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Electrocatalytic and Simultaneous Determination of Phenylhydrazine and Hydrazine Using Carbon Paste Electrode Modified With Carbon Nanotubes and Ferrocenedicarboxylic Acid

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A carbon paste electrode (CPE) modified with ferrocenedicarboxylic acid (FCD) and carbon nanotubes (CNTs), was prepared for simultaneous determination of phenylhydrazine (PHZ) in the presence of hydrazine (HZ). The electrochemical response characteristics of the modified electrode toward PHZ and HZ were investigated by differential pulse voltammetry (DPV). The results showed an efficient catalytic role for the electro-oxidation of PHZ and HZ, leading to a remarkable peak resolution (~200 mV) for two compounds. The mechanism of the modified electrode was analyzed by monitoring the CVs at various potential sweep rates and pHs of the buffer solutions. Under the optimum conditions, the calibration curve for PHZ was obtained in the range of 7.0×10^{-8} to 9.0×10^{-4} M. The prepared modified electrode shows several advantages such as simple preparation method, high sensitivity, long-time stability, ease of preparation and regeneration of the electrode surface by simple polishing and excellent reproducibility. The proposed method was applied to determination of PHZ and HZ in urine and water samples and the obtained results were satisfactory.

Keywords: phenylhydrazine, hydrazine, carbon paste electrode, carbon nanotubes, ferrocenedicarboxylic acid.

1. INTRODUCTION

Carbon paste electrode (CPE) is a special kind of heterogeneous carbon electrode consisting of mixture prepared from carbon powder (as graphite, glassy carbon and others carbonaceous materials) and a suitable water-immiscible or non-conducting binder [1–3]. The use of carbon paste as an electrode was initially reported in 1958 by Adams [4]. In afterward researches a wide variety of modifiers including enzymes [5–8], polymers (nafion, chitosan, etc.) [9–13] and nanomaterials [14–16] have been used with these versatile electrodes. CPEs are widely applicable in both electrochemical studies and electroanalysis thank to their advantages such as very low background current (compared to solid graphite or noble metal electrodes), facility to prepare, low cost, large potential window, simple surface renewal process and easiness of miniaturization [17–19]. Besides the advantageous properties and characteristics listed before, the feasibility of incorporation different substances during the paste preparation (which resulting in the so-called modified carbon paste electrode), allow the fabrication of electrodes with desired composition, and hence, with pre-determined properties [20, 21]. Since the discovery of carbon nanotubes (CNTs) in 1991 [22], numerous investigations were focused on the studies of their properties and applications [23–25]. Because of the special tube structure, CNTs possess several unique properties such as good electrical conductivity, high chemical stability and extremely high mechanical strength [26, 27]. In addition, the subtle electronic behavior of CNTs reveals that they have the ability to promote electron-transfer reaction and have a high electrocatalytic effect when used as electrode materials [28, 29]. All these fascinating properties make CNTs as a suitable candidate for the modification of electrodes [30, 31].

The electrochemical methods using chemically modified electrodes (CMEs) have been widely used as sensitive and selective analytical methods for the detection of the trace amounts of biologically important compounds [32, 33]. One of the most important properties of CMEs has been their ability to catalyze the electrode process via significant decreasing of overpotential respect to unmodified electrode. With respect to relatively selective interaction of the electron mediator with the target analyte in a coordination fashion, these electrodes are capable to considerably enhance the selectivity in the electroanalytical methods [34, 35].

The hydrazines —which are extensively used in laboratory, industrial and therapeutical settings — are toxic and can cause irreversible cellular damage [36]. A variety of toxic effects of the hydrazines have been described, including autoimmune disturbances in humans [37], human leukemogenesis 38], alterations in the liver, kidney, central nervous system [36], hemolytic anemia [39], and cancer [40]. Phenylhydrazine (PHZ) intoxication leads to hemolysis resulting in severe hemolytic anemia and reticulocytosis [39], and to hepatic and splenic iron overload [41] causing a number of pathophysiological changes, e.g. fatty liver [42] and hepatocyte necrosis [43]. PHZ-induced free iron release [6], followed by free radical generation, is a likely mechanism of its toxicity. It is known, for example, that PHZ induces oxidative damage to hemoglobin [44], to membrane phospholipids and proteins in human erythrocytes [45] and it generates free radicals and reactive oxygen species [46]. PHZ is one of the most potent carcinogens belonging to the hydrazine family of molecules. Iron often plays a central role in oxidative stress since, once released from its binding molecules, it becomes free iron which acts locally [47], as well as diffusing to adjacent cells [48], where it generates free radicals that destroy essential macromolecules. In conditions of iron overload an increased risk of cancer, e.g. hepatocellular carcinoma [49], has been noted. Similarly, human and mouse colon cancer related to dietary iron overload [50, 51] have been reported. Hence simple, rapid, highly sensitive and accurate methods are required for the determination of trace amount of hydrazine derivatives in samples. Several methods have been reported for the determination of hydrazine and phenylhydrazine. These, include titrimetry [52], spectrophotometry [53] and kinetic methods [54]. Electrochemical methods provide useful alternatives since they allow faster, cheaper and safer analysis [55, 56].

