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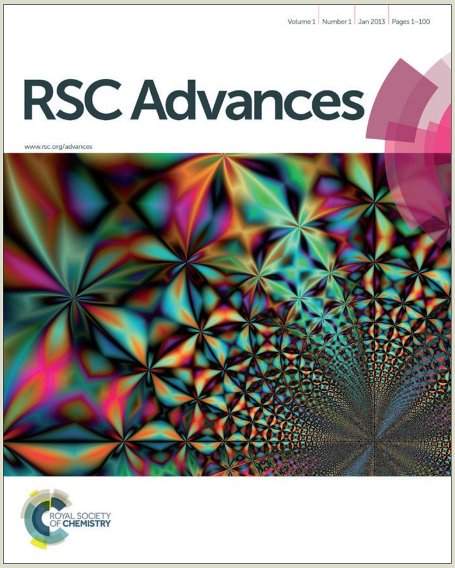

ROYAL SOCIETY OF CHEMISTRY

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Journal Name

ARTICLE

Highly Sensitive Determination of Perphenazine on a Carbon Nanocompsite Ionic Liquid Electrode

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

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Abstract Carbon nanomaterial-ionic liquid nanocomposites have proposed impressive class of materials expressly for electrochemical applications. In this work the electrocatalytic oxidation of perphenazine (PPZ) was studied at a carbon nanocomposite electrode, fabricated using multi-walled carbon nanotubes (MWCNT) and the ionic liquid 1-octylpyridinium hexaflourophosphate (OPFP). The surface of the proposed electrode was characterized by scanning electron microscopy. Adsorptive stripping voltammetry (AdSV) was applied as a highly sensitive method for quantification of sub-micromolar amounts of PPZ. Various parameters were optimized for practical applications. It was found that the electrode showed sensitive voltammetric response to PPZ. Cyclic voltammetery (CV) was also applied to obtain information about the reaction mechanism and calculating kinetic parameters. The oxidation was irreversible and exhibited adsorption controlled behavior. The electron transfer coefficient (α) value was found 0.57 from the slope of Tafel plot.The anodic peak current was linear to PPZ concentration in the ranges of 5.0×10^{-8} to 3.0×10^{-5} M and 3×10^{-5} to 1.5×10^{-4} M, with correlation coefficients of 0.9987 and 09917, respectively. The detection limit was 2.3×10^{-8} M. Also for the amperometric procedure the liner dynamic range of 1.0×10^{-5} to 1.2×10^{-4} M with detection limit of 2.1×10^{-6} M was obtained. The method was successfully applied for determination of PPZ content in pharmaceuticals and blood serum samples.

Introduction

More than half century ago the neuroleptic properties of some drugs were discovered. This discovery caused further investigation to clarify the antipsychotic drugs.[1] Phenotiazines are traditional antipsychotics used to treat mental disorders. Their derivatives are characterized by tricyclic rings with sulfur and nitrogen atoms at position 5 and 10. They often affect by blocking dopamine D_2 receptors.[1,2] Perphenazine which is chemically known as 4-[3-(2-chlorophenotiazine-10-yl) propyl] -1- piperazine-ethanol belongs to the phenothiazines family. It is usually prescribed for the treatment of psychotic symptoms such as schizophrenia, Parkinson's disease and schizoaffective psychoses in order to decrease restlessness, aggressiveness and impulsive behavior in psychotic patients.[3,4] PPZ is extensively metabolized in the liver to a number of metabolites by sulfoxidation, hydroxylation, dealkylation and glucuronidation.[5,6] Therefore, the determination of PPZ is important in medical screening, clinical diagnostics, and pharmaceutical industry for obtaining optimum therapeutic dosage in body fluids to minimize the risk of toxicity. Some efforts have been over the last several decades to develop a simple, reliable and sensitive method for determination of PPZ in pharmaceuticals and biological samples. Thought the good selectivity of high performance liquid chromatography[7-11] and gas chromatography-mass spectroscopy[12-14] methods, costly apparatus are needed and experiments conditions are greatly affected by environmental factors. Spectrophotometric methods has also been applied to determine PPZ, however the sensitivity of these methods are poor.[15,16] In addition, chemiluminescence method developed for this proposes lacks the selectivity.[17]

PPZ is an electroactive molecule and electrochemical methods that are simple, sensitive, inexpensive and fast can be applied for determination of PPZ. To the best of our knowledge only a few electrochemical methods have been reported. A glassy cabon electrode using methylene blue as mediator was employed to study and sense the elextrocatalytic oxidation of pherphenazine.[18] Graphene oxide (GO), is a low-dimensional carbon material which is extensively used for biosensing of various compounds.[19,20] was applied for modification of a glassy carbon electrode and PPZ determined in the millimolar range.[21]

Carbon ionic liquid electrode (CILE) was introduced in 2006 for the first time as a new and high performance carbon composite electrode.[22] The main idea for fabrication of this new electrode was the replacement of conventional nonconductive organic binders in carbon paste electrodes (CPEs) with a pyridinium-based ionic liquid. Some interesting features of CILE include wide potential window in aqueous solutions, low background current, renewable surface,

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resistivity toward bio-molecules fouling and a rapid electron transfer.[23]

Carbon nanotubes (CNTs) have promoted a large number of significant applications in electroanalytical chemistry, including electrochemical sensors that are reported in the literature.[24-27] CNT/IL composites have also been explored for the modification of the surface of the electrodes by drop casting[28] or layer by layer self-assembly.[29] CNTs are also incorporated into IL modified CPEs which remarkably improves the electrochemical responses.[30,31]

In this work we have employed the unique properties of multi-walled carbon nanotube modified CILE for fabrication of a PPZ electrochemical sensor. Adsorptive stripping voltammetry as a very sensitive analytical method was used. The results illustrated that the nanocomposite electrode exhibits excellent sensitivity, selectivity, stability and very low background currents for determination of PPZ.

