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Voltammetric Determination of Venlafaxine by Using Multiwalled Carbon Nanotube- Ionic Liquid Composite Electrode

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Abstract

The combination of multi-walled carbon nanotubes and ionic liquid 1-octylpyridinium hexa fluorophosphate (OPFP) yields a carbon nanocomposite (MWCNT-CILE) that can be well used as electrode for electrocatalytic oxidation of venlafaxine (VEN). The influence of various experimental parameters including pH of the solution, the amount of multi-walled carbon nanotube used for the modification of the carbon ionic liquid electrode (CILE), accumulation potential, accumulation time and scan rate on the voltammetric responses of venlafaxine were all investigated in the present research. The oxidation peak potential of venlafaxine appeared at 640 mV at the proposed electrode that is about 150 mV lower than the peak potential at traditional carbon paste electrodes (CPE). The oxidation is irreversible and exhibits adsorption controlled behavior. Under the optimum conditions, the anodic peak current has a linear relationship with venlafaxine concentration in the range of 10.0 to 500.0 μ M. The detection limit (three times signal-to-noise ratio) of 0.47 μ M was found. Finally, the proposed sensor has the features of being sensitive, reproducible, easily renewable and cost effective which might suggest promising applications for determination of VEN in pharmaceutical formulations as well as blood serum and urine samples.

Keywords: *Electrochemistry; Ionic liquid; Multi-walled carbon nanotube; Sensor; Venlafaxine.*

Introduction

Venlafaxine (1-(2-(dimethylamino)-1-(4-methoxy-phenyl) ethyl) cyclohexanol), is an antidepressant of the selective serotonin-norepinephrine reuptake inhibitor (SNRI) class [1, 2]. It has a broad application in the treatment of depression, generalized anxiety disorder (GAD), panic disorder, social phobia [3], sudden fears and agoraphobia [2]. On the other hand, an overdose of venlafaxine (VEN) might cause the symptoms of depression, serotonin toxicity, seizure, or cardiac conduction abnormalities [4]. Therefore, for avoiding toxicity and adverse effects, as well as evaluating interactions and therapeutic efficiency, the drug levels in body fluids such as urine and plasma of consumers is usually monitored [5].

To determine VEN some analytical methods have been reported, such as chromatography [6-9], Capillary electrophoresis [10,11] and fluorescence detection [12]. However, the mentioned techniques require expensive sophisticated equipment. Also they need time-consuming sample pre-treatment and some of them have sensitivity issues.

Electrochemical techniques are good candidates for the analysis of VEN because of their simplicity, low-cost, rapidity and sensitivity. Moreover, concerning the electrochemical measurements of VEN, only a few reports are available [4, 13-15]. Different kinds of mercury electrodes were used which detect VEN at low overpotential [13, 14]. But, these methods are not suitable for real sample analysis due to environmental issues of mercury. To the best of our knowledge, the electrochemical behavior of VEN was not studied at carbon paste electrodes.

Carbon ionic liquid electrodes (CILEs) [16] are the new generation of carbon paste electrodes (CPEs). In addition to the advantages of CPEs, including low cost, ease of preparation, low background current, wide electrochemical window and renewable surface, CILE has high electrocatalytic activity and antifouling effect, which has been led to the widely application of CILE in the field of electrochemical sensing [17-19].

Carbon nanotubes (CNTs) based electrochemical sensors have also been extensively reported for the considerable improvement of their electrochemical responses toward many biologically important compounds [20-24]. The ionic liquid-carbon nanotube (IL-CNT) composite electrodes have been prepared and used in biological fields with excellent performance [25, 26].

In the present study, multi-walled carbon nanotube modified CILE (MWCNT-CILE) has been applied for the sensitive determination of VEN in pharmaceuticals and biological fluids. Interestingly, the proposed sensor has the features of being sensitive, reproducible, easily renewable and cost effective.

