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Electro-Polymerized Poly(L-Arginine) Film as An Efficient Electrode Modifier for Highly Sensitive Determination of Methadone in Real Samples

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Abstract- Developing simple yet efficient sensors for drug-abuse detection is highly required from both human health and medical aspects. In this work, a glassy carbon electrode modified by a thin layer of poly L-arginine (P-L-Arg/GCE) was fabricated by electropolymerization of L-arginine monomer using cyclic voltammetry (CV) and then it was used for determination of Methadone (MET). The surface characteristics of the P-L-Arg/GCE was examined by scanning electron microscopy and electrochemical impedance spectroscopy. To study the electrochemical responses of MET on P-L-Arg/GCE and bare GCE, cyclic and differential pulse voltammetric techniques were employed. For P-L-Arg/GCE, a considerable enhancement in electrooxidation signal of MET was observed compared to bare electrode. The influence of various determinant parameters on P-L-Arg/GCE performance was studied. At optimized conditions, using DPV technique, the electrooxidation peak current was linear to MET concentration in the ranges of 0.49-2.98 µM and 2.98-11.9 µM with a detection limit of 0.32 µM. The utility of P-L-Arg/GCE was successfully validated by analyzing MET in serum and urine samples, representing its potential application for real samples analysis.

Keywords- Methadone; Arginine; Electropolymerization; Modified glassy carbon electrode; Differential pulse voltammetry

1. INTRODUCTION

Methadone, chemically named (6-dimethylamino-4, 4-diphenyl-3-heptanone (MET), is being used as a pain reliever but mainly used in opioid addiction cases for replacement therapy. It acts as a harmless substitute to heroin and other illicit opioids, with a priority of using as tablets. Suffering from personal risk, being harmful to the user in many ways especially blood borne viruses, injecting medications are less considered. Additionally, there are some social risks with employing needles and injecting apparatus in circulation among the addicts. While dispensed under supervision, MET lessens the hospitalization and death rates linked with heroin usage [1]. More importantly, MET has been listed by the World Health Organization in the list of vital drugs, proving its global usage for treating addiction [2]. However, the abuse consumption of MET interrupts the nervous system and leads to intensive mental disorders and physical dependence; hence, determination of MET in biofluids is interesting from both medical and judicial standpoints [3].

Up to now, for determining MET, some analytical methods have been introduced. Among them are some well-stablished techniques such as gas chromatography-mass spectrometry [4], high performance liquid chromatography (HPLC) [5], capillary electrophoresis [6], liquid chromatography [7], gas chromatography [8], liquid chromatography tandem mass spectrometry (LC-MS/MS) [9], atomic absorption and atomic emission spectrometry [10], and recently introduced electrochemical methods [11]. However, the electrochemical approaches have gained much attention thanks to the advantageous features such as low response time, less manipulation of sample, time-saving procedure, low-cost, and high sensitivity. As reviewed by S. Ren, et al [12], modified electrodes containing GCE, CPE, SPE, Pt, and Al with polymers, carbonaceous nanomaterials, metal and metal oxide nanomaterials have been used for illicit drug analysis. Polymers derived from natural monomers such as amino acids paved a way for easy modification of electrode surfaces and creating multifunctional surfaces. Amino acid monomers are biologically important compounds and has been utilized in different areas[13]. Polyaminoacids are designed via connecting a series of amino acids by various polymerization methods. The non-toxicity, biodegradability, biocompatibility, electrochemical stability, facile electron transfer on electrode surface, and rich functional groups are some outstanding features of these biopolymers over the chemical counterparts [14]. On the other hand, electropolymerization has been an interesting and easy route from past decades for in-situ creation of electrode modifiers and yields reproducible, stable and well-adhered films for sensing purposes [15]. Employing L-arginine solely or in combination of nanomaterials to produce polymer/composite films make environmentally friendly sensor materials, in which polymerized L-arginine film has been employed as a good sensor platform for analysis of different analytes including drugs [16], pollutants [17], and biomarkers [18]. However, there is not any report for the analysis of MET via P-L-Arg film coated GCE.

The present study aims to fabricate a stable sensing platform by electropolymerizing L-Arginine (L-Arg) on the surface of GCE to achieve the task of determination of MET. After optimizing the influential factors on sensor performance, the feasibility of the developed sensor was evaluated for determination of MET in human serum and urine as real samples.