Experimental

Reagents and chemicals

Graphite powder, ascorbic acid, uric acid, catechol, potassium dihydrogen phosphate, and dipotassium hydrogen phosphate were purchased from Merck and were used as received. Multi-walled carbon nanotube with a 95% purity, o.d.=6-9 nm and 5 µm length were obtain from Sigma-Aldrich. Perphenazine was kindly supplied by Darou Pakhsh Pharmaceutical Company (Tehran, Iran) and used without prior purification. The ionic liquid, 1-octylpyridinium hexafluorophosphate, was synthesized as described elsewhere.[32] OPFP was obtained by anion exchange of octylpyridinium iodide with ammonium hexafluorophospate. A 1.00×10^{-2} M stock solution of drug was prepared by dissolving an appropriate amount of PPZ in methanol (Merck) and stored at 4 °C. Standard solutions were prepared using this stock solution. Phosphate buffer (PBS) 0.1M, pH 2.0 was used as supporting electrolyte. All solutions were freshly prepared with double distilled water. The drug-free serum samples were kindly supplied by Blood Transfusion Organization (Fars, Iran) and were belonging to the healthy male volunteers. The serum samples were stored frozen until the assay.

Electrode Preparation

Carbon ionic liquid electrode (CILE) was fabricated by thoroughly hand-mixing the graphite powder and OPFP with a ratio of 50/50 (w/w) in a mortar and pestle, followed by packing the resulting paste firmly into the electrode cavity (1.8 mm i.d.) of a Teflon holder. In order to have better homogeneity in the composite and to lower background current, the electrode was heated for 2 min in oven, to a temperature above the melting point of IL (m.p. ~ 65 °C) prior to use.[33] A copper wire inserted into the carbon paste provided the electrical contact. Before each measurement, pushing an excess of paste out of the tube and then polishing the freshly exposed paste with weighing paper obtained a new surface. The carbon nanotube ionic liquid nanocomposite electrode (MWCNT/CLE) was prepared in similar way with the weighted amounts of graphite powder, ionic liquid and multi walled carbon nanotube (40%:50%:10%, wt %), respectively. The Multi-walled carbon nanotube paste electrode (MWCNT/CPE) was prepared by mixing 60% graphite powder, 30% mineral oil and 10% MWCNT. Classical carbon paste electrode (CPE) was made in the same way but without adding MWCNTs to the mixture.

Apparatus

Voltammetric measurements were performed using an Autolab electrochemical system (Eco-Chemie, Utrecht, The Netherlands) equipped with Autolab PGSTAT-302N, GPES software (Eco-Chemie, Utrecht, The Netherlands). The electrochemical cell was assembled with a conventional three electrode system: an Ag/AgCl/ KCl (3M) reference electrode (Metrohm) and a platinum disk as a counter electrode. The working electrodes used in this study were CILE, MWCNT/CILE, MWCNT/CPE and CPE. The cell was one compartment cell with an internal volume of 100mL. All experiments were typically conducted at 25 °C without removing the dissolved oxygen. Scanning electron microscopy (SEM) images were obtained by using a HITACHI S-4160 field emission electronic microscopy (Japan). The FTIR studies were performed using Perkin Elmer FT-IR spectrometer spectrum RX-1.

Results and discussion

To make more insight into structural characterization of the material composite FTIR spectra of the graphite powder, MWCNT, IL and the nanocomposite (graphite/MWCNT/IL) were investigated and the results have been shown in Figure S-1. The peaks appeared at 2930 cm^{-1} (aliphatic C-H stretching), 1660 cm^{-1} (stretching vibration of C=N), 1490 cm^{-1} (in-plane bending vibration of $-CH_3$, $-CH_2-$)[34,39] and 830 cm^{-1} (PF_6^-)[38] keep the same with the literature data showing the synthesis of OPFP ionic liquid. Because MWCNTs do not support a static dipole moment, a relatively weak dynamic dipole moment is responsible for the observation of infrared-active modes of MWCNTs. Thus the characteristic IR absorption peaks of MWCNTs and also graphite are very weak[38]. Therefore, the observed spectra of the composite came from only the IL. . The strong band at 833 cm^{-1} is assigned to PF_6^- anion can be seen in both IL and the composite spectra.[38]

In order to characterize and investigate the nature of MWCNT/CILE, scanning electron microscopy (SEM) images were taken from this composite together with three different pastes CPE, MWCNT/CPE and CILE. As shown in Fig. 1 the electrodes using OPFP (C,D) as binder have clearly different morphology compared to those used mineral oil as binder which has also been reported previously by other researchers.[22,33] The electrodes containing OPFP displayed uniform surface morphology and exclusive structure, indicating that IL could fill well into the space between graphite particles or CNTs.

