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Full Paper

Fabrication of a Simple Cast-deposited Nafion/multi-walled Carbon Nanotube Composite Film as a Sensor for Voltammetric Determination of Pethidine

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Abstract- The effect of surface modification of glassy carbon electrode (GCE) by multi-walled carbon nanotubes (MWNTs) and Nafion (Nafion/MWCNT/MGCE) has been studied to voltammetric determination pethidine (PTD) as a potent opiate analgesic for humans for the first time. Cyclic Voltammetry (CV) and scanning electron microscopy (SEM) were used to characterize the properties of the modified electrode. The Nafion/MWCNT/MGCE exhibited a well-defined anodic peak at pH 7.0 at a potential of ~ 0.85 V for the oxidation of PTD as compared to 1.2 V at GCE. Using differential pulse voltammetry (DPV) technique a linear calibration curve was obtained over PTD concentration range 1.0-80.0 μM in phosphate buffer solution of pH 7.0 with detection limit of 0.83 μM. The method has been found selective and successfully implemented for the determination of PTD in human urine and injection samples using standard addition method. The electrode exhibited an efficient catalytic response with good reproducibility and stability.

Keywords- Multi-walled carbon nanotubes, Nafion, Pethidine, Sensor, Voltammetry

1. INTRODUCTION

Pethidine (Meperidine hydrochloride, Dolantin) (Ethyl, 1-methyl-4-phenylpiperidine-4-carboxylate hydrochloride), PTD is a potent opiate analgesics, which has been employed in

the treatment of a variety of medical conditions [1]. Pethidine hydrochloride is also used as an illicit drug and therefore it is placed on the schedule II controlled substances list (drugs that have acceptable medical use and have high potential for abuse) in the United States and many other countries [2]. Pethidine is also prescribed as a substitute for heroin [3], and often used medically as postoperative analgesia. In sports, athletes often take far higher doses of drugs than have been given for therapeutic use or in clinical studies to excel in competition. They have been barred to use by the International Olympic Committee and other sports organizations [4]. Therefore, determination of pethidine has important practical meanings.

Many Several sophisticated analytical methods were reported to determine the pethidine such as high performance liquid chromatography (HPLC) [5,6], gas chromatography [7], gas chromatography in combination with mass spectrometry (GC-MS) [8,9], spectrophotometry [10] and potentiometric analysis [11–15]. Although these methods are sensitive and highly reliable, they often require time consuming complex pretreatment steps and their apparatus and operating cost are too expensive for routine analyses.

Carbon nanotubes (CNTs) have been one of the most actively studied electrode materials in the past few years due to their unique electronic and mechanical properties. The subtle electronic properties suggest that CNTs will have the ability to mediate electron transfer reactions with electroactive species in solution when used as the electrode material [16–18]. The coating of CNT solution or suspension on the electrode surface is a simple but effective strategy to prepare CNT-modified electrodes. These CNT-modified electrodes have been proved to have excellent electro-analytical properties, such as wide potential window, low background current, low detection limits, high sensitivities, reduction of over potentials and resistance to surface fouling [19,20].

Nafion, a perfluorinated sulphonated cation exchanger with properties of excellent antifouling capacity, chemical inertness and high permeability to cations, has been extensively employed as an electrode modifier [20]. CNTs can be homogeneously dispersed in Nafion solution because of the hydrophobic side chains and polar head groups of Nafion [21]. Nafion/CNTs composite thin film modified electrodes have their attractive effects in electroanalytical applications. Yang et al. constructed a Nafion/multi-walled carbon nanotubes modified glassy carbon electrode for determination of caffeine [22]. Tsai et al. constructed a Nafion/multi-walled carbon nanotubes thin film for Electrochemical Stripping Analysis [23].

To the best of our knowledge, no voltammetric methods are available for the determination of PTD. This paper presents an application of Nafion/multi-walled carbon nanotubes composite film on GCE (Nafion/MWCNT/MGCE) for the trace determination of PTD employing voltammetry technique. A very sensitive and well-defined anodic peak was observed at the surface of modified electrode. The Nafion/MWCNT/MGCE can intensively enhance the oxidation of PTD and strongly improve its anodic peak current due to the

attractive properties of Nafion/MWNTs. Finally, this modified electrode was used for determination of PTD in pharmaceutical and urine samples.