To our knowledge, no study has reported the electrocatalytic and simultaneous determination of PHZ and HZ by using ferrocenedicarboxylic acid modified carbon nanotube paste electrode (FCDMCNPE). Thus, in the present work, we described initially the preparation and suitability of a FCDMCNPE as a new electrode in the electrocatalysis and determination of PHZ in an aqueous buffer solution and then we evaluated the analytical performance of the modified electrode in quantification of PHZ in the presence of HZ. Finally, in order to demonstrate the catalytic ability of the modified electrode in the electrooxidation of PHZ and HZ in real samples, we examined this method for the voltammetric determination of PHZ and HZ in urine and water samples.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

All the cyclic voltammetric measurements were performed using a BHP 2063$^+$ Electrochemical Analysis System, Behpajooh, Iran potentiostat/galvanostat coupled with a Pentium IV personal computer connected to a HP laser jet 6L printer.

An Ag / AgCl / KCl 3 M, a platinum wire, and a FCDMCNPE were used as reference, auxiliary and working electrodes, respectively. A digital pH/mV meter (Metrohm model 710) was applied for pH measurements. Graphite powder, paraffin oil and reagents were analytical grade from Merck. PHZ and HZ were purchased from Merck. Multi-walled carbon nanotubes (purity more than 95%) with o.d. between 10 and 20 nm, i.d. between 5 and 10 nm and tube length from 0.5 to 200 nm were prepared from Nanostruc-tured & Amorphous Materials (USA). The buffer solutions were prepared from ortho phosphoric acid and its salts in the pH range 2.0-12.0.

2.2. Preparation of the electrode

Modified carbon nanotube paste electrodes were prepared by dissolve 0.01 g of FCD in diethyl ether and hand mixing with 89-times its weight of graphite powder and 10-times its weight of carbon nanotube with a mortar and pestle. The solvent was evaporated by stirring. A 70:30 (w/w) mixture of FCD spiked carbon nanotube powder and paraffin oil was blended by hand mixing for 20 min until a uniformly-wetted paste was obtained. The paste was then packed into the end of a glass tube (*ca.* 3.4 mm *i.d.* and 10 cm long). Electrical contact was made by inserting a copper wire into the glass tube at the back of the mixture. When necessary, a new surface was obtained by pushing an excess of paste out of the tube and polishing it on a weighing paper. Unmodified carbon paste, prepared in the same way without adding FCD and carbon nanotube to the mixture and was used for comparison purposes. Also, a modified carbon paste disk served as the working electrode for RDE experiments. The body of the carbon paste-working electrode was a Teflon rod with a hole (2mm diameter, 5mm deep) bored at one end for electrode filling. Contact was made with a platinum wire through the center of the rod, which screwed to the RDE device.

3. RESULTS AND DISCUSSION

3.1. SEM characterization

For study surface of modified electrode we used SEM. Results show that at a surface of modified electrode mediator particle and carbon nanotubes packed and dispersed homogeneously (Fig. 1).

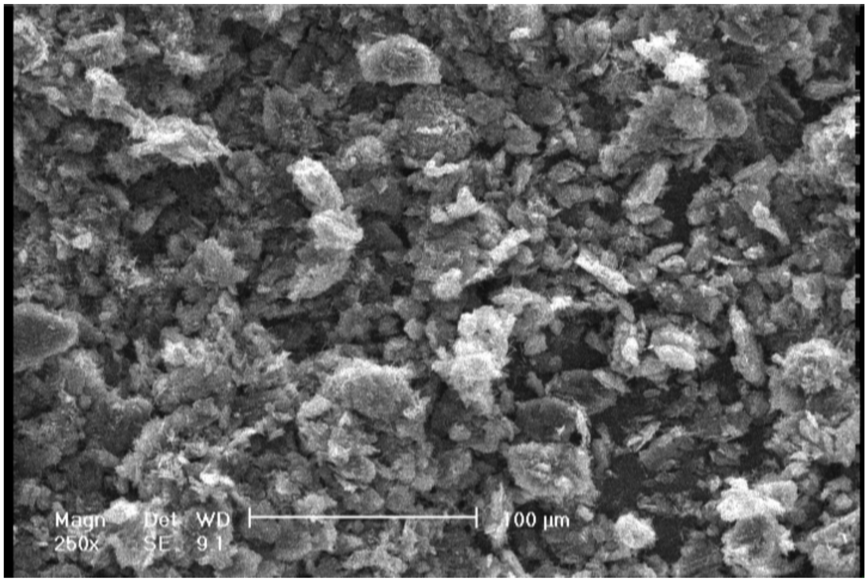

Figure 1. SEM image of FCDMCNPE

3.2. Electrochemical Behavior of FCDMCNPE

Cyclic voltammetry was employed for investigation the electrochemical properties of FCDMCNPE in a pure buffered aqueous solution (pH 5.0). The cyclic voltammogram exhibits an anodic and corresponding cathodic peaks with E_{pa}= 0.50 V and E_{pc}= 0.38 V vs. Ag / AgCl / KCl 3 M. The experimental results show well-defined and reproducible anodic and cathodic peaks related to Fc/Fc^+ redox couple with quasireversible behavior, because of 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 results from cyclic voltammetry of this modified electrode in various buffered solutions do not show any shift in the anodic and cathodic peak potentials. Therefore, the electrochemical behavior of the redox process of Fc/Fc^+ in FCDMCNPE is independent on the pH of aqueous solution.