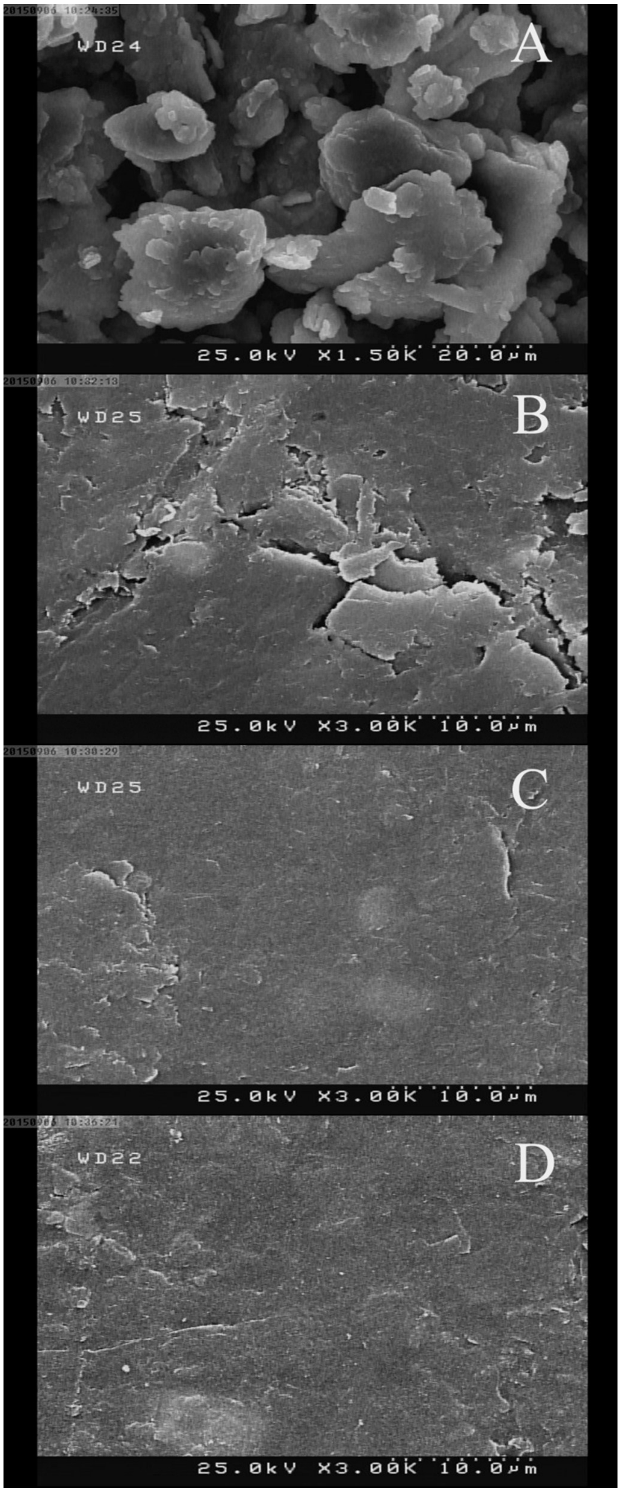


Fig. 1. Scanning electron microscopy of CPE (A), MWCNT/CPE (B), CILE (C) and MWCNT/CILE (D) surface.

The interesting behavior of MWCNT and OPFP toward oxidation of PPZ has been illustrated in Fig. 2. To elucidate the properties of the MWCNT/CILE it was essential to compare it with other electrodes. Fig. 2 shows typical cyclic voltammograms of CPE (curve a), MWCNT/CPE (b), CILE (c) and MWCNT/CILE (d) in PBS (0.1 M pH 2.0), containing 100 µM PPZ. In the absence of PPZ, no obvious peak (inset of Fig.2) was observed during the potential scan. After the addition of PPZ, three oxidation peaks appeared at about +0.73 V (peak I), +0.95 V (peak II) and +1.09V (Peak III) on all electrodes, respectively. No cathodic peak was observed on the reverse scan. The oxidation current of PPZ on both CILE and MWCNT/CILE were much higher than CPE. This result clearly shows the significant effect of IL that used as binder in both CILE and MWCNT/CILE and also increased the sensitivity of these electrodes toward oxidation of PPZ. It is interesting to note that an increase in faradic currents at CILE and MWCNT/CILE in comparison with classic CPE and MWCNT/CILE is caused by ionic conductivity of the binder (IL) which leads to a larger electroactive area. Before electron transfer occurs, some fraction of polar reactant is transferred across IL/aqueous electrolyte and electrode reaction takes place at carbon/IL interface within CILE body. This is not possible at classic CPE with nonpolar binder, where the electrode reaction occurs at the surface of carbon particles next to the aqueous electrolyte.[35]

