, the overall process was performed using multiwall carbon nanotube modified glassy carbon and pyrolytic graphite electrodes.²⁵ The other reports for electrochemical determination of IDA are based on the interaction this drug with DNA.²⁶⁻²⁸

Carbon nanotubes (CNTs) have been known as a rolled graphene sheets that exhibit excellent mechanical, electrical and electrocatalytic properties.²⁹ In the past decade, massive amount of works have been done on both multiwall carbon nanotube

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(MWCNT) and single walled carbon nanotube (SWCNT) based electrochemical sensors and biosensors.³⁰ Carbon nanotubes (CNTs) are considered as an important group of nanostructures with attractive electronic, chemical and mechanical properties.^{31, 32} Some exclusive characteristics including compatibility, functionality and electronical features make capable CNTs to immobilize a variety of species on their external and internal surfaces through a variety of electrochemical application.³³⁻³⁷ Multiwall carbon nanotubes (MWCNTs) have attracted considerable attention in electrochemical field because of their unique physical and chemical properties.^{38, 39} MWCNTs are often used in the fabrication of sensors to promote the electron transfer reactions of various molecules and increase the available electroactive surface area of various electroactive substances.⁴⁰ Due to advantages such as non-toxic, low background current, wide applicable potential range, rapid renewability and easy fabrication, modified carbon paste electrode (MCPE) has attracted considerable attention in recent years.^{41, 42} Therefore, in the present work, MWCNT was used for construction of modified carbon paste electrode to establish a robust electrochemical sensor for direct quantification of IDA.

In this paper, we exploited a differential pulse voltammetric technique for direct electrochemical determination of IDA by using a MWCNT modified graphite paste electrode with desirable analytical characteristics. This proposed sensor has a lower detection limit and wider linear range in comparison to the former report for direct electrochemical determination of IDA.²⁵ This method is convenient and useful because of its simplicity, low cost and low detection limit. Finally, the modified electrode was used for successful determination of IDA in biological samples.

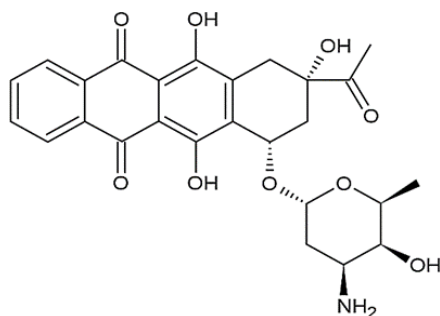


Figure 1. Chemical structure of IDA.

Experimental

Apparatus and chemicals

Cyclic voltammetric (CV) and differential pulse voltammetric (DPV) experiments were performed using an Autolab PGSTAT 302N (Metrohm Autolab BV, Netherlands) with an FRA32M impedance analysis module. The working electrode was an unmodified or modified carbon paste electrode, the auxiliary and reference electrodes were a platinum wire and standard calomel electrode (SCE), respectively. A Metrohm 710 pH meter was used for pH measurements. The surface morphology of modified electrodes was characterized with a scanning electron microscope (SEM) (Philips XL 30). All experiments were carried out at ambient temperature (25 ± 1 °C). IDA and MWCNT (outer diameter < 8 nm) were of analytical grade from Sigma-Aldrich. The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0–8.0. All other reagents were of analytical grade and solutions were freshly prepared with double distilled water.

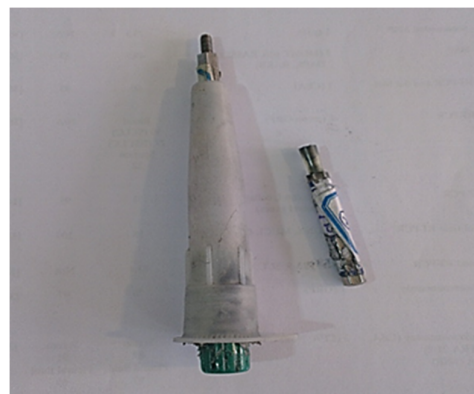


Figure 2. A typical picture of the modified electrode.