2. EXPERIMENTAL

2.1. Chemicals and reagents

Pethidine was purchased from Sigma. The MWCNT with 99% purity was obtained from Sigma. MWCNTs were chemically functionalized by ultrasonification in a mixture of sulfuric acid and nitric acid (3:1 v/v) for 8 h [24]. MWCNTs were washed with deionized water and separated by centrifuging three times. Nafion solution (5 wt%) in a mixture of lower aliphatic alcohols and 10% water was purchased from Aldrich, and diluted by alcohol to desired concentrations. MWNTs were dispersed in Nafion solution by ultrasonication in water bath for 10 min. Phosphate buffered solutions (PBS) 0.1 M were prepared by mixing stock solutions of 0.1 M H_3PO_4, NaH_2PO_4, Na_2HPO_4 and Na_3PO_4 for different pH values.

The pharmaceutical and biological samples used in this work were obtained from Gerot, Austria and Dr. Safiri Medical Diagnostic Laboratory, Babolsar, Iran, respectively. Other reagents were of analytical grade and purchased from Merck and used as received.

2.2. Apparatus

The electrochemical measurements were recorded with a potentiostat and galvanostat (Autolab, model PGSTAT30, Eco Chemie, The Netherlands) controlled by personal computer. The experimental conditions for voltammetric measurements were controlled with general purpose electrochemical system (GPES) software. The measurements were carried out using a conventional three-electrode cell using an Ag|AgCl|KCl (3 M) electrode as the reference and a Pt wire as the counter (auxiliary) electrode. A digital pH-meter (Ion Analyzer 250, Corning) with precision of ±0.001 was used to read the pH value of the buffered solutions. A glassy carbon disk electrode (Azar electrode Co., Iran) with a geometrical area of 0.0254 cm^2, bare or modified, was used as working electrode. The experiments were carried out at room temperature. All solutions were prepared in distilled deionized water.

2.3. Electrode modification

The MWNTs/Nafion suspension was achieved by dispersing MWNTs into Nafion alcohol solution in a certain ratio by ultrasonication agitation for about 10 min. Prior to modification, the GCE was mechanically polished with alumina (0.05 μm) paste of different grades to a mirror finish, rinsed and sonicated in mixture of ethanol and water (1:1 v/v) for 5 min. The GCE was modified with 5.0 μL of the MWNTs/Nafion suspension by droplet and allowed to evaporate the solvent at room temperature in the air. The Nafion film-coated GCE

(Nafion/GCE) was prepared by the same procedure explained above, but without MWNTs. The MWCNT coated GCE (MWCNT/GCE) was prepared by dispersion of 1.0 mg MWCNT in 10 mL ethanol with the aid of ultrasonic agitation to give a 0.1 mg mL^{-1} black solution. Then, 5.0 μL of the black solution was cast at the GCE surface and the solvent was evaporated at room temperature for 5 min.

2.4. Preparation of real sample

Normal urine samples (ca. 5 mL) were obtained from three healthy volunteers (male, seventeen) from single-morning urination of the volunteers. Solution of 0.1 M NaOH was added to urine solution. The mixture was vortexed for 3 min after which 3 mL ethyl acetate was added and mixture was vortexed for an additional 3 min. The mixture was centrifuged at 3000 rpm for 10 min to separate the aqueous and organic layers. The residual aqueous phase was reconstituted with phosphate buffer solution at pH 7.0 and directly used for analytical determinations.

3. RESULTS AND DISCUSSION

3.1. Characterization of Nafion/MWNTs modified electrode

Fig. 1 displays the characterization of the Nafion/MWNTs composite film on the GCE (Nafion/MWCNT/MGCE) by using SEM method. It is obvious that the Nafion/MWNTs composite film was uniformly coated on the electrode surface and formed a porous layer. The special surface morphology offered a much larger real surface area than the apparent geometric area.