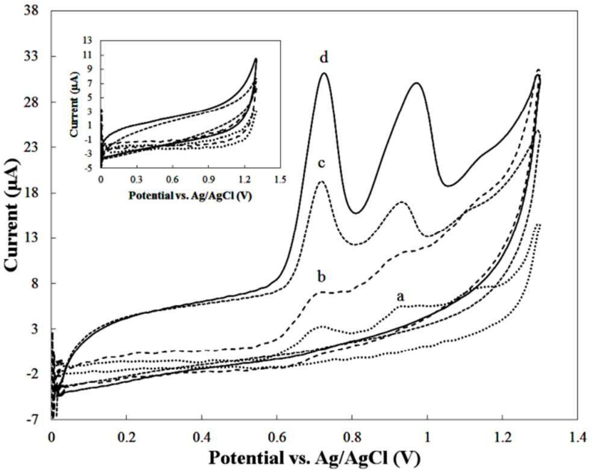
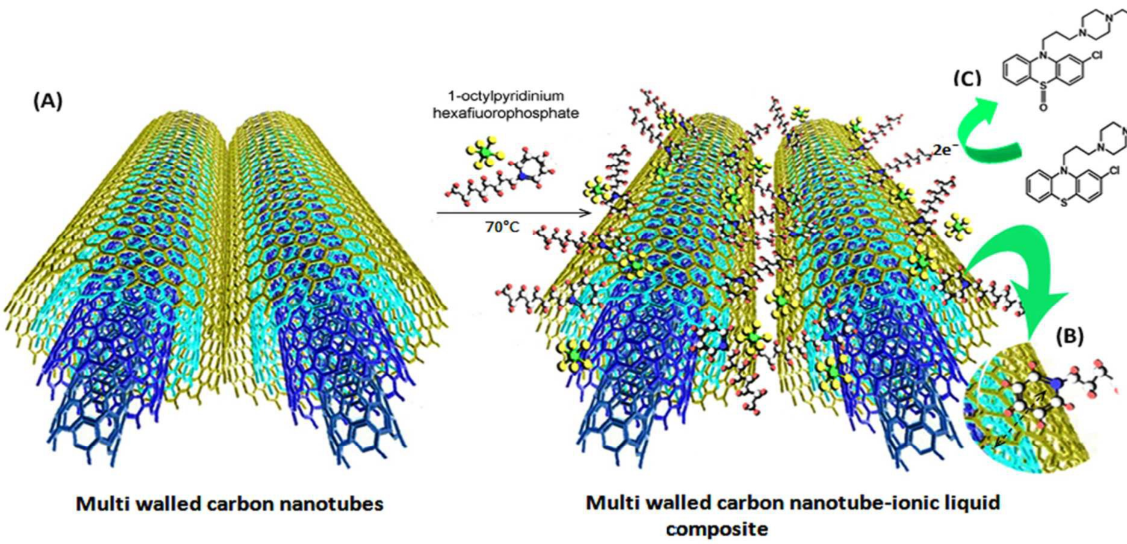


Fig. 2. Cyclic voltammograms of different electrodes in PBS with pH 2 at a scan rate of 50mVs^{-1}; using CPE (a) MWCNT/CPE (b) CILE (c) and MWCNT/CILE (d) in the presence of 100µM PPZ. Inset shows the voltammograms of corresponding electrodes in the absence of PPZ.

The use of carbon nanotubes had been confined by difficulty in handling due to their propensity to agglomerate.[36] In this regard, OPFP appeared as a suitable material for dispersion of carbon nanotubes.[37] Scheme 1 demonstrates the molecular view of fabricated nanocomposite. The strong π-π interaction between carbon nanotubes is shielded by the ionic liquid, which ultimately hinders rebundling of nanotubes.[37] A possible "cation-π" interaction between the surface of MWCNTs and the cation of ionic liquid[37] (Scheme 1B) or weak van der Waals interaction[38] are known as the reason for dispersing nanotubes by ILs such as OPFP. Therefore, the exclusive performance of



Scheme 1. Schematic representation of the dispersion process for MWCNTs in OPFP (A), the "cation-π" interaction between the surface of MWCNTs and the cation of ionic liquid. (B) and oxidation of PPZ on the nanocomposite surface (C).

the MWCNT/CILE toward oxidation of PPZ is not only because of the MWCNTs but also for the mixture of MWCNT and IL OPFP.

Consequently, this electrode was selected for further studies.

The influence of the MWCNT amounts on the voltammetric responses of the MWCNT/CILE was evaluated. Four electrodes containing different amounts of MWCNT (1, 5, 10 and 25% weight percent ratio) were prepared and examined under identical conditions. Increasing the amount of MWCNTs up to 10% caused an improvement of PPZ accessibility to the electrode surface active sites, therefore the oxidation peak currents were obviously improved. However composites with higher amounts of MWCNTs were unstable in solution and increased the background current. This can be due to low mechanical stability of the composite when it contains proportionally more nanotubes compared to the IL which results the increase in surface area of composite and increase in double layer capacitance. By decreasing the amount of MWCNT and having more of the binder, better filling between nanotubes was achieved which caused higher mechanical stability and lower background currents.[33]