2. EXPERIMENTAL

2.1. Materials

Methadone hydrochloride (MET) was obtained from Sigma–Aldrich. L-arginine and NaH_2PO_4, Na_2HPO_4 were attained from Merck. Stock solution of MET (1 mM) was prepared in water and kept in dark within refrigerator until use. Double-distilled water was used all over the experiments.

2.2. Apparatus

All electrochemical measurements were conducted using a Autolab (Eco Chemie B.V., Ultrecht, the Nether-lands) controlled by the GPES software (Version 4.9). A usual three-electrode system was employed with a saturated Ag/AgCl as reference electrode, a Pt wire as counter electrode and a GCE or P-L-Arg/ GCE as working electrode. A Metrohm pH-meter (Model: 691Herisau, Switzerland) was used to set the pH values. The surface morphologies of the bare CGE and P-L-Arg film modified GCE were observed by scanning electron microscopy (SEM) (Philips XL 30).

2.3. Preparation of P-L-Arg/GCE

The surface polishing of bare GCE was conducted by different grades of Al_2O_3 slurry (0.05–3 μm) on cloth, then, it was rinsed with water and sonicated subsequently in a 1:1 ration of DW and ethanol for 5 min. The electro polymerization of L-Arg on GCE was performed using cyclic voltammetry in 0.1 M phosphate buffer solution (pH 6.0) containing 2.5 mM L-Arg in a potential range of −2.0 to +2.0 V versus Ag/AgCl (sat. KCl) at a scan rate of 100 $mV \cdot s^{-1}$ for 15 cycles [19,20].

2.4. Real samples analysis

Human serum specimens were gained from Pastor Laboratory (Khoy–Iran) and kept at -4°C until use within microtubes. Each day prior to analysis, these samples were thawed at ambient temperature and vortexed to guarantee homogeneity. A 2 mL of it was then spiked with MET, then plasma proteins were precipitated and separated by addition of acetonitrile with a volume ratio of 2:1 and centrifugation for 5 min at 6,000 rpm. For analysis, a 2 mL of supernatant added into supporting electrolyte to reach a total volume of 10 mL and then analyzed by developed sensor.

The spiking of human urine samples as also conducted by addition of MET, followed by treatment with 0.2 mL methanol. These samples were agitated and placed in a micro centrifuge during 3 min at 6,000 rpm to remove proteins. The supernatant was removed and the remaining solution was transferred into a 10 mL PBS solution with pH 6 for doing analysis.

3. RESULTS AND DISCUSSION

3.1. Surface characterization of the P-L-Arg/GCE

L-Arg exists in zwitterionic form with the positive charge distributed over three nitrogen atoms in the guanidyl group, which is engaged in hydrogen bonds with unique feature and it can easily polymerized on electrode surface providing an interesting surface for electroanalysis [13]. The typical surface morphologies of bare GCE (Figure 1A) and P-L-Arg/GCE (Figure 1B) were evaluated by scanning electron microscope (SEM). In contrast to a smooth surface which observed for bare GCE, the P-L-Arg film appeared as snowflake like structures over the GCE surface. These observations are in accordance with previous reports [18], proving successful electro polymerization of P-L-Arg on the surface of GCE.

3.2. Electrochemical Impedance spectroscopy analysis of P-L-Arg/GCE

As an efficient approach for elucidating electrode-solution interface, electrochemical impedance spectroscopy (EIS) enables measuring the electron transfer rate at the interface of electrode and solution. As a part of EIS analysis, the Nyquist plot contains two regions: a semicircle part at high frequencies and a linear part at low frequencies, which represent to the electron transfer resistance (R_{et}) and the diffusion process on the electrode surface, respectively. In which, the diameter of the semicircle provides a rough estimation of the resistance of electron transfer on the surface of the bare and modified electrode, providing a facile approach for conductivity analysis of electrode after film formation. In Figure 1C, the Nyquist plots of GCE (a) and P-L-Arg/GCE (b) in a solution containing 1 mM of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.1 M KCl at a frequencies of 0.01 Hz to 50 kHz are shown., As seen, the semi-circular diameter for the bare GCE electrode is 10000 Ω while for the P-L-Arg/GCE is 250 Ω. This considerable electron transfer enhancement may arise from the high conductivity of the electrodeposited polymer layer onto the GCE surface, in which it facilitates the electron transfer of the redox couple. The results exhibit that P-L-Arg could enhance the electron transfer rate of $[Fe(CN)_6]^{3-/4-}$ redox probe at the electrode interface. Therefore, the EIS measurement results demonstrate that the P-L-Arg/GC sensor was effectively made-up and the presence of P-L-Arg would advance the sensitivity for detecting MET by enhancing in conductivity and electron transfer.