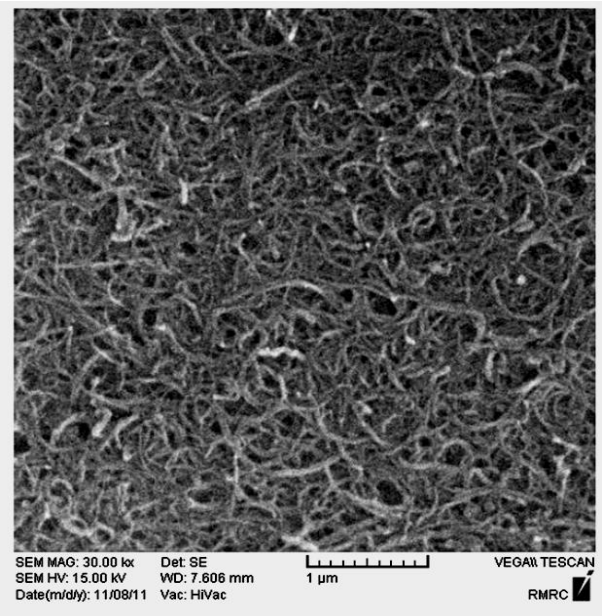

Fig. 1. SEM image of Nafion/MWNTs composite film on glassy carbon electrode

3.2. Cyclic voltammetric study of Pethidine on Nafion/MWNTs/MGCE

The direct electrochemistry of PTD on the modified electrode was investigated by cyclic voltammetry. Fig. 2 shows the cyclic voltammetric responses of 30.0 µM PTD at Nafion/MWNTs/MGCE (curve a), MWNTs/GCE (curve b), Nafion/GCE (curve c) and bare GCE (curve d) at a scan rate of 50 mVs^{-1}. As it can be seen in Fig. 2, electrochemical oxidation of PTD on the surface of bare GCE shows a relatively weak and irreversible peak with a peak potential of approximately 1.2 V *vs*. Ag|AgCl|KCl (3M) (curve d). The corresponding anodic peak current was significantly higher at the surface of the Nafion/MWNTs/MGCE than those at the MWNTs/GCE or the Nafion/GCE. Moreover, compared with the Nafion/GCE, the oxidation potential at the MWNTs/GCE or Nafion/MWNTs/MGCE was negatively shifted from 0.95 to 0.85 V. This phenomenon may be an evidence of catalytic effect of MWNTs toward PTD oxidation. The reasons for the notable sensitivity of the PTD determination at the Nafion/MWNTs/MGCE may be attributed to (1) the presence of Nafion as a cation exchanger that can improve electrostatic interactions, (2) the origin of electro-catalytic properties of MWCNTs assigned to the embedded metal impurities in CNT samples and edge-plane-like defects which are present at the open ends of nanotubes [25–27] and (3) the improvement of the porosity on the electrode surface.

