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Electrochemical detection of Methadone by use of poly-l-arginine/carbon nanotubes composite modified carbon paste electrode (P-L-Arg/CNTs/CPE) in human urine

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The current study focused on the easy synthesis of poly-l-arginine and carbon nanotubes composite on carbon paste electrode (P-L-Arg/CNTs/CPE) based on electropolymerization technique as wide range electrochemical sensor for determination of methadone in biological samples for doping monitoring between athletes. Structural analyses using SEM and XRD indicated that the p-L-Arg/CNTs/CPE surface had been covered with a homogenous structure of rod-like snowflakes. The nano-sized twisted bundled CNTs network with excellent surface area served as nucleation sites for the electropolymerization of L-Arg monomers. Study of electrochemical response of P-L-Arg/CNTs/CPE using CV and amperometery techniques illustrated considerable improvement of sensitivity and selectivity of P-L-Arg/CNTs/CPE due to synergistic effect of P-L-Arg and CNTs. Results showed that linear response was from 0 to 600 μM with a correlation coefficient of 0.99990, and the sensitivity and limit of detection of P-L-Arg/CNTs/CPE were obtained to be 0.02961 μA/μM and 22 nM, respectively. The comparison between the obtained sensing parameter of P-L-Arg/CNTs/CPE was compared with the other reported methadone sensors in the litereture revealing the broad linear range to the determination of methadone and an appropriate limit of the detection value of P-L-Arg/CNTs/CPE toward the other reported methadone sensors. The P-L-Arg/CNTs/CPE as a proposed sensing system for analysis of methadone in real samples prepared from the urine of four athletes was evaluated. Results exhibited that the RSD values (3.56% to 4.41%) and recovery (98.86% to 99.91%) satisfied appropriate validity and promising potential of the proposed sensor in the practical sample analysis.

Keywords: L-Arginine, CNTs; Nanocomposite; Methadone; Electropolymerization; Amperometry

1. INTRODUCTION

Methadone (6-(dimethylamino)-4,4-diphenylheptan-3-one) is a drug under the controlled substances act that traded in the brand names Methadose and Dolophine. It is an artificial opioid

agonist that is prescribed for moderate to severe pain. Methadone exhibits full agonist acting at the μ-opioid receptor. While μ-opioid receptor agonism is the main mechanism of act for pain treatment, methadone also performs as an agonist of σ- and κ-opioid receptors in the peripheral and central nervous system [1, 2]. It is also usually used to treat opiate habits, particularly habit to Heroin. Methadone reacts on similar opioid receptors as heroin and morphine to steady patients and minimalize withdrawal signs into the case of a habit [3, 4].

According to recent studies, long-term methadone treatment can affect cognitive functioning, such as learning and memory, and can change the brain, and treatment may affect the nerve cells in the brain [5, 6]. Side effects of long-term methadone treatment include agitation, and anticholinergic properties (fast heart rate, palpitation and dry mouth) cardiac arrest [7]. Drowsiness is one of methadone's characteristic side effects [8]. Extreme fatigue is common after taking methadone because the medication stimulates changes in brain chemistry, which can interfere with sleep regulation. Sports life can lead to substance abuse for some reasons, counting performance improvement, self-medication of untreated mental illness, and coping with stressors, like retirement, injuries, pressure to perform, and physical pain from sports. Moreover, it is also reported methadone is recreational medicine used by student-athletes, and new concerns are illicit drug use, prescription stimulant drug abuse, and narcotic drugs [9, 10].

As a result, population-level opioid-related harms or the opioid crisis is essential for identification and determination of methadone levels in biological and pharmaceutical fluids samples [11]. Gas chromatography-mass spectrometry assay [12], liquid chromatography-tandem mass spectrometry [13], high-performance liquid chromatography technique [14], radioimmunoassay [15], capillary electrophoresis [16], spectrofluorimetric method [17] and electrochemical analyses [18-24] are the analytical techniques which have been studied for determination of methadone level in clinical samples. However, many of these techniques need rather high-priced instrumentation, and require comprehensive, time-consuming, and costly pre-treatment and sample preparation. Between these techniques, electrochemical analyses have been presented with low cost and ease of sample preparation and analysis process with accurate outcomes for wide ranges of chemical inorganic and organic materials [25-27]. The novelty and advantage of this work is represented an accessible synthesis of P-L-Arg/CNTs/CPE that could create natural polysaccharides with an amorphous structure and thus improve the selectivity and sensitivity methadone sensors in a biological sample.