Preparation of modified electrode

The unmodified and modified graphite paste were prepared by thoroughly mixing analytical grade graphite and paraffin oil in the absence and presence of modifier, afterward, each paste was mixed in a mortar for at least 10 min to become homogeneous. The obtained pastes were packed into the end of a metallic spacer which after inserting in a holder was used as a working electrode (see Figure 2). The surface of each electrode was polished using a butter paper to produce reproducible working surface and then was used for electrochemical studying of IDA by voltammetric techniques. To obtain the optimum composition, the modified graphite paste electrode was prepared by mixing different percentages of graphite powder, paraffin oil, and modifier, as the best results were obtained at 53:32:15% of graphite, paraffin oil and modifier, respectively.

Analytical procedure

The modified electrode was first activated in a 10 ml volume of 0.1 M phosphate buffer by potential scanning from -0.25 to 1 V vs. SCE at a scan rate of 100 mV s^{-1} , until a low and steady background was obtained. Differential pulse voltammetric experiments were conducted under the instrumental conditions of 50 mV pulse amplitude, 40 ms pulse width and 60 mV s^{-1} scan rate. For recording the cyclic voltammograms, the potential was scanned from the 0 to 1 V using the scan rate of 100 mV s^{-1} .

Preparation of serum sample

For determination of IDA in human serum sample, a serum sample of a healthy volunteer was stored frozen until assay. For the preparation of serum samples, 2 mL of methanol was added to a 1.5 mL serum sample to remove serum proteins. After vortexing of the serum sample for 2 min, the precipitated proteins were separated by centrifugation for 3 min at 6000 rpm. The clear supernatant layer was filtered and diluted to a definite volume. The urine sample used for measurement was centrifuged and diluted 10 times without any further pretreatment. For both of real samples, known amounts of analyte was added to the phosphate buffer solution (pH 3) containing deliberate amounts of the real sample.

Results and discussion

Characterization of the modified electrode

The electron transfer properties of the MWCNT/CPE was characterized by electrochemical impedance spectroscopy (EIS) using $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as the redox probes. In EIS experiments, the semicircle diameter of Nyquist plot is equals to the electron transfer resistance (R_{et}), which controls the electron transfer

kinetics of the redox probe at the interface of electrode/electrolyte. Figure 3 presents the Nyquist diagrams of the bare CPE (curve a, with $R_{et} = 290 \Omega$) and the MWCNT/CPE (curve b, with $R_{et} = 110 \Omega$) in 3 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and 0.1 M KCl. MWCNT/CPE shows a smaller semicircle at the high frequency region in compare to the bare CPE, indicating lower electron transfer resistance. This can be attributed to the presence of MWCNTs with good conductivity and large effective surface area in the modified electrode, which could effectively increase the rate of electron transfer between electrode surface and $[\text{Fe}(\text{CN})_6]^{3-/4-}$ and decrease the interfacial electron transfer resistance.

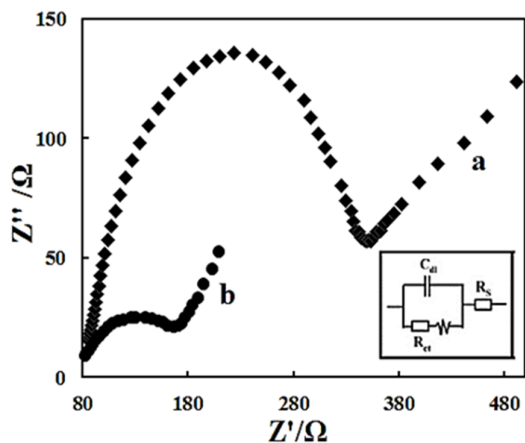


Figure 3. EIS for (a) CPE and (b) MWCNT modified carbon paste electrode in 3 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ with 0.1 M KCl over the frequency range of 10^5 -0.1 Hz and $E_{ac} = 10$ mV.