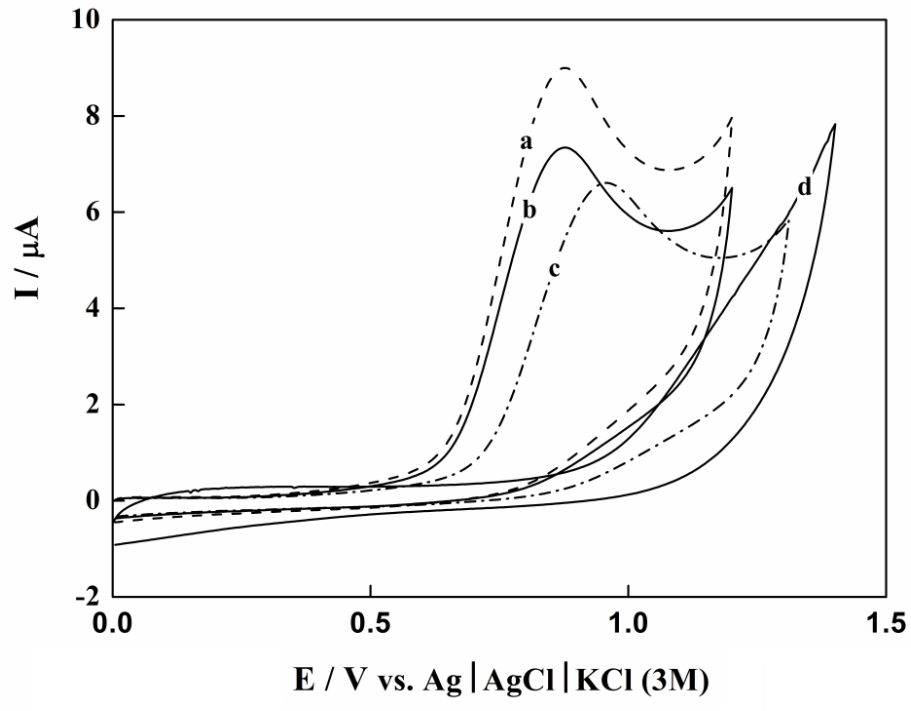

Fig. 2. Cyclic voltammograms of the **(a)** Nafion/MWNTs/MGCE, **(b)** MWNTs/GCE, **(c)** Nafion/GCE and **(d)** GCE containing 30.0 µM PTD in 0.1 M phosphate buffered solution (pH 7.0); scan rate 50 mVs^{-1}

3.3. Effect of scan rate on the electrooxidation of PTD

Useful information involving electrochemical mechanism can be usually acquired from the relationship between peak current (I_p) and scan rate of potential (υ) in the cyclic voltammetry. Therefore, the effect of scan rates on the peak current of PTD at the Nafion/MWNTs/MGCE in 0.1 M phosphate buffer (pH 7.0) was investigated by cyclic voltammetry (Fig. 3A). There is a good linear relationship between peak current and square root of scan rate (Fig. 3B), which confirm the diffusion-controlled process for electro-oxidation of PTD on the surface of modified electrode in the studied range of potential sweep rates.

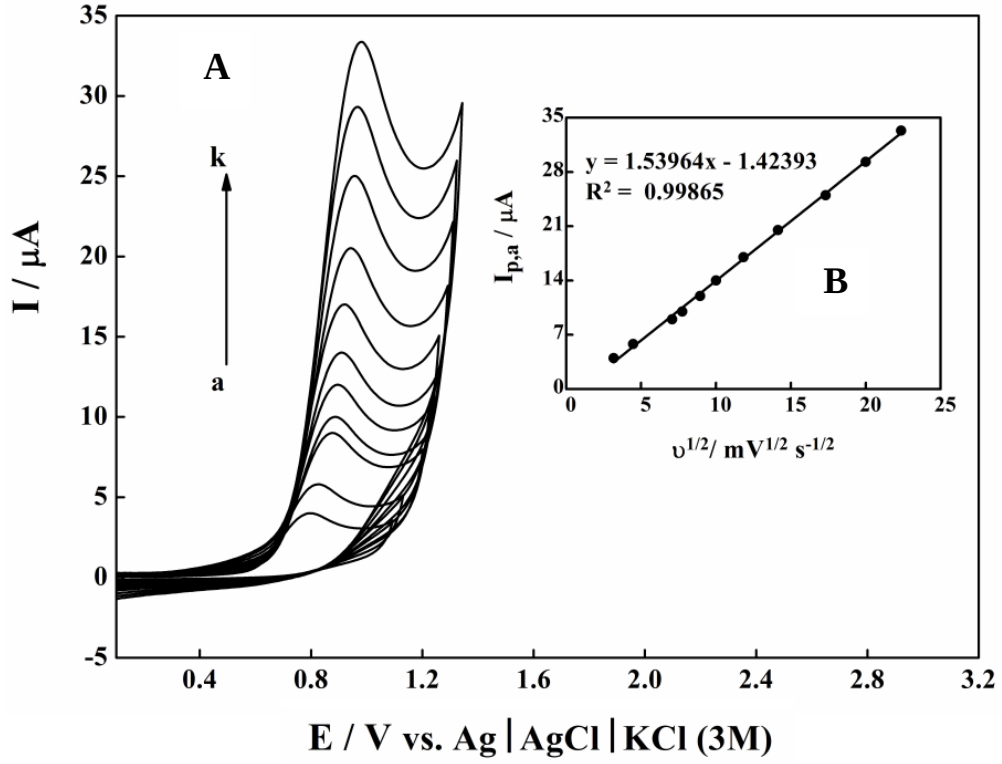

Fig. 3. (A) Cyclic voltammograms of 25.0 mM PTD at various scan rates: (a) 10, (b) 20, (c) 50, (d) 60, (e) 80, (f) 100, (g) 140, (h) 200, (i) 300, (j) 400 and (k) 500 mVs^{-1} in 0.1 M phosphate buffer solution (pH 7.0) at Nafion/MWNTs/MGCE. **(B)** Plot of $I_{p,a}$ *vs.* $\upsilon^{1/2}$ for cyclic voltammograms of (A)