The electrode capability for the generation of a reproducible surface was examined by cyclic voltammetric data obtained in optimum solution pH from five separately prepared FCDMCNPEs (Table 1). The calculated RSDs for various parameters accepted as the criteria for a satisfactory surface reproducibility (1 – 4%). This degree of reproducibility is virtually the same as that expected for the renewal or ordinary carbon paste surface.

Table 1. Cyclic voltammetric data obtained for constructed FCDMCNPE in 0.1 PBS (pH 5.0) at 20 mVs^{-1}.

E_{pa} (V) [a]	E_{pc} (V) [a]	$E_{1/2}$ (V) [a]	ΔE_{pa} (V) [a]	I_{pa} (μA)	I_{pc} (μA)	Γ_a (mol cm^{-2})	Γ_c (mol cm^{-2})
0.50 (0.68) [b]	0.38 (0.76) [b]	0.44 (0.62) [b]	0.12 (0.73) [b]	1.91 (1.94) [b]	0.81 (1.86) [b]	2.37×10^{-9} (2.92) [b]	2.13×10^{-9} (3.17) [b]

[a] Versus Ag / AgCl / KCl 3 M as reference electrode
[b] The values in paranthesis indicate the calculated RSD

In addition, the long term stability of the FCDMCNPE was tested over a three-week period. The cyclic voltammetry of PHZ at the surface of FCDMCNPE after the modified electrode was stored in an atmosphere at room temperature shows the oxidation peak potential of PHZ was unchanged and the anodic peak current was only decreased less than 1.81 % of the initial oxidation peak current. The antifouling properties of modified electrode toward PHZ and its oxidation product were investigated by recording the cyclic voltammograms of this modified electrode before and after using in the presence of PHZ.

The cyclic voltammetry of PHZ at the surface of FCDMCNPE after 15 repetition cycles at a scan rate 20 mVs^{-1} shows the oxidation peak potential of PHZ was not changed and the anodic peak current was decreased by less than 2.6 %. However we regenerated the surface of FCDMCNPE before each experiment.

3.3. pH Optimization of the Solution

The electrochemical behavior of PHZ is dependent on the pH value of the aqueous solution, whereas the electrochemical properties of Fc/Fc^+ redox couple are independent on pH. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of PHZ. Thus we studied the electrochemical behavior of PHZ in 0.1 M phosphate buffered solutions in different pH values ($2.0< pH<12.0$) at the surface of FCDMCNPE by cyclic voltammetry. It was found that the electrocatalytic oxidation of PHZ at the surface of FCDMCNPE was more favored under acidic conditions than in neutral medium. This appears as a gradual growth in the anodic peak current and a simultaneous decrease in the cathodic peak current in the cyclic voltammograms drawn at the surface of FCDMCNPE. The results show the anodic peak potential of PHZ at the surface of FCDMCNPE was shifted to a less-positive potential. Also, the anodic peak current and the shifted potential value for electrooxidation of PHZ are high at an acidic pH. Thus, the pH 5.0 was chosen as the optimum pH for electrocatalysis of PHZ oxidation at the surface of FCDMCNPE.

3.4. Electrochemistry of PHZ at FCDMCNPE

Fig. 2 depicts the cyclic voltammetric responses from the electrochemical oxidation of 350 µM PHZ at FCDMCNPE (curve f), FCD modified CPE (FCMCPE) (curve e), CNPE (curve d) and bare CPE (curve a).

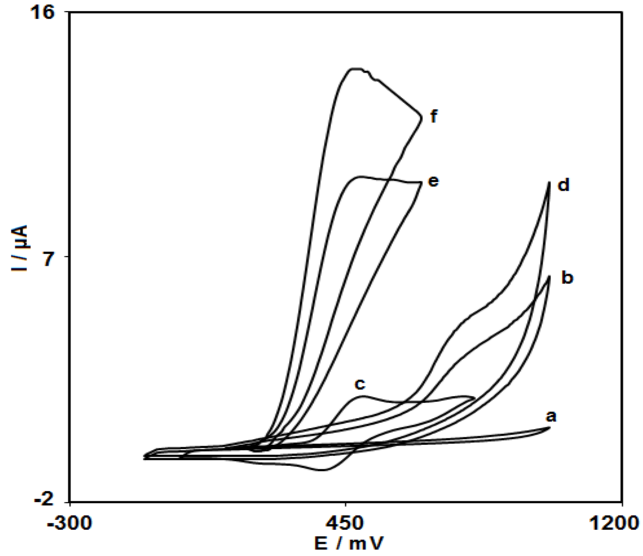

Figure 2. Cyclic voltammograms of (a) CPE in 0.1M PBS (pH 5.0) at scan rate 10 mV s^{-1} and (b) as (a) + 350 µM PHZ; (c) as (a) and (d) as (b) at the surface of FCDMCNPE and CNPE respectively. Also, (e) and (f) as (b) at the surface of FCDMCPE and FCDMCNPE respectively.