Figure 4 displays the SEM images taken at the surface of CPE (A) and MWCNT/CPE (B). It can be seen that on the surface of CPE (Figure 4A), the layer of irregular flakes of graphite powder is presence and isolated with each other. After multiwall carbon nanotube added to the carbon paste matrix, it can be seen that multiwall carbon nanotubes were dispersed on the electrode with special three-dimensional structure (Figure 4B).

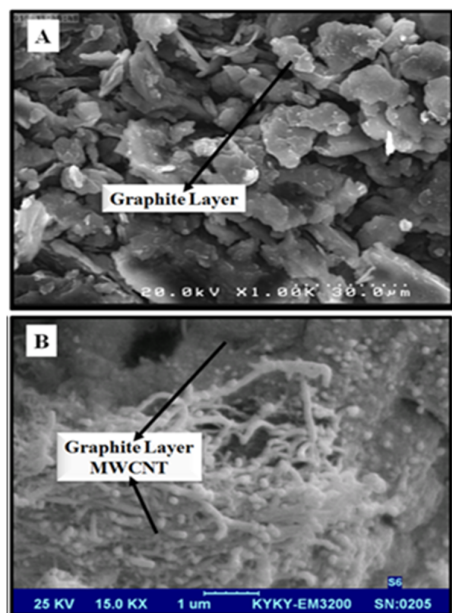


Figure 4. SEM images of (A) bare CPE and (B) MWCNT/CPE. Electrochemical behavior of IDA at MWCNT/CPE.

The voltammograms of the phosphate buffer solution (pH 3) were recorded in the absence (curves a and b) and presence (curves c and d) of 0.5 μM IDA at the surface of the bare CPE and MWCNT/CPE and the results are shown in Figure 5. For both CPE and MWCNT/CPE, one couple of redox peaks were appeared in the presence of IDA. These anodic and cathodic peaks are attributed to the electrooxidation and reduction of the hydroquinone/quinone moiety in the structure of IDA (as are specified in Figure 8). At the bare CPE (Figure 5, curve c), a pair of poor anodic and cathodic peaks at 0.76 and 0.60 V with peak-to-peak potential separation (ΔE_p) of 160 mV and the peak current ratio (I_{pc}/I_{pa}) nearly 0.82 were observed which might be ascribed to low electrical conductivity and low surface area of the bare CPE. The modification of CPE by MWCNT not only decreased the peak-to-peak potential separation ($\Delta E_p = 120$ mV) but also enhanced the sensor response and increased the peak current ratio to about one (curve d). Furthermore, the anodic peak potential of IDA was shifted towards less positive values while the cathodic peak potential shifted towards more positive values (relative to those obtained at the bare CPE) that may be due to faster electron transfer at the surface of modified electrode. In the other words, the reversibility of electroic reaction increased and the overpotential for electrooxidation and electroreduction of IDA decreased. These observations exhibit that MWCNT enhances the conductivity, surface area, and also facilitates the electron transfer between the drug and the electrode surface.

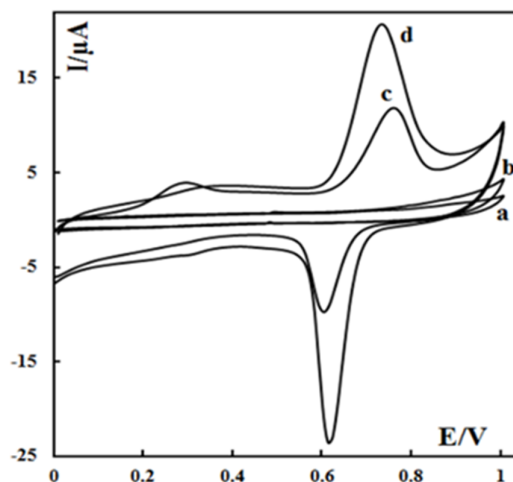


Figure 5. Cyclic voltammograms of 0.5 μM IDA in phosphate buffer (pH 3) at (c) bare CPE, (d) MWCNT/CPE, (a) absence of IDA in solution on bare CPE and (b) absence of IDA in solution on MWCNT/CPE, scan rate: 100 mV s^{-1} .