2. EXPERIMENT

2.1. Synthesis of P-L-Arg/CNTs/CPE

To prepare the CPE, graphite powder (90%, Qingdao Furuite Graphite Co., Ltd., China) and paraffin oil (99%, Hebei Tianhao Plastic Additives Co., Ltd., China) of high purity were mixed in the ratio of 7:3. After thoroughly grinding, the mixture was packed into the cavity of Teflon tube (1.8 mm diameter). A copper wire as electrical contact was established via a stainless steel handle. The electrode was polished on a clean butter paper to achieve a smooth surface. For modification the CPE surface with P-L-Arg/CNTs, the CPE was subjected to cyclic potential sweeps, between -1.0 and + 0.9

V vs. Ag/AgCl at a scan rate of 20 mV/s for 20 cycles in support electrolyte 0.1 M phosphate buffer solution (pH 6.0) containing 3 mM L-Arg (99%, Xi'an Faitury Bio-Tech Co., Ltd., China) and 0.5 mg/ml CNTs (99%, Guangzhou Hongwu Material Technology Co., Ltd., China) [28]. The electropolymerization was conducted on potentiostat/galvanostat (Autolab® model PGSTAT 10N, Eco Chemie, Netherlands) which equipped with a Pt wire as the counter electrode, an Ag/AgCl electrode as the reference electrode and CPE as the working electrode.

2.2. Real samples preparation

The urine sample was taken from a healthy volunteer athlete. The sample was filtered and centrifuged, and then used as electrolyte in electrochemical cell. Before analyses, the Methadone tablet (Neogen NV, Belgium) which contains 2.5 mg methadone hydrochloride was dissolved in 100 mL prepared urine samples (72.2 μM). The conventional addition approach was also utilized in the study of real sample.

2.3. Instrumentation

Scanning electron microscopy (SEM; Hitachi 3000, Tokyo, Japan) and an X-ray diffraction (XRD) with D8-Advance Bruker diffractometer was employed to analyze the morphology and crystal structure of nanostructures. Cyclic voltammetry (CV) and amperometry measurements were achieved on a potentiostat/galvanostat. An electrochemical analysis was done in a 0.1M phosphate buffer solution(PBS) with a pH 7.0 that was prepared by combining 0.1M KH_2PO_4-K_2HPO_4 and 0.1M NaCl solutions.

3. RESULTS AND DISCUSSION

3.1. Structural analyses

Figure 1 displays SEM images of CNTs/CPE, P-L-Arg/CPE and P-L-Arg/CNTs/CPE. Figure 1a's SEM image of CNTs/CPE exhibits that a smooth and randomly entangled surface morphology of pure CNTs network was covered the CPE. It can be observed from the SEM image that CNTs formed as bundles and some bundles are twisted together. The CNTs have approximately 70nm in diameter. SEM image in Figure 1b indicates that the p-L-Arg/CNTs/CPE surface has covered with a homogenous structure of rod-like snowflakes. The nano-sized twisted bundled CNTs network with excellent surface area serves as nucleation sites for the electropolymerization of L-Arg monomers. As seen, the P-L-Arg is uniformly coated over the CNTs and forms a high aspect ratio structure. The -COOH and -NH_2 groups of amino acid show a main role into the electro-polymerization procedure onto the CNTs and electrode surface [29-31]. The enhanced roughness and porosity in p-L-Arg/CNTs/CPE surface, thereby improving the catalytic activity and surface area of the modified electrode [32, 33].

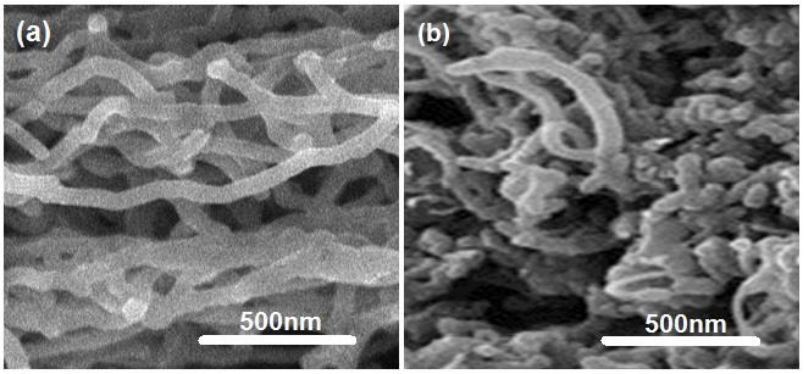

Figure 1. SEM images of (a) CNTs and (b) P-L-Arg/CNTs.