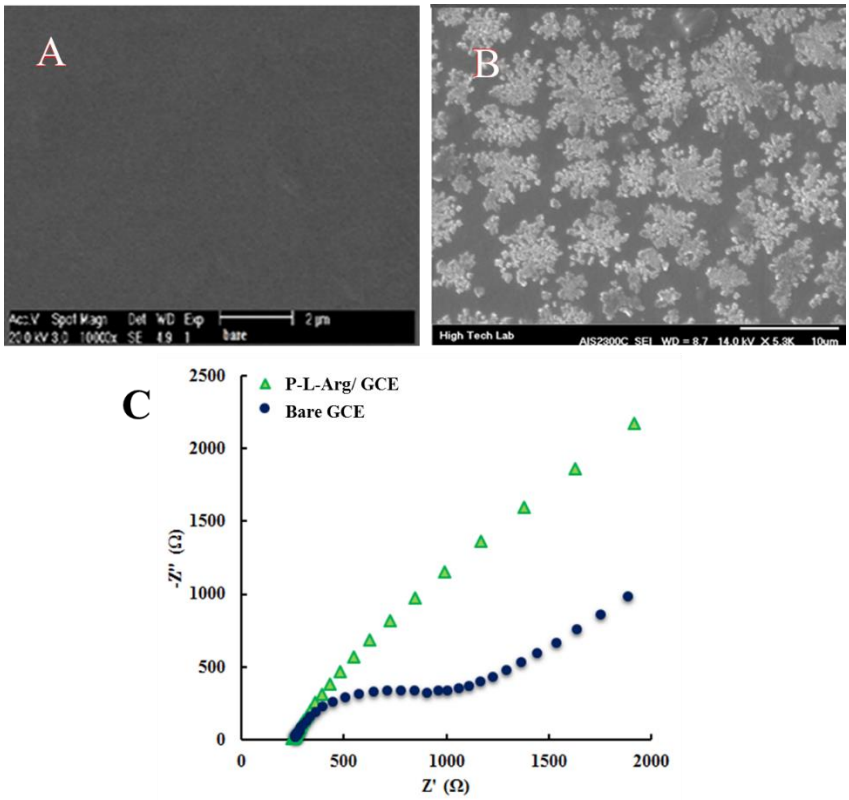


Figure 1. SEM images of (A) bare GCE electrode, (B) P-L-Arg/GCE, and (C) Nyquist plots in a solution containing 1 mM of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.1 M KCl at a frequency range of 0.01 Hz to 50 kHz

3.3. Electrochemical response of MET on P-L-Arg film modified electrode

The cyclic voltammetry was utilized to examine the electrochemical behavior of MET on GCE and modified electrode. Figure 2A shows the cyclic voltammograms of 4.9 µM MET in 0.1 M PBS (pH 6.0) at the surface of GCE (a) and P-L-Arg/GCE (b) electrodes, with starting from 0.4 V and reversing at 1.0 V. As seen on both electrodes, one anodic peak was observed without any accompanying cathodic peak in the reversed scan. As it is clear, modification of the GCE electrode with polymer film boosts the oxidation peak current of MET. The improved electrical conductivity and electrocatalytic activity resulted from the P-L-Arg film facilities the kinetic of oxidation process, resulting an increase in the sensitivity of the sensor. Figure 2B illustrates the differential pulse voltammograms of MET at GCE and P-L-Arg modified glassy carbon electrodes in phosphate buffer (pH 6.0). A wide peak with low current response was observed for MET on bare electrode at a higher potential around 0.8 V. In contrast, for P-L-Arg modified electrode a negative shift of the oxidation potential to 0.76 V and an increase in peak current were observed, clarifying the strong electrocatalytic activity of P-L-Arg modified electrode toward oxidation of MET.