The effect of scan rate on the electrochemical response of 0.5 μM IDA was also investigated over the range of 10 to 500 mV s^{-1} and the corresponding voltammograms are shown in Figure 6. As is shown, with the increase of scan rate, the redox peak current increased gradually along with the slight shift of redox potential. The relationship of redox peak currents with the scan rates was constructed and the results showed the redox peak currents were proportional to the scan rate (ν) over the range of 10 to 500 mV s^{-1} , indicating that the electron transfer reaction of IDA on the MWCNT/CPE is a adsorption-controlled process. The electrochemical parameters of the IDA on the MWCNT/CPE were further calculated. From Figure 6, it can be seen that the redox peak potentials were also moved in positive direction with the increase of scan rate and the peak-to-peak separation increased.

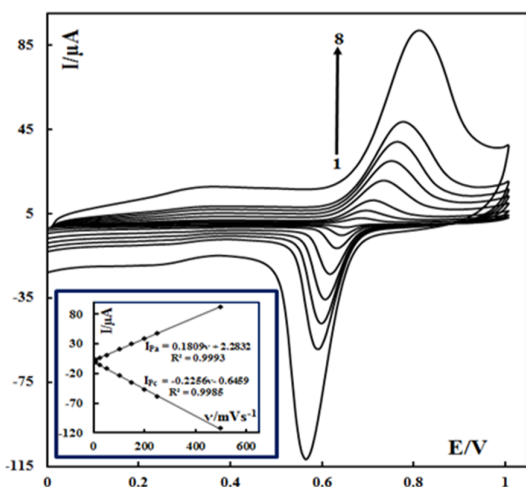


Figure 6. Cyclic voltammograms of 0.1 M phosphate buffer (pH 3) containing 0.5 μM IDA at different scan rates. The numbers of 1–8 correspond to 10, 25, 50, 100, 150, 200, 250 and 500 mV s^{-1} respectively. Inset: variation of the I_{pa} and I_{pc} with v .

The relationship of the peak potentials with scan rate was also sketched, which could be used for the calculation of the electrochemical parameters of IDA. According to the Lavron's equation:⁴³

$$E_{PC} = E^{0'} - \frac{RT}{\alpha nF} \ln v \quad (1)$$

$$E_{Pa} = E^{0'} + \frac{RT}{(1-\alpha)nF} \ln v \quad (2)$$

where α is the charge transfer coefficient, n the number of electron transfer, v the scan rate, $E^{0'}$ the formal potentials and F the Faraday's constant. The plot of the E_p with the $\ln v$ was established and two straight lines were got with two linear regression equations as E_{pa} (V) = 0.0328 $\ln v$ + 0.5895 and E_{pc} (V) = -0.0226 $\ln v$ + 0.7108. According to the Equations (1) and (2) the values of α and n were calculated to be 0.59 and 1.91 (≈ 2), respectively. So, the electrooxidation reaction of IDA on MWCNT/CPE was a two-electron process. The related calculations are given below:

$$\begin{aligned} \text{Slope of } E_{PC} \text{ vs. } \ln v \text{ graph} &= -\frac{RT}{\alpha nF} \Rightarrow -0.0226 \\ &= -\frac{RT}{\alpha nF} \end{aligned}$$

$$A) 0.0226 = \frac{8.314 \times 298}{\alpha n \times 96485} \Rightarrow \alpha n = 1.13$$

$$\begin{aligned} \text{Slope of } E_{Pa} \text{ vs. } \ln v \text{ graph} &= \frac{RT}{(1-\alpha)nF} \Rightarrow 0.0328 \\ &= \frac{RT}{(1-\alpha)nF} \end{aligned}$$

$$B) 0.0328 = \frac{8.314 \times 298}{(1-\alpha)n \times 96485} \Rightarrow 0.78 = n - \alpha n$$