Figure 2 shows the XRD patterns of powders of CNTs, P-L-Arg and P-L-Arg/CNTs. It is observed from XRD pattern of CNTs, there is a prominent characteristic XRD peaks at 2θ=26.07° and 43.31, which indexed to the (002) and (100) planes of CNTs [34-36]. The P-L-Arg/CNTs XRD pattern displays additional diffraction peaks at 2θ= 11.14°, 18.39°, 19.21°, 23.10° and 27.60°, which a natural polysaccharide has an amorphous structure according to JCPDS card No. 00–030-1527 [37-39], that indicate to successful electropolymerization of P-L-Arg on the CNTs surface.

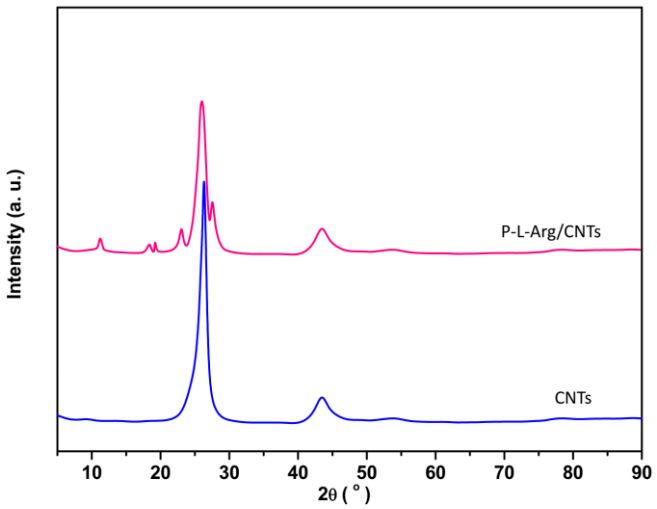

Figure 2. XRD patterns of powders of CNTs and P-L-Arg/CNTs.

3.2. Study of electrochemical response

The CV curves of P-L-Arg/CPE, CNTs/CPE,and P-L-Arg/CNTs/CPE at scanning rate of 15 mV/s for a potential range from 0.0 to 1.2V into 0.1M PBS of pH 7.0 are displayed in Figure 3. The CV curves are shown in both conditions of absence and presence of methadone solutions in electrochemical cells. It can e found that any peak is not appeared for CV of all electrodes in lack of methadone solution. After adding the methadone in electrolyte solution, the anodic peaks at 0.90 V, 0.87 V and 0.82 V appeared in CV curves of P-L-Arg/CPE, CNTs/CPE, and P-L-Arg/CNTs/CPE, respectively, that it can be attributed to the probable oxidation mechanism of methadone as shown in

Figure 4 [40-42]. In addition, Figure 5 depicts that the CV peak current of P-L-Arg/CNTs/CPE is more significant than that of P-L-Arg/CPE and CNTs/CPE, and anodic peak is observed at a substantially lowest positive potential. It reflects to considerable improvement of P-L-Arg/CNTs/CPE electrocatalytic activity due to synergistic effect of P-L-Arg and CNTs. The spaghetti-like structure of CNTs with oxygen functional groups like −COOH, −OH, =O, and −O− as active sites enhance the electrocatalytic performance because of increased adsorption capacity as well as the high surface area of CNTs [43-45]. L-Arg as amino acid possesses the positive charge dispersed on three N atoms in a guanidyl group, and its multidentate characteristics assist L-Arg in shaping electrostatic interactions and long-range hydrogen-bonding with methadone groups [46, 47]. The electrodepolymerized L-Arg on electrode surface could act as tiny centers to accelerate the electron transfer between the analyte and the electrode [48, 49]. Therefore, further electrochemical tests were conducted on P-L-Arg/CNTs/CP.