As Fig. 2 shows, the electrochemical reaction of PTD is an irreversible reaction at the surface of Nafion/MWNTs/MGCE. For the irreversible electrochemical reaction, the kinetic information for an analyt (here PTD) can be obtained from the relationship between the peak potential E_p and the scan rate υ according to the following equation expressed by Laviron [28]:

$$E_p = E^\circ + (RT/\alpha_a nF) \ln (RTk_s/\alpha_a nF) - (RT/\alpha_a nF) \ln \upsilon \quad (1)$$

Where α_a is the transfer coefficient, k_s, the standard rate constant of the surface reaction, n is the number of electrons involved in the reaction and E° is the formal potential. According to Eq. (1), the plot of E_p *vs*. ln υ is linear with a slope that allows $n\alpha_a$ to be determined, and an intercept from which k_s can be calculated if the value of E° is known. The value of E° in Eq. (1) can be obtained from the intercept of the E_p *vs*. υ curve by extrapolation to the vertical axis at υ=0.

Fig. 4 shows the plot of E_p *vs*. lnυ obtained from a 0.1 M phosphate buffer solution containing 25.0 µM PTD by varying the scan rate from 10-500 mVs^{-1} (Fig. 3). The value of $n\alpha_a$ was found to be 0.54 using the slope of resulting straight line. Also, the value of E° was found to be 0.85 (not shown). From slope 0.0472, intercept 1.0164, E° 0.85 and $n\alpha_a$ 0.54 values, the value of k_s=696 s^{-1} was calculated. Since α_a is assumed to be 0.5 in a totally irreversible electrode process, these results demonstrate that one electron is involved in the oxidation of PTD.

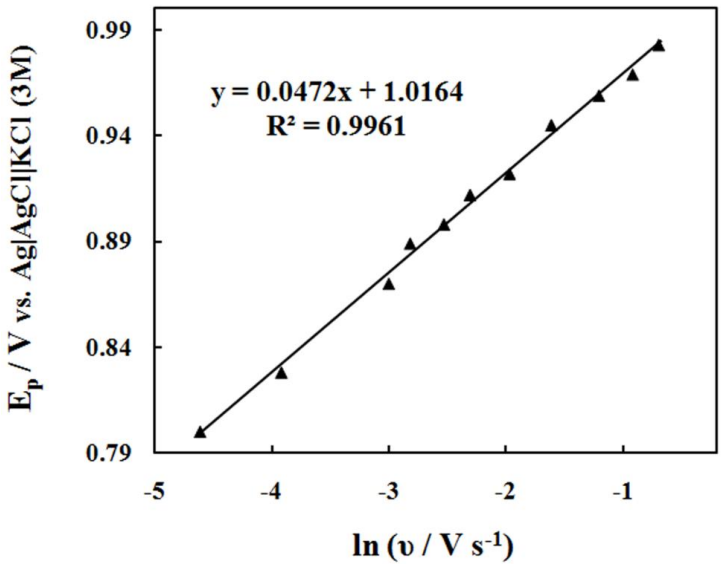

Fig. 4. Dependence of peak potential E_p on the potential scans rate lnυ. Data obtained from Fig. 3

3.4. Chronoamperometric measurements

Chronoamperometry, as well as other electrochemical methods, was employed for the investigation of electrode processes at the surface of Nafion/MWNTs/MGCE. The current-time curve reflects the change in the concentration gradient in the vicinity of the surface. This involves a gradual expansion of the diffusion layer associated with the depletion of reactant, and hence decreased slope of the concentration profile as time progresses. Accordingly, current decays with time (Cottrell behavior) [29]. Fig. 5A shows the current–time curves of Nafion/MWNTs/MGCE obtained by setting the working electrode potential at 0.8 V versus