$$A \text{ and } B \Rightarrow n = 1.91 \text{ and } \alpha = 0.59$$

The electrochemical behavior of IDA is dependent on pH value of the aqueous solution. Therefore, pH optimization of the solution seems to be necessary. Thus, the influence of the pH ranging from 2.0 to 8.0, using phosphate buffers on the peak current of 0.42 μM of IDA was studied (Figure 7A). Furthermore, for a better resolution, the cyclic voltammograms in the pH ranging from 2.0 to 6.0 are given in a distinct Figure (Figure 7B). The maximum current at the MWCNT modified CPE was obtained in pH 3.0 and thus, this pH was chosen as the optimum pH for electrooxidation of IDA at the surface of MWCNT/CPE.

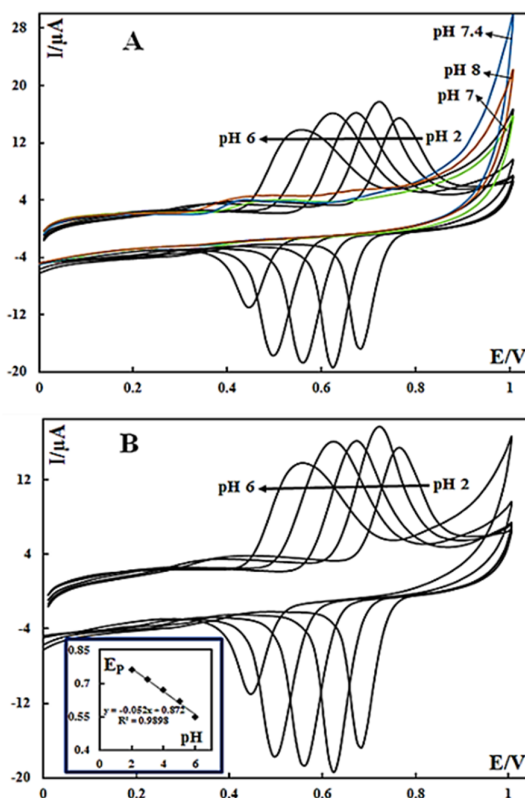


Figure 7. Effect of pH on cyclic voltammograms of 0.1M phosphate buffer solution containing 0.5 μM IDA at 100 mV s^{-1} : A: in the pH ranging from 2 to 8 (pHs: 2, 3, 4, 5, 6, 7, 7.4 and 8). B: in the pH ranging from 2 to 6 (pHs: 2, 3, 4, 5 and 6). Inset B: Variation of the peak potential with pH in the range of pH 2 to 6.

Furthermore, our study showed that by increasing the pH the anodic peak potential of IDA was shifted towards less positive values. The variations of anodic peak potential relative to pH of the solution was linear in the pH ranging from 2.0 to 6.0 (inset of Figure 7B). The linear regression equations for anodic peak is given by:

$$E_{pa}(\text{V}) = -0.052\text{pH} + 0.872 \quad (r^2 = 0.9898) \quad (3)$$

The slope value of about -52 mV/pH was obtained which is nearly equal to the theoretical value of 59 mV, suggesting the participation of equal number of protons and electrons in the oxidation of IDA at the modified CPE. According to the above results the electrooxidation reaction of IDA on MWCNT/CPE was a two-electron two-proton process. This observation is a well-known behavior of hydroquinones.⁴⁴⁻⁵⁰ The mechanism of electrooxidation of IDA has been previously reported by other researchers (see Figure 8).²⁵

Optimization of the amount of modifier in the electrode

Primarily experiments show that the amount of MWCNT modifier in carbon paste electrode influences on the anodic peak current of IDA. Therefore, five electrodes containing different percent of MWCNT (0, 5, 10, 15 and 20%) were prepared and examined for detection of 0.5 μM IDA under identical conditions. The maximum peak current was obtained when the amounts of the graphite powder, paraffin oil and MWCNT in the paste were 53:32:15%, (w/w).