Accumulation step is mainly a simple and effective way to enhance the sensitivity of the determination. The effect of accumulation potential on the peak current of PPZ (100 µM) was examined over the range of -0.1 to 0.6 V by keeping the accumulation time of 100s. The oxidation peak current increased up to 0 V. An increase in the accumulation time improves the sensitivity of the determination. The accumulation time was studied in the range of 0 to 450s. The peak height was increased with increasing the accumulation time up to 100s and then levelled off. It could be concluded that the adsorption of PPZ on MWCNT/CILE became saturated. Thus all experiments were performed under accumulation potential of 0V and accumulation time of 100s. In addition optimum conditions for DPV response were recognized by measuring the current dependence on some instrumental parameters such as modulation amplitude, step potential, interval time and modulation time to obtain maximum signal to noise ratio and the optimum amounts were 70 mV, 10 mV, 0.1 s and 0.05 s, respectively.

In general, pH is one of the most important variables that can influence the current and shape of voltammograms. Therefore, the electro-oxidation of PPZ was studied over the pH range of 2.0-7.5 using PBS as electrolyte. At pH values higher than 7.5 the solubility of the drug is partially decreased due to the hydrophobic behaviour of deprotonated molecule of PPZ. Differential pulse voltammograms of 100 µM PPZ at different pH in the mentioned range were recorded. The effect of pH on the peak currents is shown in Fig. 3A. With raising the pH of solution, the peak current decreased. The greatest anodic peak current was obtained in the pH 2. Therefore, phosphate buffer with pH 2.0 was used as the supporting electrolyte in all voltammetric determinations. The second oxidation peak was disappeared at pH 7.5. According to the previous work the cation radicals of phenothiazine derivatives are more stable in acidic solution.[40]

To acquire more insight about the mechanism of electrocatalytic oxidation of PPZ, the dependence of peak potential versus pH was studied. As shown in Fig. 3B, both catalytic peak potentials shifted toward less positive potentials with an increase in pH of the solution. This behaviour suggests the involvement of protons in the overall electrode reaction. A plot of peak potential versus pH for both anodic peak I and II is shown in Fig. 3B. For peak I a linear portion was observed in the pH range of 2.0 to 7.5, with the slope of 0.0292 V/pH. The following equation displays correlation between peak potential and pH: E_{pa}(V)=0.7729–0.0292pH; and R^2=0.9898. The slope of 0.0296 V/pH implying in the electrode reaction the number of electron transferred was as two times as that of proton according to the following equation.[41]

$$0.0592(h/n)\text{V/pH} \qquad (1)$$

where *h* and *n* are the number of protons and electrons involved in the electrode process, respectively. For the peak II the linear relation with the slope of 0.0541 V/pH was observed which indicates that equal number of electrons and protons involved in redox process. This agrees with the mechanism proposed for the anodic oxidation of phenothiazines in aqueous solutions (Scheme 2).[42]

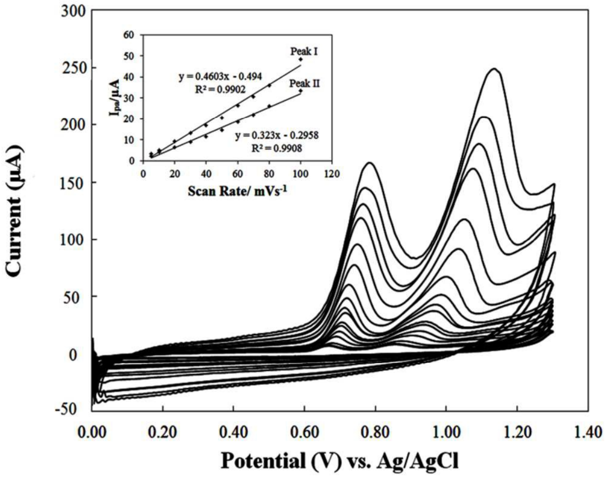


Fig.4. Cyclic voltammograms of MWCNT/CILE in PBS (pH 2.0) containing 100 µM PPZ at various sweep rates; Sweep rate: 5, 10, 20, 30, 40, 50, 60, 70, 80, 100, 200, 300, 400, 500, 600, 800 mVs^{-1}. Inset: linear dependence of peak current I and II to sweep rate in the range of 5-100mVs^{-1}.