Ag|AgCl|KCl (3M) for various concentration of PTD in phosphate buffered solutions (pH 7.0). The diffusion coefficient (D_{app}) for oxidation of PTD at the surface of modified electrode can be estimated using Cottrell's equation[30]:

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

Where D_{app} and C_b are the diffusion coefficient (cm^2s^{-1}) and the bulk concentration (mol cm^{-3}), respectively. At a mass transport limited rate condition, a plot of I *vs.* $t^{-1/2}$ will be linear, and the value of D_{app} can be calculated from the slope of this line. Fig. 5B shows the obtained experimental plots for different concentrations of PTD. The mean value of D_{app} was found to be 7.1×10^{-5} cm^2s^{-1} using the slopes of resulting straight lines plotted versus the PTD concentrations (Fig. 5C).

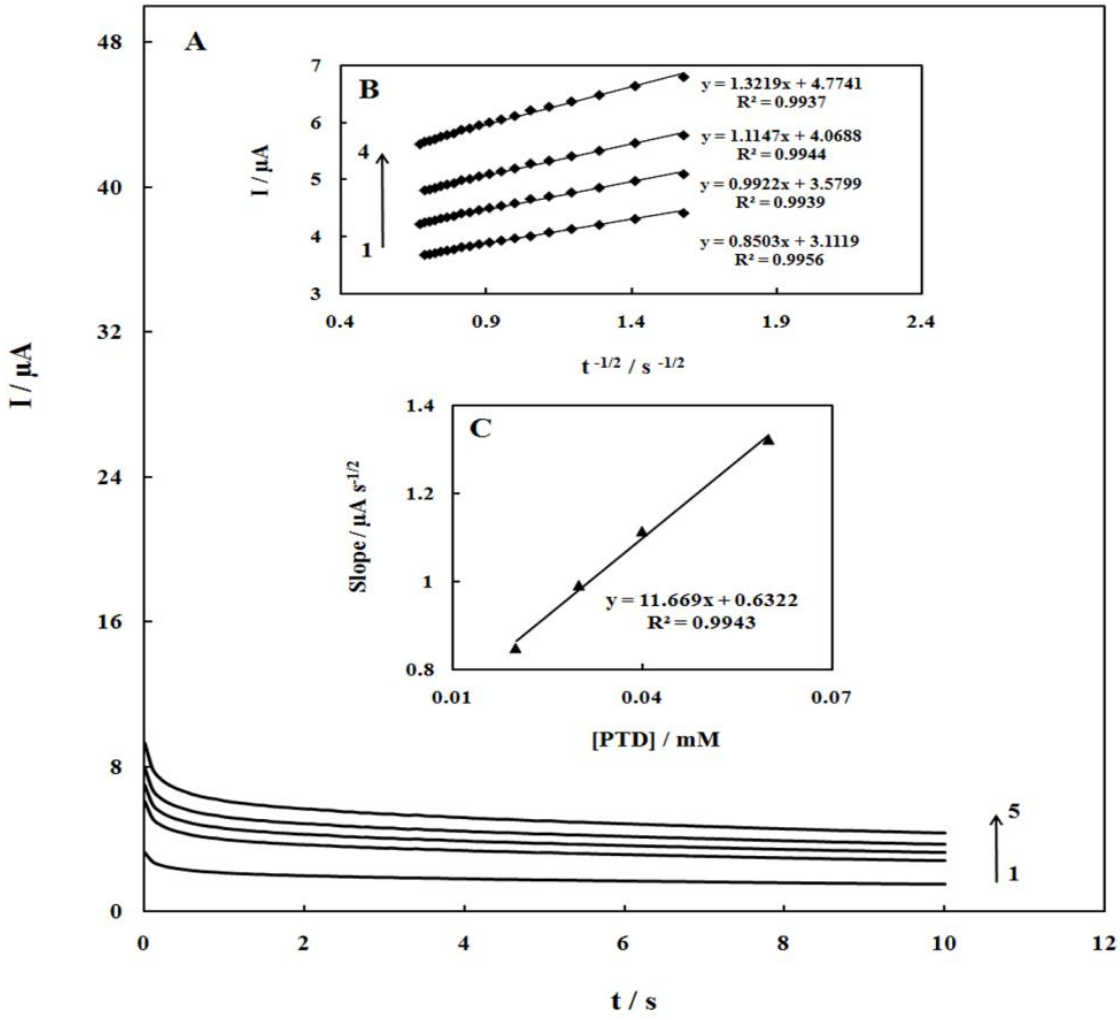

Fig. 5. (A) Chronoamperograms obtained at Nafion/MWNTs/MGCE in 0.1 M phosphate buffer solution (pH 7.0) for different concentration of PTD. The numbers 1-5 correspond to 0.0, 0.02, 0.03, 0.04 and 0.06 mM of PTD. Insets: **(B)** Plots of I *vs.* $t^{-1/2}$ obtained from chronoamperograms 2-5. **(C)** Plot of the slope of the straight lines against PTD concentration

3.6. Calibration curve

In order to validate the accuracy of this method for PTD quantitative analysis, the variation of peak current with different concentration PTD was studied using differential pulse voltammetry (DPV) under the optimum instrumental conditions. The