Experimental

Materials

The pure form of venlafaxine hydrochloride used in the present work was kindly supplied by Farabi pharmaceutical company (Isfahan, Iran). Multi-walled carbon nanotube (MWCNT) with a 95% purity (o.d.= 6-9 nm and 5 μm length), graphite powder (particle size $<100 \mu\text{m}$) (Aldrich), were used as received. All other chemicals were of analytical grade. The ionic liquid, 1-octylpyridinium iodide, was synthesized as described elsewhere [27]. 1-Octylpyridinium hexafluorophosphate (OPFP) was prepared by anion exchange of 1-octylpyridinium iodide with ammonium hexafluorophosphate. For preparing all solutions, doubly distilled water was used. A 1.00×10^{-2} M stock solution of drug was prepared and stored at 4 °C. Fresh blood samples were obtained from Central Blood Transfusion Organization (Shiraz, Iran) and urine samples were collected from healthy individuals and, then, it was stored frozen until assay.

Fabrication of electrodes

Graphite powder and OPFP were hand mixed effectively in a mortar with a ratio of 50/50 (w/w) in order to make CILE. Then, a portion of the obtained paste was packed tightly into the cavity (1.8 mm i.d.) of a Teflon holder. For the purpose of making a good homogeneous composite, as well as decreasing background current, the electrode should be heated (e.g. by using a hair drier) to a temperature above the melting point of IL (m.p. $\sim 65 \text{ }^\circ\text{C}$) prior to use it [16]. The electric contact was established with a copper wire to the carbon composite. MWCNT/ CILE was prepared in the same way as CILE with graphite powder, ionic liquid and multi walled carbon nanotube weight percentage of (40%:50%:10%, wt %), respectively. Also, 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 similar way but without adding MWCNTs to the mixture.

Apparatus

Electrochemical measurements were done with a galvano potentiostat Behpajoooh Co. model BHP2063+ (Isfahan, Iran). The electrochemical cell was assembled with a conventional three-electrode system; a saturated calomel electrode (SCE) and a platinum disk electrode were used as reference and counter electrodes, respectively. CILE, MWCNT/CILE, CPE and MWCNT/CPE were applied as the working electrodes. All experiments were typically carried out at room temperature. The quantitative determination of VEN was achieved by measuring their oxidation peak current after background subtraction.

Results and discussion

The electrocatalytic behaviors of the MWCNT/CILE towards oxidation of VEN

Figure 1 shows the cyclic voltammograms of CPE, MWCNT/CPE (10%, wt), CILE and MWCNT/CILE (10%, wt) in the 0.1 M phosphate buffer solution (PBS), pH 9.0, containing 1mM VEN. None of these electrodes show any peak in the absence of VEN (inset of Figure 1). The decrease in overpotential due to electrocatalytic activity of CILE is more pronounced for the oxidation of VEN. It can be seen that the oxidation peak potential of VEN on both CILE (curve c) and MWCNT/CILE (curve d) shifted to a more negative potential (0.66 V and 0.64 V vs. SCE, respectively) in comparison with CPE (curve a) and MWCNT/CPE (curve b) and the peak currents are much higher. The negative shift in the peak potential (ca.150 mV) and also the higher peak current demonstrated that the MWCNT/CILE composite electrode made the electron transfer easier.

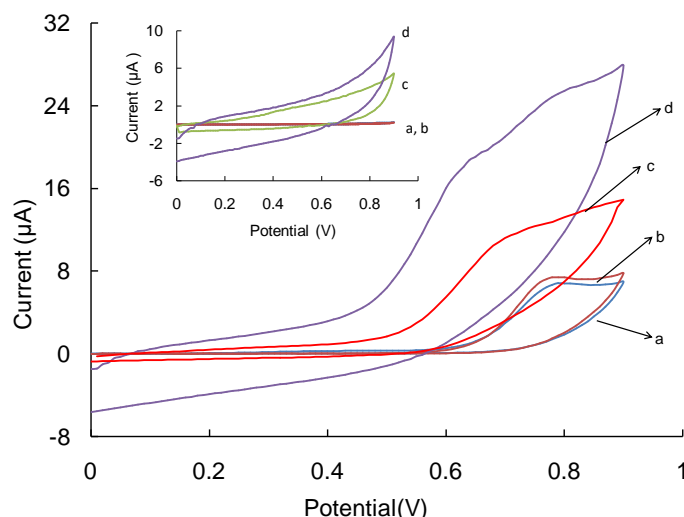


Figure 1. Cyclic voltammograms of (a) CPE, (b) MWCNT/CPE, (c) CILE, (d) MWCNT/CILE in the presence of 1mM VEN in 0.1 M PBS (pH 9.0) at a scan rate of 50 mVs⁻¹. Inset shows the voltammograms of corresponding electrodes in the absence of VEN.