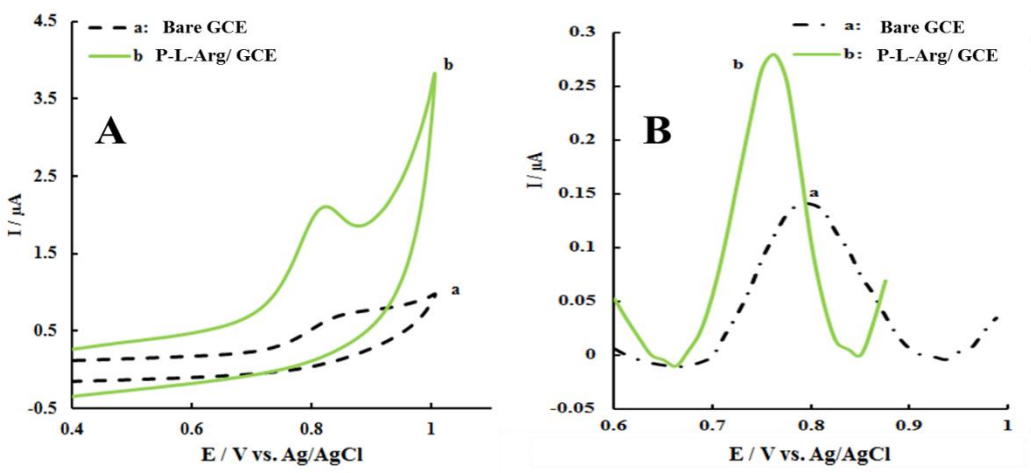


Figure 2. (A) Cyclic voltammograms, (B) Differential pulse voltammograms for 4.9 µM MET on bare GCE and P-L-Arg/GCE (in 0.1 M PBS pH 6.0, scan rate = 100 mV/S)

3.4. Effect of pH on the peak current of MET on P-L-Arg/GCE

The effect of pH in the range of 4.0 to 10.0 was investigated on the anodic peak potential (E_{pa}) and anodic peak current (I_{pa}) in electro oxidation of MET on P-L-Arg/GCE using cyclic voltammetry (CV) from 0.4 to 1.2 V (Figure 3A). The I_{pa} gradually increased from pH 4.0 to 6.0, and then decreased with the further increase in pH (Figure 3B). Due to the high current at pH 7.0 and its proximity to the physiological pH, it was chosen for the electrochemical determination of MET in this paper. The E_{pa} shifted negatively and linearly with the increase in pH from 4.0 to 10.0, with a slope of -65.8 mV/pH (Figure 3B), revealing that equal number of protons and electrons (two electrons, two protons) are involved in the MET oxidation [11, 21].

3.5. Optimization of the electro polymerization process

Keeping the other conditions constant, the only factor which impact on polymeric film formation is the number of cycles applied to the electrode during the electropolymerization. Hence, in a series of experiments electrodes were fabricated with increasing numbers of CV cycles to get the optimal cycle number in electropolymerization process. As shown in Figure 3C, the current response for 5.9 µM of MET reached a maximum of 0.58 µA within 15 cycles, and then lessened with further increasing cycle number. It can be explained that in low cycle numbers, the little electrochemical responses can be ascribed to the thin or insufficient P-L-Arg film formation on GCE surface. On the other hand, the excessive number of cycles can lead to passivation of electrode surface, resulting in lower voltammetry response for MET electrooxidation.