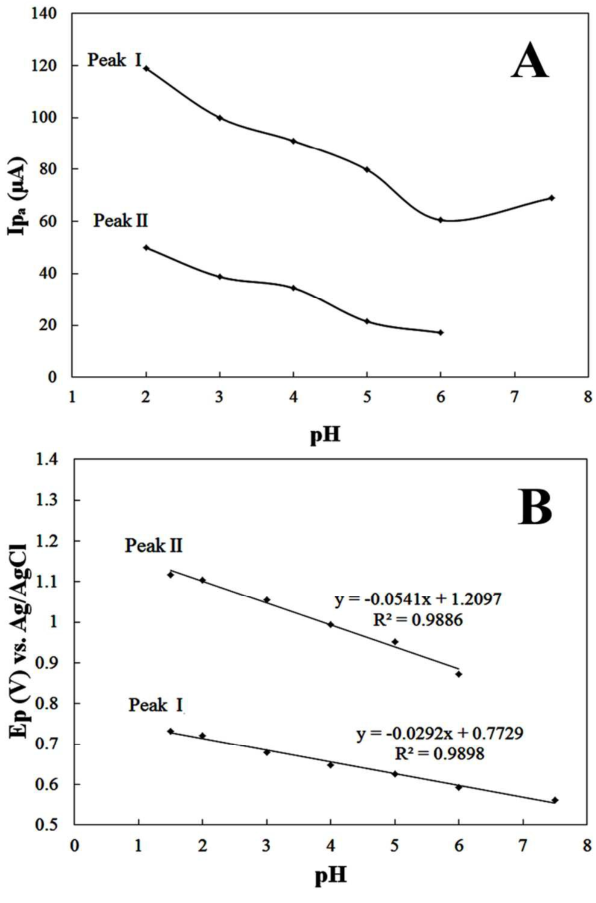


Fig. 3. (A) Plot of I_{pa} (peak I, peak II) versus pH. (B) plot of E_{pc} (peak I, peak II) versus pH. Recorded for a 100 µM concentration of PPZ under optimized conditions. pH values: 2.0, 3.0, 4.0, 5.0, 6.0, 7.5.

To study the prevail type of mass transport, effect of sweep rate on the electrooxidation of PPZ at the MWCNT/CILE was investigated by cyclic voltammetry. Fig. 4 shows the cyclic voltammograms corresponding to the response of MWCNT/CILE in presence of 100 µM PPZ at different sweep rates. Both anodic peak currents were linearly proportional to the sweep rate over the range of (5-100mVs-1) which indicates that the oxidation is controlled by an adsorptive process (Fig. 4 inset). In addition with increasing sweep rate, oxidation peak potential shifts to more positive values. This positive shift in peak potential also confirms the irreversibility of the electrooxidation process. Plot of log anodic peak current versus log sweep rate was also linear over the range of (5-100mVs^{-1}) for both anodic peak I and II. A slope of 1.00 is expected for an ideal reaction of surface species and the slopes of 0.91 and 0.89 for anodic peak I and II, respectively, are close to the expected value establishing that the PPZ electrooxidation reaction is an adsorption controlled process.

There is also a linear relation between the peak potential and log of sweep rate with a correlation coefficient of 0.9958 by the following equation: E_{pa}= 0.0354 log v + 0.6141. The Tafel slope (b) can be obtained from the slope of anodic peak potential versus log of sweep rate using Equation 1:[43]

$$E_{pa} = b/2 \log v + \text{constant} \qquad (2)$$

The Tafel slope was found to be 70 mV. By using the number of electrons that involved during PPZ oxidation in 0.01M PBS at MWCNT/CILE and the Tafel slope, the value of transfer coefficient was calculated according to following equation:[44]

$$b = (2.303RT)/[(1\text{-}\alpha)n\text{F}] \qquad (3)$$

The value of α was found to be 0.57.

After optimization of operating conditions, the calibration curve for PPZ was characterized by differential pulse voltammetry. Fig. 5A illustrates the differential pulse voltammograms obtained for a series of PPZ solution with different concentrations in PBS (pH=2) and the respective analytical curve (inset).

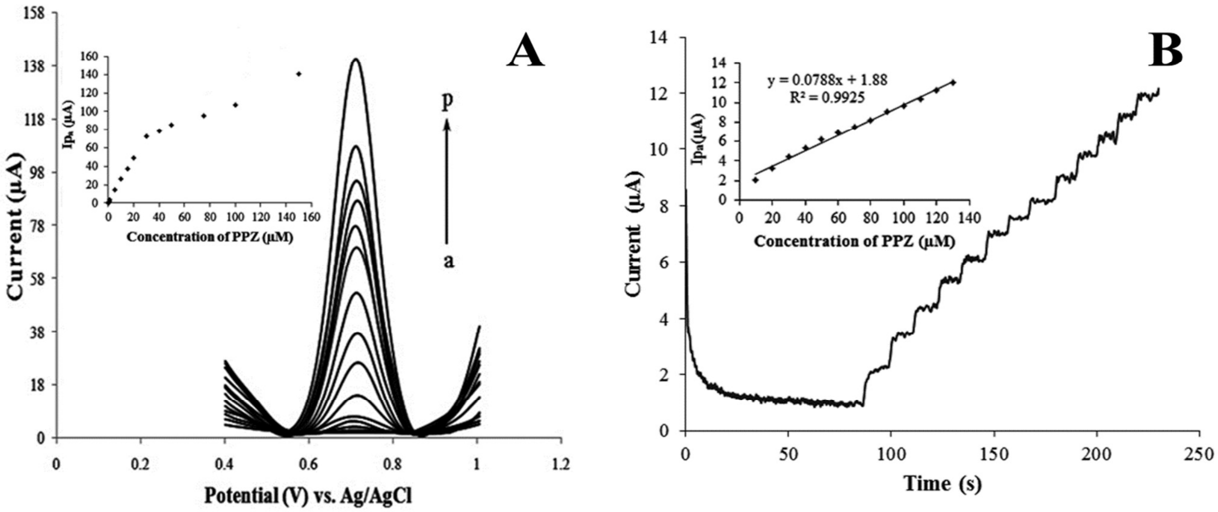
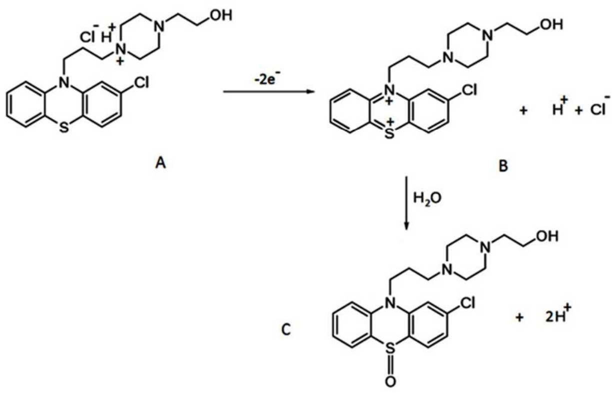