Also, the peak potential of VEN oxidation at MWCNT/CILE was less than observed on the glassy carbon electrode covered by a Nafion/ carbon nanotube composite film (NAF-CNT-GCE) (ca. ~ 0.78 V vs. Ag/AgCl) [4]. At mercury electrodes the oxidation peak potential of VEN was appeared at significantly lower potentials (ca. -0.1 v vs. Ag/AgCl) [13]. However, mercury toxicity complicated the use of mercury electrodes in practical analytical laboratories. By using IL as suitable binder in CILE and MWCNT/CILE, the faradic currents increased compared to the CPE and MWCNT/CPE. This caused by ionic conductivity of the IL which leads to a larger electroactive area. In addition, CNTs have been examined for their excellent electrocatalytic abilities and antifouling properties and, thus they increase electron transfer rate in electrochemical reactions when used as an electrode material [28]. Moreover, MWCNTs can interact with the cation of ionic

liquid [29] through the π - π or/and π -cationic, or weak van der Waals interaction [30] which is resulted in a stable MWCNT/CILE composite modified electrode. Therefore, MWCNT/CILE was selected for further studies.

Effect of pH

The effect of pH was recognized as an important factor for the determination of VEN at the nanocomposite electrode. Therefore, the influence of pH on the cyclic voltammetric responses of 1mM VEN, employing the Britton-Robinson buffer was investigated in the pH range from 5.0 to 9.0 and the corresponding voltammograms were shown in Figure 2. The oxidation peak current of VEN was not observed below pH 5.0. As seen, with raising pH of the solution, the peak currents increased. However, at pH values higher than 9.0 the solubility of the drug is partially decreased due to the hydrophobic behaviour of deprotonated molecule of VEN. Therefore, phosphate buffer with pH 9.0 was used as the supporting electrolyte in all voltammetric determinations.

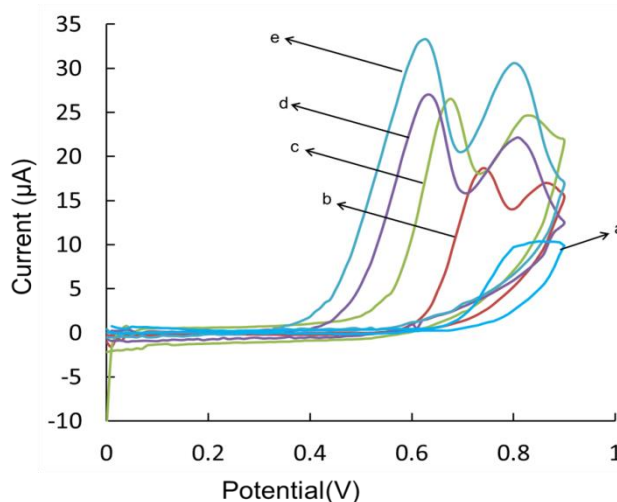
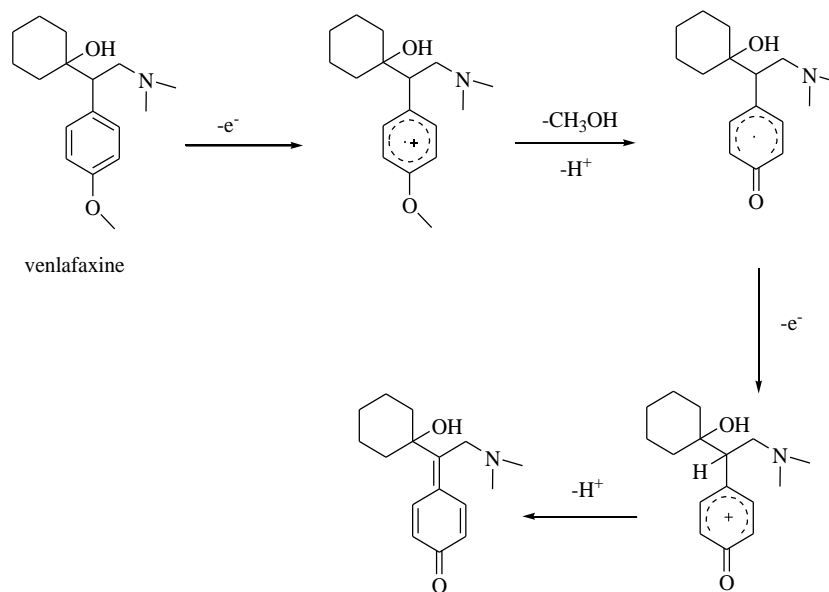