results showed that the plot of peak current *vs*. PTD concentration is linear for 1.0 – 80.0 μM of PTD, with a regression equation of $I_p(\mu A)=(0.2955)C_{PTD}$ +(1.1584) (R^2=0.9985), where C is μM concentration of PTD and I_p is the net peak current (Fig. 6). According to the following equation [31]:

$$LOD = 3.3\ (s_{y/x}/b) \qquad (3)$$

Where $s_{y/x}$ is the residual standard deviation and b is the slope of the calibration plot. This method usually implies a decision about controlling both false positive and false negative errors (α=β=0.05). The limit of detection was calculated to be 0.83 μM.

In order to evaluate the selectivity of the proposed method for the determination of PTD, the influence of various foreign species on the determination of 25.0 μM PTD was investigated. The tolerance limit was taken as the maximum concentration of the foreign substances, which caused an approximately ±5% relative error in the determination. The results after the experiments revealed that neither 400-fold of glucose, sucrose, lactose, fructose, and citric acid nor 400-fold of methanol, ethanol, Ca^{2+}, Mg^{2+}, SO_4^{2-}, NH_4^+ and F^- nor 200 alanine, phenylalanine, methionine and glycine nor-100 tryptophan, urea and acetaminophen affected selectivity; nor did saturation of starch solution interfere and 50-fold of cysteine were interfered with the determination of PTD. Those results confirm the suitable selectivity of the proposed method for PTD determination.