Fig. 5. (A) Differential pulse voltammograms for various concentrations of PPZ (a) 0.05, (b) 0.1, (c) 0.2, (d) 0.4, (e) 0.8, (f) 1, (g) 5, (h) 10, (i) 15, (j) 20, (k) 30, (l) 40, (m) 50, (n) 75, (o) 100, (p) 150 μM, at MWCNT/CILE in 0.1M PBS (pH=2) and accumulation time of 100s. Inset: Dependence of peak I currents on the concentration of PPZ. (B) Typical hydrodynamic amperogram of MWCNT/CILE at constant electrode potential of 0.7 V by sucessive addition of 10μM PPZ. Inset: corresponding calibration graph.

Table1. Comparison between different electrochemical methods used for determination of PPZ.

Method	Linear range (M)	Calibration sensitivity (μA/μM)	Detection limit (M)	Reference
Stripping voltammetry (Gold electrode modified with decanethiol SAM)	$6.0 \times 10^{-9} - 5.0 \times 10^{-7}$ and $5.0 \times 10^{-7} - 3.0 \times 10^{-6}$	0.79 and 0.65	NR	[45]
Cyclic voltammetry (Glassy carbon electrode plus methylene blue)	$5.0 \times 10^{-6} - 2.1 \times 10^{-4}$	NR	2.08×10^{-6}	[18]
Amperometery (Carbon fiber micro-disk bundle electrode)	$1.0 \times 10^{-7} - 1.0 \times 10^{-4}$	NR	5.0×10^{-8}	[3]
Differential pulse voltammetry and apmerometery (Glassy carbon electrode modified with graphene oxide nanosheet)	$8.0 \times 10^{-4} - 8.0 \times 10^{-3}$ And $7.0 \times 10^{-4} - 7.0 \times 10^{-3}$	NR	4.7×10^{-5} And 3.8×10^{-5}	[21]
Differential pulse voltametry (MWCNT/CILE)	$5.0 \times 10^{-8} - 3.0 \times 10^{-5}$ And $3.0 \times 10^{-5} - 1.5 \times 10^{-4}$	2.41 and 0.55	2.3×10^{-8}	This work



Scheme 2. The proposed mechanism for electrooxidation of PPZ.

Because the relationship between peak currents of peak II and the concentration of PPZ was not linear in any range, the data of peak I were selected. The anodic peak current was linear to PPZ concentration in the ranges of 5.0×10^{-8} to 3.0×10^{-5} M and 3×10^{-5} to 1.5×10^{-4} M, respectively. The regression equations were: i_p (μA) = $2.4186C_{PPZ}$ (μM) + 0.8527 for the range of 5.0×10^{-8} to 3.0×10^{-5} M (R^2=0.9987) and i_p (μA) = $0.5520C_{PPZ}$ (μM) + 55.72 for the range of 3×10^{-5} to 1.5×10^{-4} M (R^2=0.9917). It is clear good linearity was found at lower concentrations compared with linearity observed at higher concentrations. The difference between the slopes of the two calibration lines can be attributed to the change of accumulation efficiency.[45]

The sensitivity of differential pulse voltammetry was quantified on the basis of the values obtained for detection and quantification limits. The limit of detection (LOD) and limit of quantification (LOQ) were calculated by the parameters obtained from the analytical curve, using LOD = $3S_b/s$ and LOQ = $10S_b/s$, where S_b is the standard deviation of blank (n=7) and s is the slope of calibration curve. Under the given conditions, the calculated LOD and LOQ of PPZ were found to be 2.32×10^{-8} and 7.73×10^{-8} M, respectively. The relative standard deviation (RSD) of seven times repetitive measurement of 5×10^{-6} M PPZ with the same electrode was 1.9%, whereas the RSD was 2.4% for 5 different electrodes.

The analytical properties of the proposed methods were compared with the previously reported ones for the determination of PPZ in Table 1. As stated before, the electrochemical determination of PPZ was rarely reported. However, the MWCNT/CILE can be applied conveniently for this purpose with the higher calibration sensitivity and wider linear range compared with previous works. The easy preparation and low cost material of the proposed electrode should also be considered.