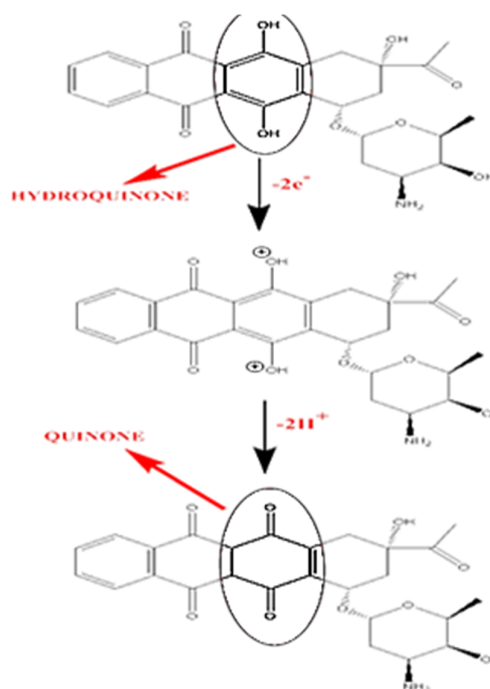


Figure 8. Mechanism oxidation of IDA.

Analytical performance

Differential pulse voltammetry (DPV) (with pulse amplitude of 50 mV, pulse width of 40 ms and scan rate of 60 mV s^{-1}) was used to measure IDA. The DPV signals obtained for tested different concentrations of IDA as well as the plotted calibration curve is shown in Figure 7. Two linear relationships are obtained over IDA concentrations ranging from 1 to 100 (Figure 9, inset A) and 100 to 1000 nM respectively (Figure 9, inset B). The linear regression equations are:

$$I_p(\mu\text{A}) = 0.9067 + 0.0527C_{IDA}(\text{nM}) \quad (r^2 = 0.9945) \quad \text{A}$$

$$I_p(\mu\text{A}) = 5.9074 + 0.003C_{IDA}(\text{nM}) \quad (r^2 = 0.9764) \quad \text{B}$$

The limits of detection (LOD) and quantitation (LOQ) were calculated using the relation ks/m ,⁵¹ where $k=3$ for LOD and 10 for LOQ, s representing the standard deviation of the peaks current of the blank ($n=10$) and m representing the slope of the first calibration curve for IDA. Both LOD and LOQ values were found to be 0.2 and 1 nM respectively, which these values indicated the sensitivity of the proposed method. In comparison to other IDA electrochemical sensors that has been reported in literature,^{25, 27, 28} the proposed sensor provides an acceptable limit of detection and linear range (Table 1). Comparing the results of this study with the earlier study performed for direct electrochemical determination of IDA (reported by Ozkan et al.²⁵) clarified that the sensitivity of IDA determination by this proposed electrode is significantly higher. The corresponding results of this research showed sharp and well-known peaks for IDA as a hydroquinone

Table 1. Comparison of the performances of various electrodes for IDA determination.

Electrode	Method	Linear range	LOD (m)	Ref.
Multiwalled carbon nanotubes modified glassy carbon electrode	Adsorptive stripping differential pulse voltammetry	$9.36 \times 10^{-8} - 1.87 \times 10^{-6}$ M	1.87×10^{-8}	25
Multiwalled carbon nanotubes modified edge plane pyrolytic graphite electrode	Adsorptive stripping differential pulse voltammetry	$9.36 \times 10^{-8} - 9.36 \times 10^{-7}$ M	3.75×10^{-8}	
Polyaniline-DNA based Glassy carbon electrode	Voltammetry	$1 \times 10^{-9} - 1 \times 10^{-4}$ M	0.2×10^{-9}	27
Poly(NR) and polycarboxylated thiacax[4]arene-DNA based glassy carbon electrode	Voltammetry	$1 \times 10^{-9} - 1 \times 10^{-3}$ M	0.5×10^{-9}	28
	Impedimetry	$1 \times 10^{-9} - 1 \times 10^{-4}$ M	1×10^{-9}	
This work	Differential Pulse Voltammetry	$1 \times 10^{-9} - 1 \times 10^{-6}$ M	0.2×10^{-9}	-

compound. In addition, in comparison of the electrochemical sensors which are based on the interaction of IDA with DNA [26-28], this proposed sensor has a simple strategy and fast response (there is no incubation of electrode in analyte solution as a time consuming process).