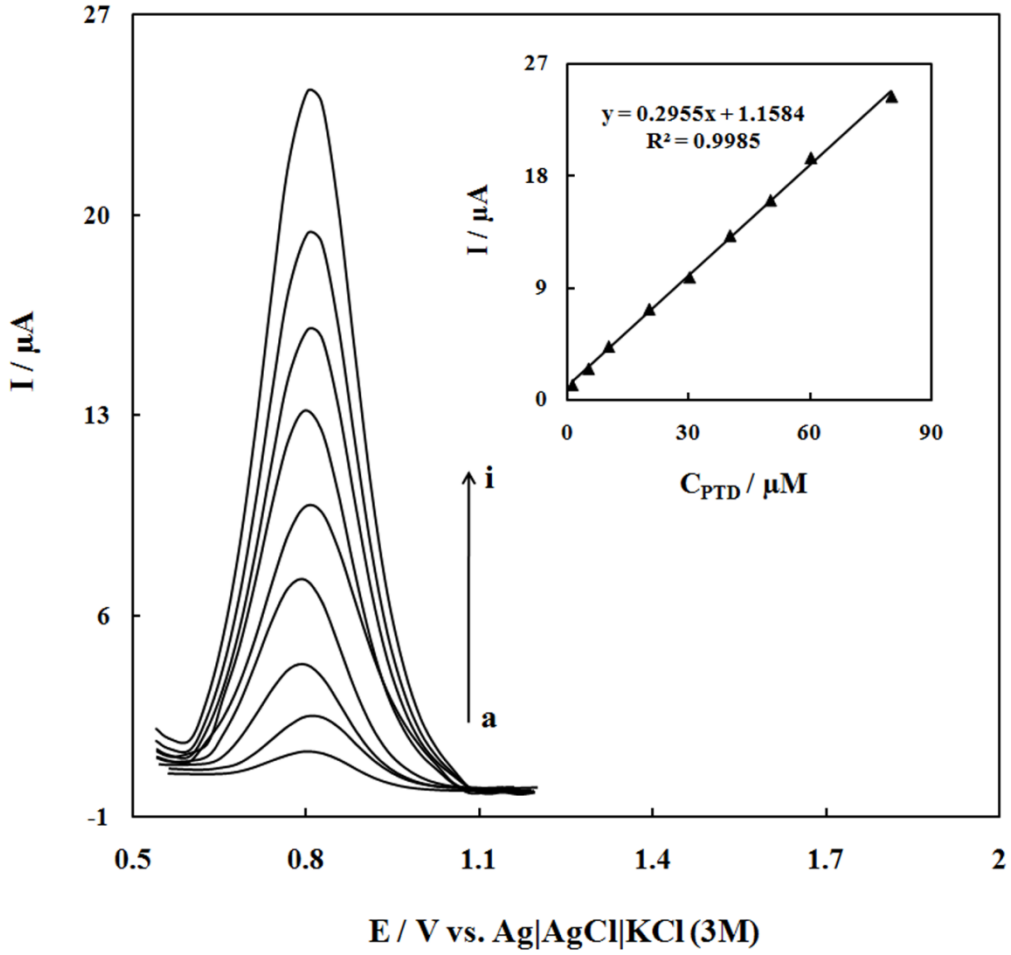

Fig. 6. Observed differential pulse voltammograms for increasing concentration of PTD [curves were recorded at (a)=1.0, (b)=5.0, (c)=10.0, (d)=20.0, (e)=30.0, (f)=40.0, (g)=50.0, (h)=60.0 and (i)=80.0 µM concentration] using Nafion/MWNTs/MGCEin 0.1 M phosphate buffer solution (pH 7.0). lnset: plot of the peak currents as a function of concentration of PTD in linear range 1.0-80.0 µM

3.7. Determination of PTD in real samples

To illustrate the modified electrode application in practical analysis, it was used for determination of PTD in injection and urine samples. The detection of PTD in two biological and pharmaceutical samples were analyzed by standard addition method (n=3). The pharmaceutical sample was performed as follows: 2.0 mL injection solution (pethidine hydrochloride, Gerot, Austria, 50 mg mL^{-1}) was diluted to 50 mL with distilled water, and then different amounts of this diluted solution was diluted to volume of 25 mL with phosphate buffer solution (pH 7.0) and was placed in the electrochemical cell. The obtained data are listed in Table 1.

Table 1. Determination of PTD in biological and pharmaceutical samples (n=3)

Sample	PTD added (μM)	PTD found (μM)	Recovery (%)	RSD (%)
1[a]	0.0	0.0	-	-
	5.0	4.87	97.4	2.8
2[a]	0.0	0.0	-	-
	25.0	25.4	101.6	1.6
3[a]	0.0	0.0	-	-
	35.0	35.2	100.5	1.9
4[b]	0.0	10.0	-	1.9
	5.0	14.9	99.3	2.3
5[b]	0.0	40.0	-	1.2
	5.0	44.9	99.7	3.1

[a] Urine sample.

[b] Pethidine hydrochloride injection sample

4. CONCLUSIONS

It has been unfold that Nafion/MWNTs/MGCE shows an appealing voltammetric performance towards determination of Pethidine. The modified electrode showed a large current response for the electroactive substrate due to its enhanced conductivity and biocompatible interface. The DPV currents of PTD at Nafion/MWNTs/MGCE increased linearly with the PTD concentration in the range from 1.0 to 80.0 μM with a detection limit of 0.83 μM. This electrode could be used to voltammetric determination of PTD without any interference in biological and pharmaceutical samples with satisfactory results. Finally, the reliability and fast analytical determination of PTD on the Nafion/MWNTs/MGCE makes it a suitable sensor for routine analysis of pethidine in clinical and pharmaceutical samples.

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