As can be seen, the anodic peak potential for the oxidation of PHZ at FCDMCNPE (curve f) and FCDMCPE (curve e) is about 500 mV, while at the CNPE (curve d) peak potential is about 800 mV, and at the bare CPE (curve b) peak potential is about 845 mV for PHZ. From these results it is concluded that the best electrocatalytic effect for PHZ oxidation is observed at FCDMCNPE (curve f). For example, the results are shown that the peak potential of PHZ oxidation at FCDMCNPE (curve f) shifted by about 300 and 345 mV toward the negative values compared with that at a CNPE (curve d) and bare CPE (curve b), respectively. Similarly, when we compared the oxidation of PHZ at the FCDMCPE (curve e) and FCDMCNPE (curve f); there is a dramatic enhancement of the anodic peak current at FCDMCNPE relative to the value obtained at the FCDMCNPE. In the other words, the data obtained clearly show that the combination of carbon nanotube and mediator (FCD) definitely improve the characteristics of PHZ oxidation. The FCDMCNPE in 0.1M phosphate buffer (pH 5.0), without PHZ in solution, exhibits a well-behaved redox reaction (curve c) upon the addition of 350 µM PHZ, the anodic peak current of mediator was greatly increased, while the corresponding cathodic peak was disappear on the reverse scan of the potential (curve f). This behavior is typical of that expected for electrocatalysis at chemically modified electrodes [57].

The effect of scan rate on the electrocatalytic oxidation of 350 μM PHZ at the FCDMCNPE was investigated by liner sweep voltammetry (Fig. 3).

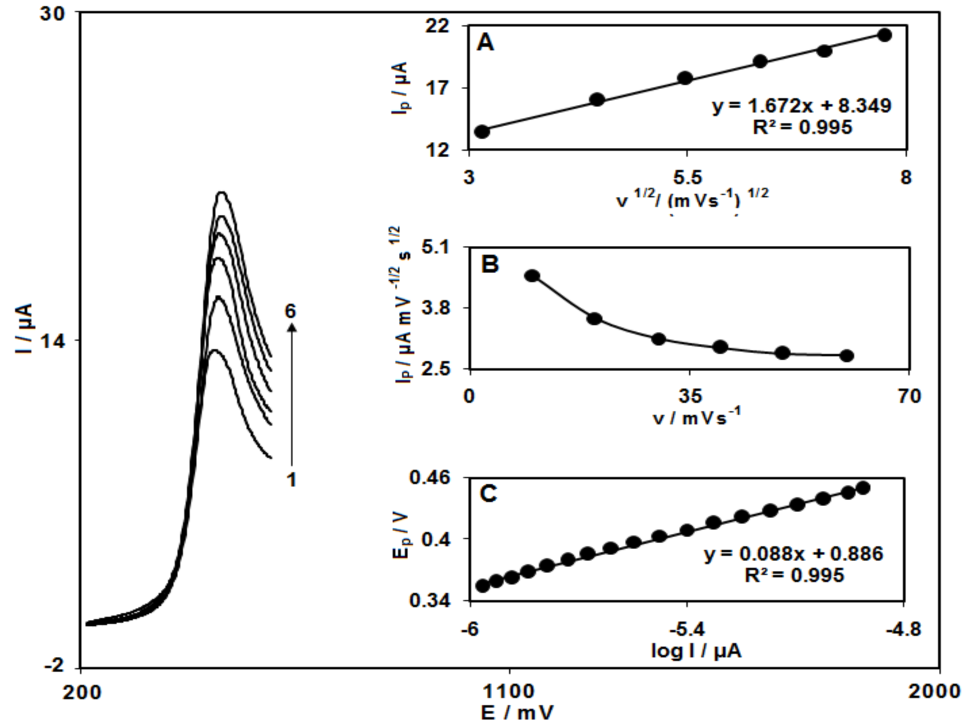

Figure 3. Linear sweep voltammograms of the FCDMCNPE in the presence of 350 μM PHZ at various scan rates; The numbers 1-6 correspond to 10, 20, 30, 40, 50, and 60 mVs^{-1} scan rates, respectively Insets: (A) The variation of the anodic peak currents vs. $v^{1/2}$. (B) Variation of the scan rate- normalized current ($I_p/v^{1/2}$) with scan rate (C) Tafel plot derived from the rising part of voltammogram recorded at a scan rate 10 mVs^{-1}

The oxidation peak potential shifts with increasing scan rates towards a more positive potential, confirming the kinetic limitation of the electrochemical reaction. Also, a plot of peak height (I_p) against square root of scan rate ($v^{1/2}$), in range of 10-60 mVs^{-1}, was constructed (Fig. 3.A), which was found to be linear, suggesting that at sufficient overpotential the process is diffusion rather than surface controlled. A plot of the sweep rate normalized current ($I_p/v^{1/2}$) versus sweep rate (Fig. 3.B) exhibits the characteristic shape typical of an EC_{cat} process [57].

Fig. 3.C, shows a Tafel plot that was drawn from data of the rising part of the current–voltage curve recorded at a scan rate of 10 mVs^{-1}. This part of voltammogram, known as Tafel region [39], is affected by electron transfer kinetics between PHZ and FCD, assuming the deprotonation of substrate as a sufficiently fast step. In this condition, the number of electron involved in the rate determining step can be estimated from the slope of Tafel plot. A slope 0.088Vdecade^{-1} is obtained indicating a one electron transfer to be rate limiting assuming a transfer coefficient of $\alpha = 0.33$.