Figure 2. Background subtracted cyclic voltammograms of 1mM VEN at the MWCNT/ CILE at different pH; a \rightarrow e (5.0 \rightarrow 9.0) at 50 mVs⁻¹, accumulation time of 240 s.

The relationship between the oxidation peak potential and pH was also investigated in the pH range from 5.0 to 9.0. With increasing pH, peak potentials shifted toward negative potentials with the regression equation of E_p (V)=1.115-0.0595 pH. This indicates the involvement of protons in the overall electrode reaction. A slope of 0.059 V/ pH suggests that the number of electron transferd is equal to the proton number involved in the electrode reaction process [31].

The probable oxidation mechanism of VEN is given in Scheme 1[4]. The lone pair of electrons in the quaternary nitrogen of VEN is more easily oxidised than that in oxygen and aromatic ring. However, preferential oxidation of aromatic pi electrons in VEN take place as the nitrogen present in the side chain has no other group for stabilization after the loss of electrons whereas the loss of

electron from aryl ring produces radical cation which can be stabilized by the oxygen attached to the ring.



Scheme 1. Mechanism of venlafaxine oxidation.

Effect of the weight percent of the modifier

The influence of the MWCNT amounts on the voltammetric responses of the MWCNT/CILE was investigated. Four electrodes were prepared by varying the percentage of MWCNT (0, 5, 10 and 15% weight percent) in the composite electrode and examined under identical conditions. The results showed that by increasing the amount of MWCNTs up to 10%, the anodic peak current increased, whereas higher content of the modifier caused a decrease in the peak current. The observed increase in the anodic peak current, using MWCNT/CILE, represents the improvement of VEN accessibility to the electrode surface active sites. However, at higher amounts of MWCNT, capacitive background current was increased, leading to worsen the signal to noise ratio in the electrochemical measurements. Therefore, MWCNT/CILE (10%, w/w) was used in further studies.

Effect of accumulation potential and time

The effect of accumulation potential on the peak current of VEN was also investigated over the range of -0.5 to 0.4 V by keeping the accumulation time of 240s. The results revealed that the peak current was independent of accumulation potential and the maximum peak current was obtained at open circuit potential. Hence, accumulation step was performed under open circuit potential. The accumulation time is an effective factor which affects the response sensitivity. The accumulation time varied between 0 to 300 s under open circuit potential. It was observed that the peak current increased with increasing the accumulation time up to 240s and then levelled off, indicating

saturated surface adsorption. Therefore, an accumulation time of 240s was chosen as the optimal time for further experiments.

Effect of scan rate

The effect of potential scan rate on the peak current of 1mM VEN at MWCNT/CILE was investigated by cyclic voltammetry (Figure 3). With increasing the potential scan rate, the oxidation peak potentials gradually shifted to more positive values suggesting a kinetic limitation in the reaction of VEN at the modified electrode. A plot of \log (peak current) vs. \log (scan rate), was found to be linear, with the following equation: $\log I (\mu\text{A}) = 0.703 \log v + 2.1251$; $R^2 = 0.9898$, indicating that the electrode process of VEN is an adsorption controlled.