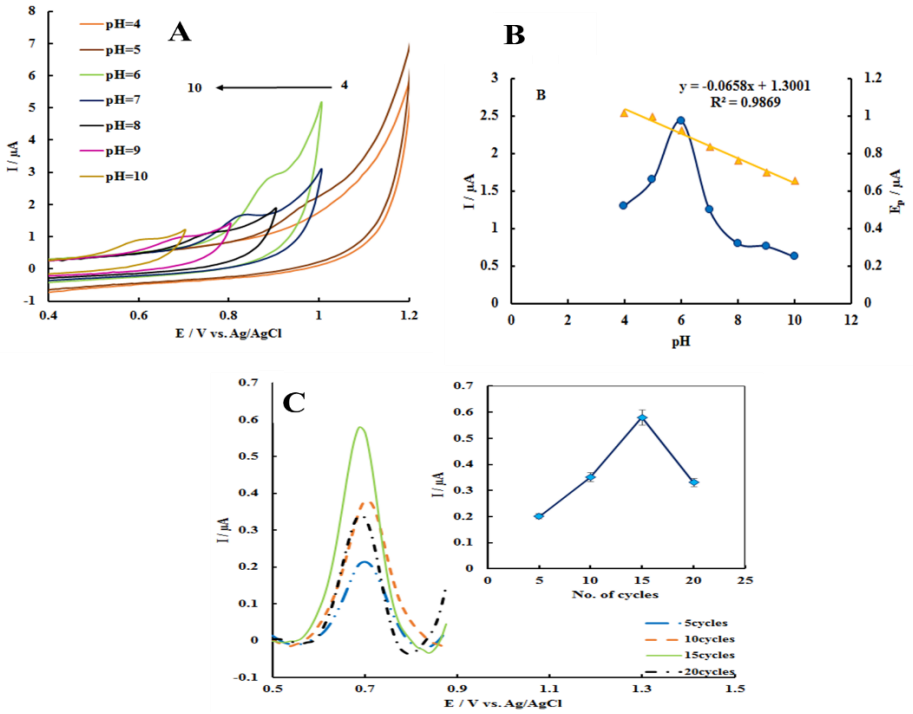


Figure 3. (A) Effect of pH on cyclic voltammograms, (B) I and Ep *vs.* pH, (C) Effect of number of cycles on performance of P-L-Arg/GCE in voltammetric determination of 5.9 µM of MET

3.6. Effect of scan rate on electrooxidation of MET on P-L-Arg/GCE

To get evidence about electrooxidation mechanism of MET on P-L-Arg/GCE, cyclic voltammograms were performed at different scan rates. Figure 4A demonstrates the impact of scan rate on the cyclic voltammograms for 3.98 µM MET on the surface of P-L-Arg/GCE in PBS buffered solution at pH 6.0 in the range of 10-120 mV s^{-1}. As shown in Figure 4B, the anodic peak currents are linearly related to the square root of scan rates, signifying a diffusion-controlled process involved in the electrocatalytic oxidation of MET on P-L-Arg modified GCE. Moreover, the association between the oxidation peak potential and Logarithm of scan rate is exposed in Figure 4C. As seen, the oxidation peak potential moves toward positive potentials with increasing scan rate and the respective linear relationship between E_p and the logarithm of the scan rate is given in Eq. (1). Such a behaviour suggests the kinetic limitation of the electrochemical oxidation for MET on P-L-Arg/GCE. Moreover, even at low scan rates, only one oxidation peak was observed, showing irreversible reaction of MET on this electrode surface.

$$E_{pa}\,(V) = 0.0539 \log \nu\,(mVs^{-1}) + 0.782\ (R^2 = 0.9896) \qquad (1)$$

Such a behaviour is in good agreement with the equation for irreversible electrochemical processes [22]:

$$E_p = \left(\frac{b}{2}\right)\log \upsilon + \text{Constant} \qquad \left(b = \frac{2.303RT}{(1-\alpha)n_\alpha F}\right) \qquad (2)$$

In which α depicts the electron transfer coefficient, n_a depicts the number of electrons participating in the rate-determining step, F is the Faraday constant, R is the gas constant and T is the absolute temperature. Based on Eq. (2) the slope of E_p versus log *v* is b/2, where b designates the Tafel slope and the slope of E_p versus log *v* is $\partial Ep/\partial \log v$, which was found to be 53.9 mV so b = 107.8 mV. This slope designates a two-electron transfer mechanism as the rate limiting step, considering a transfer coefficient of α=0.27. So, in accompany with the results of section 3.4, as seen in Scheme 1, a two electrons- two protons mechanism is assumed for electrooxidation of MET on P-L-Arg/GCE surface.