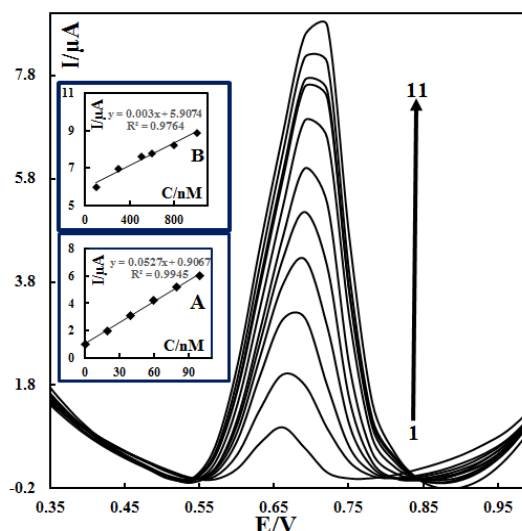


Figure 9. Differential pulse voltammograms of 0.1 M phosphate buffer (pH 3) containing different concentrations of IDA. The numbers of 1–11 correspond to 5, 20, 40, 60, 80, 100, 300, 500, 600, 800 and 1000 nM IDA. Insets show the plots of the peak current as a function of IDA concentration in the range of (A) 1–100 nM and (B) 100–1000 nM.

Reproducibility, repeatability and the long term stability of the modified carbon paste electrode were investigated. The reproducibility of the proposed electrode was investigated by using DPV. The obtained results of five replicate measurements (for a 0.5 μM IDA solution) showed a standard deviation less than ± 0.94 for DPV currents. In repeatability investigations, the recovery of analyzed target (0.5 μM IDA solution) in 10 replicate measurements was calculated to be above 96%, and the relative standard deviation (RSD) was lower than 3.4%. Also, the proposed electrode retained 93% of its initial activity after three weeks, demonstrating proper stability of the developed sensor. These results indicated the acceptable properties for the proposed electrode.

Analytical application

In order to demonstrate the application of the proposed method for voltammetric determination of IDA in real sample, it was used for voltammetric determination of IDA in serum and urine samples. After sample preparation as described in Section 2.4, the DPV method was applied to the IDA determination by the standard addition method and the results was summarized in Table 2. The results in Table 2 show that the recoveries of the spiked samples are acceptable. Thus the modified electrode can be efficiently used for determination of IDA in real samples with different matrices.

Table 2. Voltammetric determination of IDA in real samples and recovery data obtained (n = 5).

Sample	Added (nM)	Found (nM, 95% CI*)	Recovery (%)	RSD (%)
Urine	300	310	103	3.4
	400	405	101	2.6
	500	480	96	3.8
	600	630	105	3.9
	700	660	94	2.5
Serum	200	195	97	3.5
	400	420	105	3.9
	600	580	96	2.4
	800	850	106	2.8
	1000	940	94	3.6

*Confidence interval

Interference studies

For possible analytical application of the method, the effects of some common interferences on the determination of 0.5 μM IDA was examined. The tolerance limit was defined as the maximum concentration of the substances that caused an error of less than 5% in IDA determination. The results showed that when a 700-fold Mg^{2+} , Li^+ , Al^{3+} , Na^+ , K^+ , NH_4^+ and dopamine were present in the synthetic mixture, the average recovery in the determination of IDA was 102.6%.

Conclusions

The MWCNT/CPE has been successfully applied as a sensitive voltammetric sensor for fast and accurate determination of IDA in biological samples. The proposed sensor exhibited a high sensitivity, good selectivity and reproducibility. The performance characteristics of the modified electrode in conjunction with the simplicity of its preparation and the renewability of its surface by simple polishing make the system useful in constructing simple devices for determination of IDA.

Acknowledgments

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