3.5. Chronoamperometric measurements

The chronoamperometry as well as the other electrochemical methods was employed for the investigation of electrode processes at chemically modified electrodes. Chronoamperometric measurements of PHZ at FCDMCNPE were done by setting the working electrode potential at 600 mV for various concentrations of PHZ (Fig. 4).

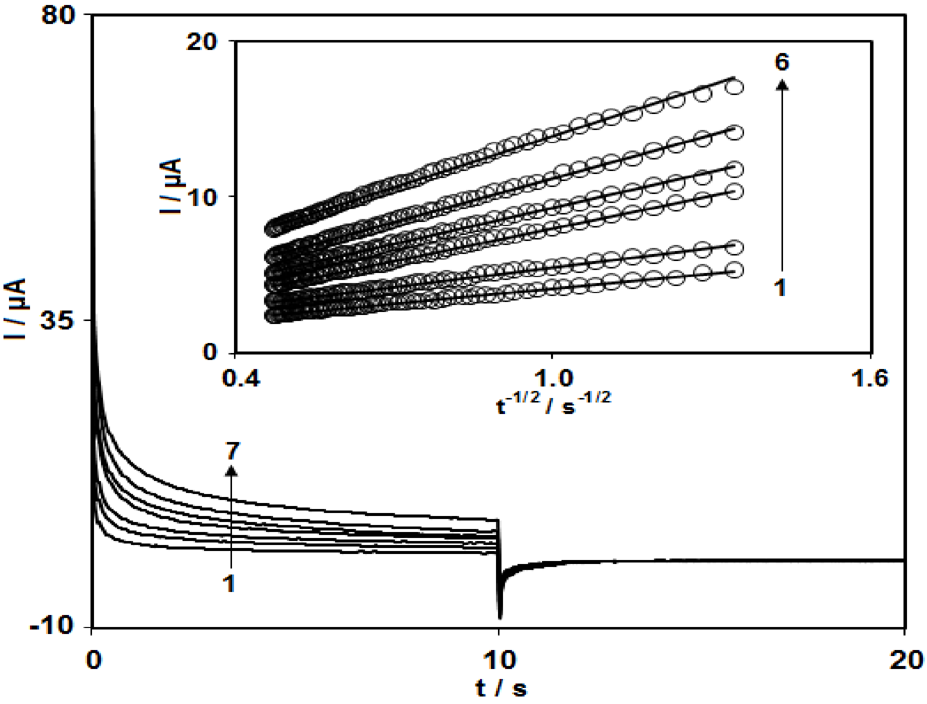

Figure 4. (A) Chronoamperograms obtained at FCDMCNPE in 0.1M phosphate buffer solution (pH 5.0) for different concentration of PHZ. The numbers 1–7 correspond to 0.0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 mM of PHZ. Inset: plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 2–7

For an electroactive material (PHZ in this case) with a diffusion coefficient of D, the current for the electrochemical reaction (at a mass transport limited rate) is described by the Cottrell equation [39]. Under diffusion control, a plot of I versus $t^{-1/2}$ will be linear, and from the slope the value of D can be obtained (Fig. 4, inset). The mean value of the D was found to be 5.23×10^{-6}cm^2/s.

Also, chronoamperometry can also be employed to evaluate the catalytic rate constant, k, for the reaction between PHZ and the FCDMCNPE according to the method of Galus [58]:

$$I_C / I_L = \pi^{1/2}\gamma^{1/2} = \pi^{1/2}(kC_bt)^{1/2} \qquad (1)$$

where t is the time elapsed and C_b is the bulk concentration of PHZ. The above equation can be used to calculate the rate constant of the catalytic process k. Based on the slope of the I_C / I_L versus $t^{1/2}$ plot; k can be obtained for a given PHZ concentration. Such plots were obtained from the chronoamperograms in Fig. 4 (Not shown). From the values of the slopes an average value of k was found to be k = 4.08×10^{3} $M^{-1}s^{-1}$.

The value of k explains as well as the sharp feature of the catalytic peak observed for catalytic oxidation of PHZ at the surface of FCDMCNPE. Finally, the heterogeneous rate constant (k′) of catalytic reaction was calculated as k′=9.67×10^{-3} cms^{-1}.

3.6. Calibration plot and limit of detection

Differential pulse voltammetry (DPV) was used to determine the concentration of PHZ. Voltammograms clearly show that the plot of peak current versus PHZ concentration is constituted of two linear segments with different slopes (slope: 0.142μA.μM^{-1} for first linear segment and 0.016 μA.μM^{-1} for second linear segment), corresponding to two different ranges of substrate concentration, 0.07 to 30.0 μM for first linear segment and 30.0 to 900.0 μM for second linear segment.