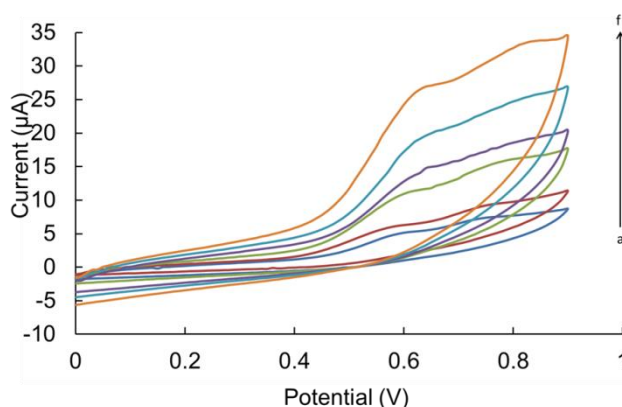


Figure 3. Cyclic voltammograms of MWCNT/CILE in 0.1 M (pH 9.0) containing 1mM VEN at various sweep rates; sweep rate: (a) 8.0, (b) 10.0, (c)30.0, (d)50.0, (e)70.0, (f) 90.0 mVs^{-1} .

There is also a linear relationship between the peak potential and \log of sweep rate with a correlation coefficient of 0.9967 by the following equation: $E_{\text{pa}} = 0.0741 \log v + 0.7159$. The Tafel slope (b) can be obtained from the slope of anodic peak potential versus \log of scan rate using the following equation [32]: $E_{\text{pa}} = b/2 \log v + \text{constant}$. The slope of the Tafel plot is equal to $2.3 RT/n(1 - \alpha) F$, which comes up to $0.148\text{V decade}^{-1}$. We obtained the electron transfer coefficient (α) as 0.6 which indicates that the activation free energy curve is not symmetrical in such an irreversible oxidation process [33].

Analytical features

After optimization of operating conditions, the relationship between the anodic peak current and the concentration of VEN have been studied. Figure 4 shows the cyclic voltammograms obtained for a series of VEN solutions with different concentrations in PBS 0.1 M (pH= 9) and the respective analytical curve (inset of figure. 4). For the concentration range of 10–500 μM VEN, the regression

equation is $Y (\mu\text{A}) = 0.0513 X(\mu\text{M}) + 2.2709$ and a correlation coefficient of $R^2 = 0.9960$ was obtained. The limit of detection (LOD) was calculated according to the definition of $\text{LOD} = 3s_b/m$ where s_b is the standard deviation of the peak current of the blank ($n = 7$) and m is the slope of the calibration curve for determination of VEN. The LOD is $4.7 \times 10^{-7} \text{M}$.

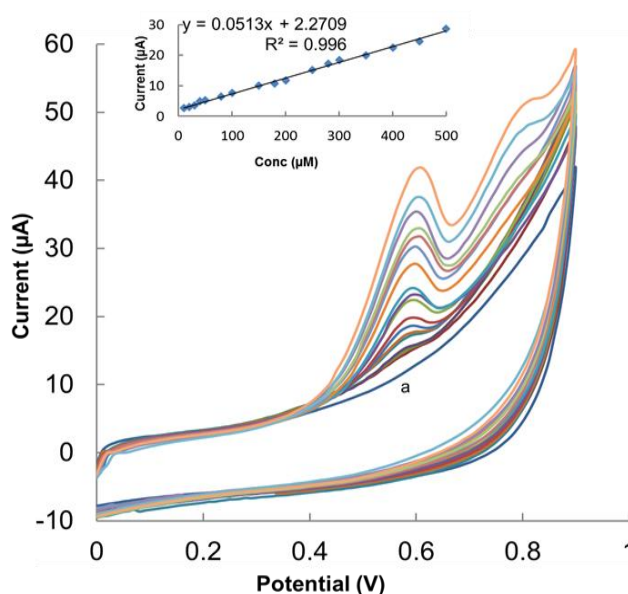


Figure 4. Cyclic voltammograms of VEN at MWCNT/ CILE in 0.1 M PBS (pH 9.0) and accumulation time of 240s in the presence of various concentrations of VEN. Curve a shows the baseline of the electrode. Inset shows the calibration curve of peak current vs. VEN concentration.

The electrochemical responses of MWCNT/CILE in terms of linear range and detection limits were compared to the other modified electrodes reported in the literature (Table 1).

Table1. Electrochemical detection of VEN reported at various electrodes.