The hydrodynamic amperometry was also applied for evaluation of the nanocomposite electrode susceptibility as an amperometric sensor for PPZ. Fig. 5B shows the typical steady-state amperometric response of the MWCNT/CILE with the successive addition of 10 μM PPZ into the continuously stirred PBS solution. The applied potential was kept at 0.7 V during the measurement. The linear regression equation was: i_p (μA) = $0.0788C_{PPZ}$ (μM) + 1.88 (R^2=0.9925) over the range of 10-120 μM with the detection limit of 2.1 μM. This demonstrates the applicability of the MWCNT/CILE as a chromatographic detector.

Table 2. Determination of PPZ in pharmaceutical formulations.

No.	PPZ added (μM)	PPZ found[a] (μM)	Recovery (%)
1	0	4.61(±0.11)	-
2	2	6.48(±0.12)	93.5
3	4	8.72(±0.10)	102.7
4	6	10.83(±0.12)	103.7
5	8	12.47(±0.09)	98.3

[a] Average of three determination

Table 3. Recovery study of PPZ human blood serum sample

No.	PPZ added (μM)	PPZ found (μM)	Recovery (%)
1	0	ND*	-
2	2	1.93(±0.12)	96.5
3	4	4.05(±0.13)	101.25
4	6	5.98(±0.11)	99.7
5	8	7.63(±0.14)	95.4

*Not detected

To investigate the contaminant effect, various species especially biological compounds commonly existing in serum including glucose, ascorbic acid and uric acid; were examined. The tolerance limit was defined as the maximum concentration ratio of interfere/PPZ caused an error less than ±5.0% for the determination of PPZ. The DPV curves of the electrode toward 2 μM PPZ (PBS 0.1 pH=2) in the presence of different concentrations of ascorbic acid (AA) and uric acid (UA) has been shown in the Supporting Information (Figures S-2 and S-3). As reported previously[46], the oxidation overpotential of ascorbic acid and uric acid significantly decreases at carbon ionic liquid electrodes due to the electrocatalytic effect of IL used as pasting binder for construction of these electrodes. Therefore, the anodic peak for oxidation of ascorbic acid and uric acid occurred at a less positive potential (0.13 V for ascorbic acid and 0.50 V for uric acid) compared to the corresponding anodic peak for PPZ oxidation (0.73 V). As shown in Figure S2 and S3, there is a significant difference between their peak potentials, and it is quite possible to detect PPZ oxidation responses with minimal interferences from ascorbic acid and uric acid. As illustrated in Figure S-2 increasing the concentration of AA up to 100 fold of the drug concentration did not affect the electrode response toward oxidation of PPZ and also increasing the concentration of UA up to 50 fold of PPZ concentration did not interfere with PPZ determination. The effect of catechol (1,2-dihydroxybenzene) on the electrochemical response of PPZ was also studied. The oxidation of catechol was characterized by a well-defined peak at the potential of 450 mV (Figure S-4). As seen, increasing the concentration of catechol up to 40 fold of the drug concentration did not affect the electrode response toward oxidation of PPZ. The results demonstrated good selectivity for the proposed electrode.

To evaluate the applicability of the proposed method for the analysis of real samples, it was applied for determination of PPZ content in pharmaceutical preparations (nominal contain 4 mg PPZ/tablet). Five tablets of PPZ (Daruo Pakhsh Pharmaceutical Company, Tehran, Iran) were accurately weighed and triturated to fine powder in a mortar. Then, definite amount of the powdered sample corresponding to a solution of 1×10^{-4} M PPZ was dissolved in methanol by sonication for 10 min filtered into a 50 mL volume calibrated flask and diluted with double distilled water. A known volume of this solution was spiked into a 25 mL aliquot of the supporting electrolyte in the volumetric flask, followed by spikes of the standard PPZ solution. The standard addition method was used in these experiments. The amounts of PPZ obtained in pharmaceutical formulations agree well with the label contents (Table 2). Furthermore, the MWCNT/CILE was applied for the analysis of the human blood serum sample. A 10 ml human blood serum sample was deproteinized by adding 2 ml of 10% (w/w) trichloroacetic acid. Then the solution was centrifuged and diluted 10 times with 0.01M PBS with pH 2.0. The appropriate amount of this diluted sample was transferred to the electrochemical cell for determination of PPZ. The sample was spiked by drug and the DPV measurements were done as described before. The results are presented in Table 3. The results showed good quantitative recoveries which imply successful applicability of the proposed method for real sample analysis

Conclusions

Using the unique properties of ionic liquid-MWCNT nanocomposites, we have introduced an effective electrochemical sensor for determination of PPZ. The electrode exhibited excellent sensitivity toward oxidation of PPZ over wide concentration ranges of 0.05-30 μM and 30-150 μM with a detection limit of 23 nM and high calibration sensitivity of 2.41 μA/μM in lower concentrations. In addition the MWCNT/CILE exhibited good response using hydrodynamic amperemetery which makes it applicable as a chromatographic detector. Low background current, high selectivity and reproducibility, associated with easy and rapid preparation made this electrode ideal for the determination of PPZ in pharmaceuticals and blood samples.

Acknowledgements

The authors acknowledge the support of this work by Islamic Azad University, branch of Marvdasht.

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