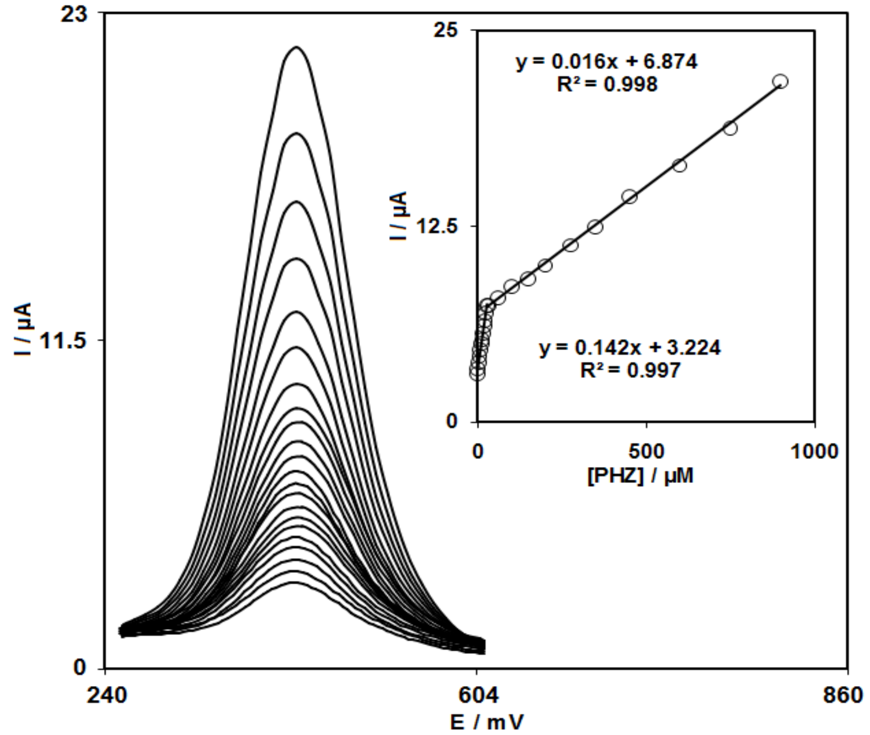

Figure 5. Differential pulse voltammograms of the FCDMCNPE in 0.1 M PBS (pH 5.0) containing different concentrations of PHZ, from inner to outer correspond to 0.07, 1.5, 4, 7, 9, 12, 15, 17, 20, 23, 26, 30, 60, 100, 150, 200, 275, 350, 450, 600, 750 and 900 μM of PHZ. Inset: Plot of the electrocatalytic peak current as a function of PHZ concentration in the range of 0.07 to 900 μM

The decrease of sensitivity (slope) in the second linear range is likely to be due to kinetic limitation. The detection limit (3σ) for PHZ in the lower range region was found to be 40 nM.

3.7. Simultaneous determination of PHZ and HZ at FCDMCNPE

One of the main objectives of the present study was the development of a modified electrode capable of the electro-catalytic oxidation of PHZ and separation of the electrochemical responses of PHZ and HZ. Using FCDMCNPE as the working electrode, the analytical experiments were carried out by varying the HZ concentration in the presence of 800 µM PHZ in 0.1M phosphate buffer (pH 5.0) and slope was 0.03 µA.µM^{-1} (Fig. 6). Results show that, an increase in the peak current of HZ is observed with increasing HZ concentration and the voltammetric peak of PHZ is almost unchanged during the oxidation of HZ.

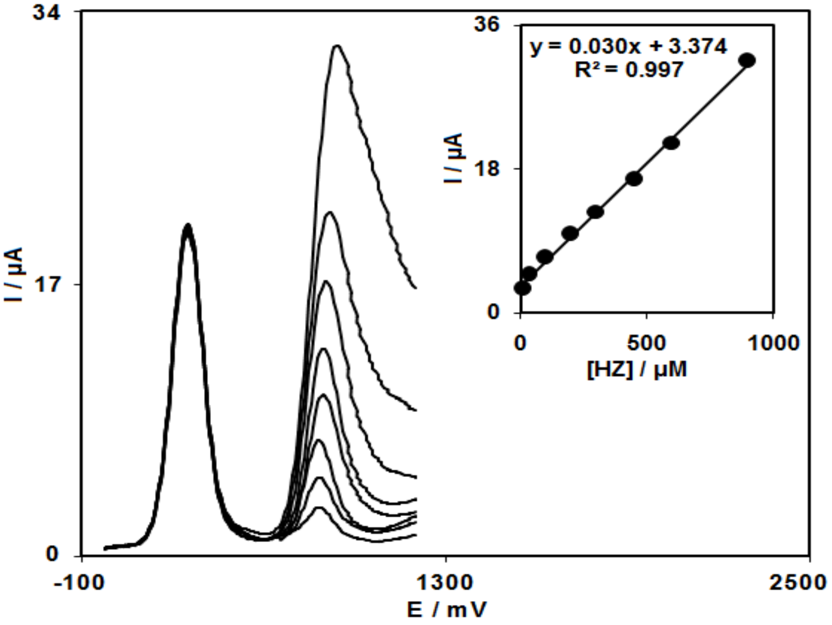

Figure 6. Differential pulse voltammograms of FCDMCNPE in 0.1M phosphate buffer solution (pH 5.0) containing 800 µM PHZ and different concentrations of HZ (from inner to outer): 10, 30, 100, 200, 300, 450, 600 and 900 mM. Inset: plot of the electrocatalytic peak current as a function of HZ concentration.