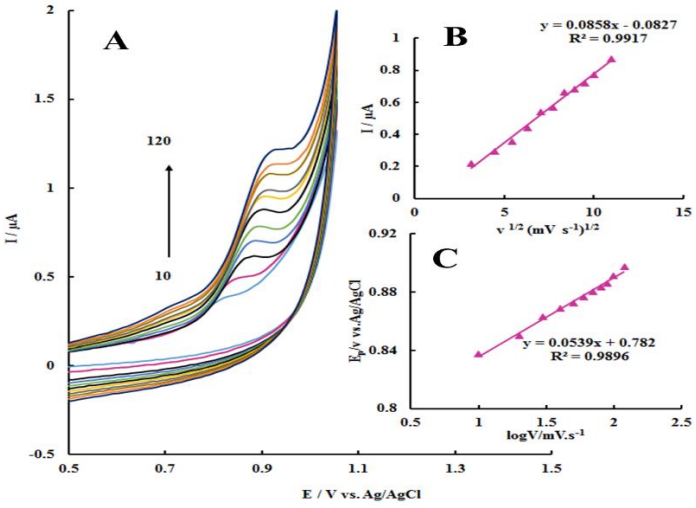
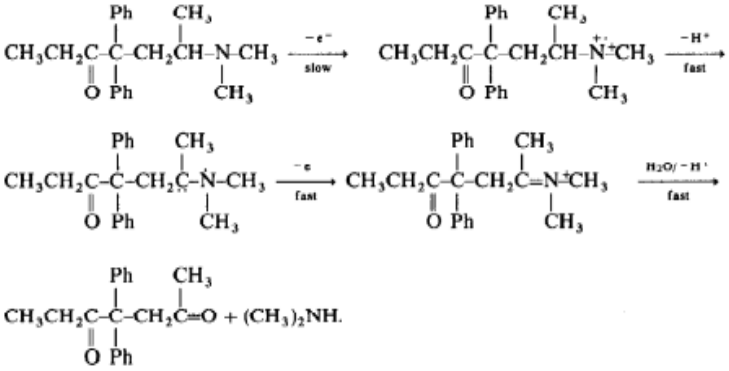


Figure 4. (A) Cyclic voltammograms for MET in phosphate buffer 0.1 M with pH = 7 in the presence of 3.98 µM MET at scan rates in the range of 10-120 mV s^{-1}. Diagram of changes in the anodic peak current *vs.* the square root of scan rate (B) and Log of scan rate (C)



Scheme 1. The electrochemical oxidation mechanism of methadone on P-L-Arg/GCE

3.7. Analytical features of P-L-Arg/GCE in determination of MET

To extract analytical features of P-L-Arg/GCE in analysis of MET, calibration graph of MET was derived from the DPV curves. The differential pulse voltammograms of incremental concentrations of MET were recorded on P-L-Arg/GCE and the results are given in Figure 5.

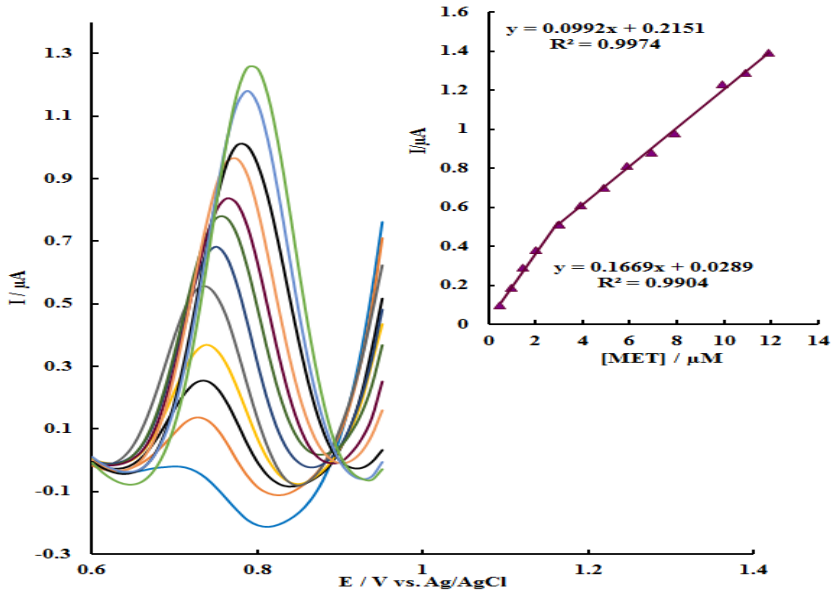


Figure 5. DPVs of methadone with different concentrations in 0.1 M BPS (pH 6.0) on P-L-Arg/GCE (1 → 13): 0.49, 0.97, 1.49, 1.99, 2.98, 3.9, 4.9, 5.9, 6.9, 7.9, 9.9, 10.9, 11.9 µM. Inset: Calibration curve of the anodic peak current *vs.* methadone concentration

Table 1. Comparison of analytical features of various electrochemical sensors in determination of MET