Electrode	Linear range (M)	LOD (M)	Reference
HMDE ^a	$7.96 \times 10^{-7} - 3.91 \times 10^{-6}$	3.95×10^{-7}	[13]
MFE ^b	$1.27 \times 10^{-6} - 2.43 \times 10^{-5}$	8.0×10^{-7}	[14]
MFE	$1.5 \times 10^{-6} - 6.0 \times 10^{-4}$	8.0×10^{-7}	[15]
NF-CNT-GCE ^c	$3.81 \times 10^{-8} - 6.22 \times 10^{-5}$	1.24×10^{-8}	[4]
MWCNT/CILE	$1. \times 10^{-5} - 5.0 \times 10^{-4}$	4.7×10^{-7}	This work

a: Hanging mercury drop electrode, b: Mercury film electrode, c: Nafion carbon nanotube composite –glassy carbon electrode.

As stated before, the electrochemical determination of VEN was rarely reported. The easy preparation and low cost material of the proposed electrode should also be considered.

Stability, repeatability and reproducibility of the modified electrode

The electrochemical stability of the modified electrode was investigated in a solution of 30 μM of VEN after every few days. MWCNT/CILE showed high stability for VEN detection, and retained 89.7% of its original response to VEN after 30 days of storage. Furthermore, the relative standard deviation (RSD) of seven repetitive measurements of 30 μM VEN with the same electrode was 1.63%. Using five different electrodes, the RSD value of 30 μM VEN was 3.05%. These results showed that the MWCNT/CILE electrode had good repeatability and reproducibility. The contaminant effects of various species, especially biological compounds commonly exist in serum including glucose; ascorbic acid and uric acid, were investigated. The tolerance limit was defined as the maximum concentration ratio of interfere/VEN caused an error less than $\pm 5.0\%$ for the determination of VEN. The results showed that 100-fold excess of glucose; 75-fold excess of uric acid and 50- fold excess of ascorbic acid did not interfere with the analysis of 30 μM VEN. The results demonstrate good selectivity for the proposed electrode.

Real sample analysis

In order to evaluate the analytical applicability of the proposed nanocomposite electrode, it was also applied for the determination of VEN content in pharmaceutical formulations. Five tablets of VEN were accurately weighed and triturated to fine powder in a mortar. Then, definite amount of the powdered samples corresponding to a solution of 1×10^{-2} M VEN was dissolved in a 50.0 mL volume calibrated flask. A known volume of this solution was spiked into a 10-mL aliquot of the supporting electrolyte in the volumetric flask, followed by spikes of the standard VEN solution. The standard addition method was used in these experiments. The amounts of VEN obtained in pharmaceutical formulations were fully in agreement with the label contents (Table 2). Furthermore, the MWCNT/CILE was applied for the analysis of VEN in biological samples such as serum and urine. The blood serum sample was deproteinized by adding 2 ml of 10% (w/w) trichloroacetic acid solution to a 10 ml sample and the solution was centrifuged [34]. The sample was then diluted 10 times with 0.1M PBS pH 9. The dilution step was also done for the urine sample and then appropriate amount of the diluted samples were transferred to the electrochemical cell for the determination of VEN. The results are presented in Table 2 that confirm the efficiency of MWCNT/CILE for the determination of VEN in real sample analysis.

Table2. Determination of VEN in tablet, serum and urine samples using MWCNT/CILE.

Sample	Added(μM)	Found (μM)	Recovery (%)
Tablet	0	49.71(± 0.11)	-
	50	99.43(± 0.09)	99.4
	60	108.66(± 0.12)	98.2
	70	120.79(± 0.11)	101.5
Blood serum	0	N.D.*	-
	50	49.69(± 0.12)	99.4
	60	61.82(± 0.14)	103.0
	70	69.84(± 0.12)	99.8
Urine	0	N.D.	-
	50	50.14(± 0.12)	100.3
	60	59.72(± 0.13)	99.5
	70	70.14(± 0.11)	100.2

*Not detected

Conclusion

In this work, the benefits of multi-walled carbon nanotube were shown for modification of a carbon ionic liquid electrode in order to study the electrochemical oxidation of VEN. The electrode exhibited a good electrocatalytic activity for the anodic oxidation of VEN compared to the conventional electrodes. The MWCNT/CILE was used for determination of VEN. The results showed that the proposed nanocomposite electrode has advantages such as high stability and reproducibility as well as the ease of preparation, low cost and surface renewal which might suggest promising applications in real sample analysis.

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