The utilization of the FCDMCNPE for the simultaneous determination of PHZ and FA was demonstrated by simultaneously changing the concentrations of PHZ and HZ. The DP voltammetric results show two-well-defined anodic peaks with a 200 mV separation of both peaks (Fig. 7), while the bare carbon paste electrode only gave an overlapped and broad oxidation peak. Fig. 7 insets A and B show the dependence of DPV peak currents on the concentration of PHZ and HZ respectively. The sensitivity towards PHZ in the absence and presence of HZ was found to be 0.016 (absence of HZ) and 0.016 (presence of PHZ) µA.µM^{-1}. It is very interesting to note that the sensitivities of the modified electrode towards PHZ in the absence and presence of HZ, are virtually the same, which indicate the fact that the oxidation processes of PHZ and HZ, at the FCDMCNPE are independent and therefore simultaneous or independent measurements of the two analytes are possible without any interference. If the PHZ signal was affected by the HZ, the above-mentioned slopes would be different.

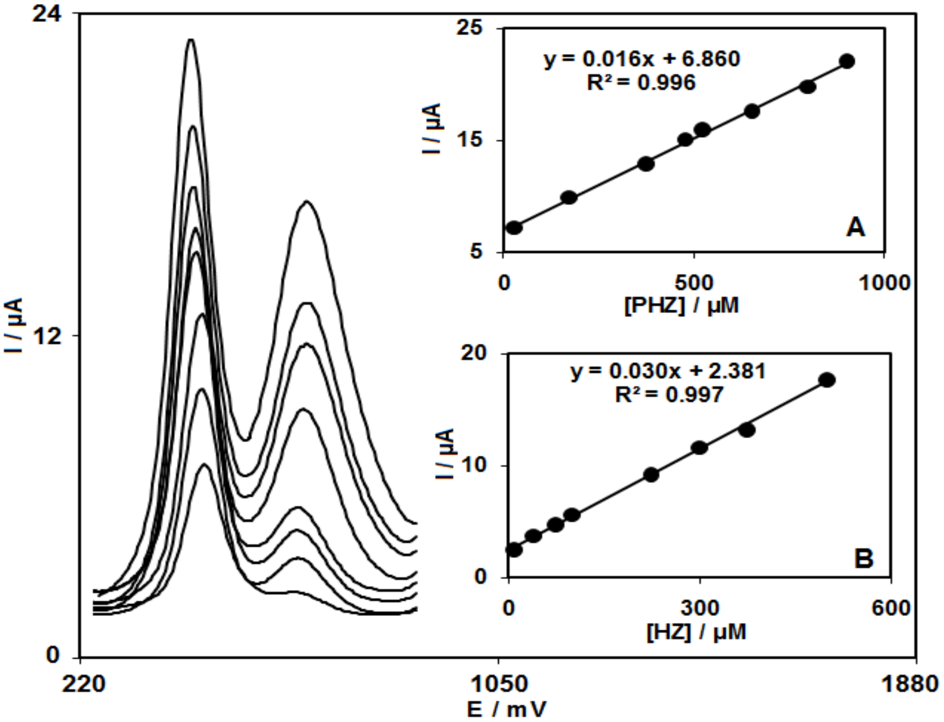

Figure 7. Differential pulse voltammograms of FCDMCNPE in 0.1M PBS (pH 5.0) containing different concentrations of PHZ and HZ (from inner to outer) mixed solutions of 30+10, 175+40, 375+75, 475+100, 525+225, 650+300, 800+375 and 900+500respectively, in which the first value is concentration of PHZ in µM and the second value is concentration of HZ in µM. Insets: (A) and (B) Plots of the peak currents as a function of PHZ and HZ concentrations respectively.

3.8. Rotating disk electrode (RDE) voltammetry

To our knowledge, no paper has used rotating disk electrode (RDE) voltammetry technique for PHZ with carbon paste and specially carbon nanotube paste electrodes and in this paper, we used this technique for the first time by this kind of electrodes for PHZ. Thus, the electrocatalytic activity of FCDMCNPE toward oxidation of PHZ was also evaluated by RDE voltammetry technique. The steady-state I–E curves were recorded for the oxidation of PHZ at EBNBHCNPE under various experimental conditions. A typical example of the I–E curves (RDE voltammograms) at rotation speed range 500–2500 rpm is shown in Fig. 8. A for 0.5 mM solution of PHZ.

In the case that the oxidation of PHZ at the surface FCDMCNPE is controlled solely by the mass transfer process in the solution, the relationship between the limiting current and rotating speed should obey the Levich equation [57]:

$$I_l = I_{Lev} = 0.620nFAD^{2/3}v^{-1/6} W^{1/2}C \quad (2)$$

where D, *v*, *W* and C are the diffusion coefficient, the kinematics viscosity, the rotation speed and the bulk concentration of the reactant in the solution, respectively, and all other parameters have their conventional meanings. Based on Eq. (2), the plot of limiting current I_l as function of the $W^{1/2}$ should be a straight line.