Electrode*	**Detection method**	**Linear range (µM)**	**LOD (µM)**	**Sensitivity** (µA/µM)	**Stability in weeks (RSD for signal)**	**Ref.**
MWCNT/GCE	SWV	0.5–100.0	0.28		NR	[11]
GNPs/MWCNT/CPE	SWV	0.5-300.0	0.005	-	8	[23]
MWCNT/PGE	DPV	0.1–15.0	0.087	-	NR	[24]
(Gr/Ag NPs)2/GCE	DPV	1.0–100.0	0.18	0.084	NR	[25]
AuNPs/PPyox/SPE	SWV	1.0-120	0.45	0.36	NR	[26]
Thioglycolic Acid-CdSe	DPV	0.1-20 µM	0.05	0.069	NR	[27]
Doped Graphene Oxide/Graphite		20-323 µM		0.032		
P-L-Arg/GCE	DPV	0.49-2.98; 2.98-11.9	0.032	0.167, 0.099	1 (< 4.1%)	This Work

* Abbreviations:
MWCNT/GCE: Multi-walled carbon nanotubes/glassy carbon electrode
GNPs/MWCNT/CPE:gold nanoparticles/multi-walled carbon nanotube/carbon paste electrode
MWCNT/PGE: Multi-walled carbon nanotubes/pencil graphite electrode
(Gr/Ag NPs)2/GCE: Two layers of Ag nanoparticles decorated graphene/glassy carbon electrode

It was found that the peak current increases linearly with the concentration of MET in two ranges of 0.49-2.98 µM and 2.98-11.9 µM. The peak current (I_p) is proportionate to the concentration of MET from 0.49-2.98 µM by I_p =0.1669 C + 0.0289, R^2=0.9904 and for range 2.98-11.9 µM by I_p =0.0922C+0.2151, R^2=0.9974. The limit of detection (LOD) was calculated to be 0.032 µM based on the signal-to-noise ratio of 3. The previous works show a wider linear range in most cases compared to the proposed method (Table 1), but the fabricated electrode

showed advantageous features including low detection limit and high sensitivity or comparable values, simple and reproducible fabrication process and good stability. In addition, the sensor can be prepared easily by means of low-cost equipment in a short time with a facile approach.

3.8. Reproducibility, stability and interference studies

Reproducibility and stability as two requirements for a desired sensor were further studied for P-L-Arg/GCE. The relative standard deviations (RSD) for 3 times determination of 2.98 µM MET were obtained as 2.56%, verifying the high reproducibility of the developed sensor. To assess the stability of P-L-Arg/GCE, DPVs were recorded under the same conditions on the first day and a week later. The change in peak currents of MET was less than 4.1%, which specified good stability of P-L-Arg/GCE.

The effect of some possible interference substances in real samples on determination of MET was investigated. Negligible interference appeared for 3.97 µM MET detection except in the presence of 500-fold ascorbic acid, glucose, acetaminophen, sucrose, epinephrine, glysine, cysteine, which may be due to its oxidation on the modified electrode at the same time, signifying the excellent selectivity of this sensors toward MET.

3.9. Analysis of Real Samples

To evaluate the analytical utility of the proposed sensor for real sample analysis, its performance was assessed in measurement of spiked MET in serum and urine samples.

Table 2. Employment of the proposed P-L-Arg/GCE sensor for voltammetric determination of MET in serum and urine samples (n = 3)

No.	Added (µM)	Expected (µM)	Found (µM)	Recovery (%)
Urine				
1	0		Not detected	
2	0.99	1	1.05	106
3	1.99	2	1.92	96.4
4	3.96	4	3.96	100
5	7.89	8	7.88	99.8
Serum				
1	0		Not detected	
2	0.99	1	0.974	98.3
3	1.9	2	1.92	101
4	2.9	3	2.88	99.3

The results of triplicate measurements of MET by differential pulse voltammetry are given in Table 2. These results prove that the P-L-Arg/GCE retained its efficiency for de-termination of MET in real samples with satisfactory recovery values within 96.4 – 106%.

4. CONCLUSION

In this work, P-L-Arg film was electropolymerized on GCE to develop a sensitive sensor for quantification of MET in biological samples. The electro oxidation of MET on the modified electrode was explored by cyclic voltammetry and differential pulse voltammetry. The P-L-Arg film showed electrocatalytic action toward the electrooxidation of MET, describing by the enhancement of the peak current and decrease in oxidation potential. The electro oxidation of MET was found to be an irreversible two-electron and two-proton process with diffusion character. The method presented here is sensitive, selective, fast, convenient and cost-effective compared to some previous reports which involve multistep modification of solid electrodes. In addition, the proposed sensor can be utilized for measurement of MET in real samples with acceptable recoveries.

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