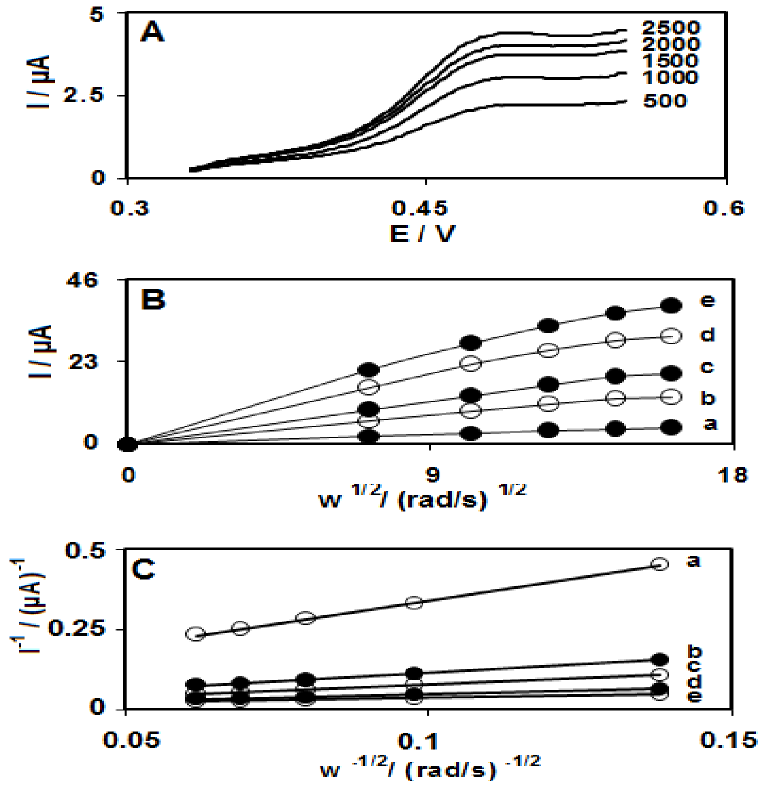

Figure 8. (A) Voltammograms of rotating disk FCDMCNPE in 0.1 M phosphate buffer (pH 5.0) containing 0.5 mM PHZ at the various rotation rates indicated for each voltammogram. Scan rate: 10 mV s^{-1}. (B) Levich plots constructed from the modified RDE voltammograms of solution with (a) 0.5, (b) 1.5, (c) 2.5, (d) 3.5 and (f) 4.5 mM PHZ. (C) Koutecky–Levich plot obtained from Levich plots shown in A.

According to the Levich plots (Fig. 8. B), the current increases with increasing electrode rotation speed, but were found to be nonlinear, including kinetic limitations. For irreversible reaction, relation between the limiting current and rotating speed has been given by Koutecky–Levich equation [57]:

$$[I_l]^{-1} = [nFACK\Gamma]^{-1} + [0.620nFAD^{2/3}v^{-1/6} CW^{1/2}]^{-1} \quad (3)$$

where A, C, K, A, D, v and W are the electrode area (cm^2), substrate concentration (mol cm^{-3}), catalytic rate constant (cm^3 mol^{-1} s^{-1}), surface coverage (mol cm^{-2}), diffusion coefficient (cm^2 s^{-1}), kinematics viscosity (cm^2 s^{-1}) and rotation speed (rad s^{-1}), respectively. It can be seen that the intercepts of all linear plots are positive, clearly indicating the kinetic limitation of the electrode process. In addition, the slopes and intercepts are inversely proportional to the bulk concentration of PHZ, suggesting that the current is not limited by the rate of electron transport within the electrode. According to Eq. (3) the plot of $[I_l]^{-1}$ vs. $W^{-1/2}$ gives a straight line (Fig. 8. C). The rate-determining step must be catalytic process at electrode surface or electron diffusion process within the electrode. The value of the rate constant for the catalytic reaction K can be obtained from the intercept of the Koutecky– Levich plot. The value of heterogen rate constant for FCDMCNPE by using intercepts of the Koutecky–Levich was found to be 9.41×10^{-3} cm s^{-1} for PHZ concentration in the range 0.5 – 4.5 mM. Diffusion coefficient of PHZ ,D, may be obtained from the slop of Koutecky– Levich plots. The mean value of D was found to be 5.65×10^{-6} cm^2 s^{-1}.

3.9. Determination of PHZ and HZ in urine and water samples

To evaluate the applicability of the proposed method to real samples, it was applied to the determination of PHZ and HZ in human urine and water samples. The samples tested were found to be free from PHZ and, thus, synthetic samples were prepared by adding known amounts of PHZ and HZ to the urine and water samples.

The results for determination of the two species in real samples are given in Table 2. Satisfactory recovery of the experimental results was found for PHZ and HZ. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

4. CONCLUSIONS

The results obtained in this work demonstrated the potentiality of the FCDMCNPE for simultaneous determination of PHZ and HZ. The modified electrode exhibits highly electrocatalytic activity for the oxidation of PHZ and HZ associated with negative shifts in anodic peak potentials. Thus, large peak separations obtained with this electrode allow it to simultaneously detect these compounds. Moreover, good sensitivity, high selectivity, low detection limits with the low cost of the sensor, makes this method very suitable for accurate determinations in real samples. The proposed method could be applied to the determination of PHZ and HZ in urine and water samples with satisfactory results.

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