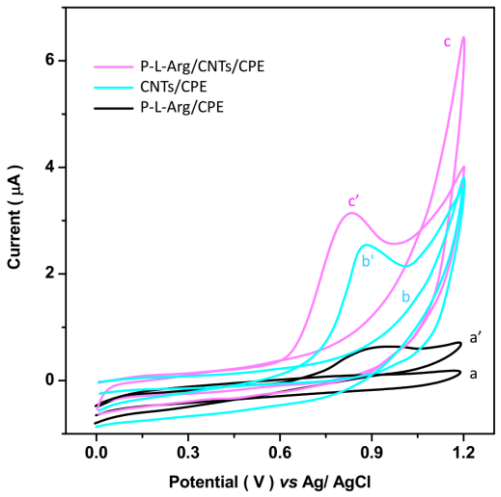

Figure 3. The CV curves of (a and a') CNTs/CPE, (b and b') P-L-Arg/CPE and (c and c') P-L-Arg/CNTs/CPE at scanning rate of 15 mV/s for a potential range from 0.0 to 1.2V into 0.1M PBS of pH 7.0.

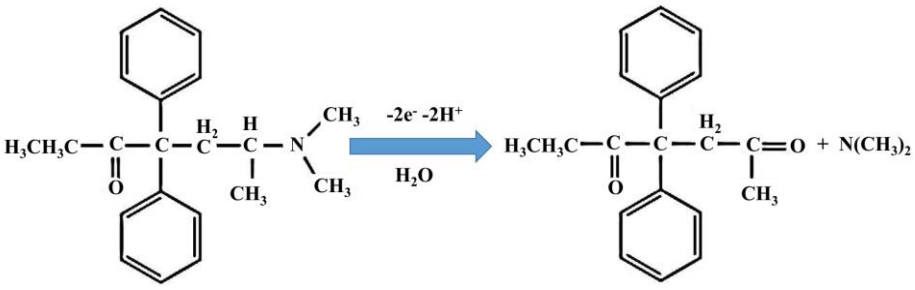

Figure 4. The probable oxidation mechanism of methadone [40].

Figure 5a depicts the obtained amperometric of P-L-Arg/CNTs/CPE through successive injections of a solution containing 30 μM methadone in electrochemical cell containing 0.1 M PBS (pH 7.0) electrolyte solution at an applied potential of 0.82 V. A well-defined and stable amperometric response is obtained in consecutive addition of methadone. With methadone addition in each step, the

response current increased and a steady state current response was observed. The fast reaction of proposed methadone sensor can be mainly attributed to the combination P-L-Arg and CNTs that provide an efficient conductive pathway to transfer electrons [50, 51]. The corresponding calibration graph in Figure 5b shows that there is a linear response from 0 to 600 µM with a correlation coefficient of 0.99990.

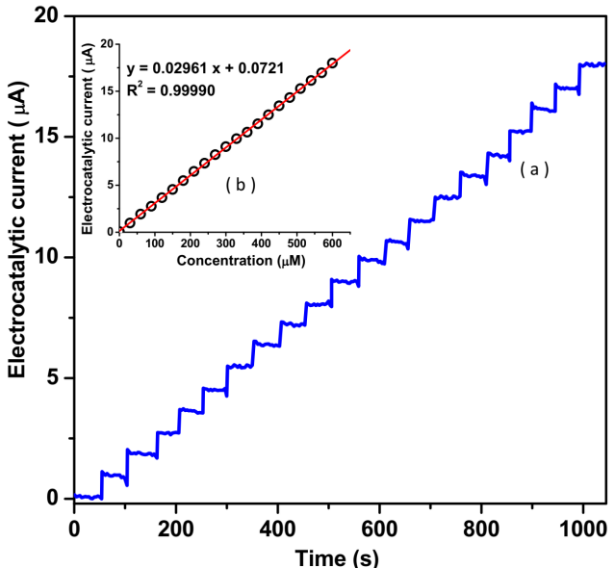

Figure 5. (a) Obtained amperogram of P-L-Arg/CNTs/CPE through successive injections of a solution containing 30 µM methadone in electrochemical cell containing 0.1 M PBS (pH 7.0) electrolyte solution at an applied potential of 0.82 V, and (b) the corresponding calibration graph.

Table 1. Comparison between the P-L-Arg/CNTs/CPE electrocatalytic performance and other reported methadone sensors

Electrode	**Technique**	**LOD (nM)**	**Linear range (µM)**	**Ref.**
P-L-Arg/CNTs/CPE	Amperometry	22	0 to 120	This work
MWCNT/pencil graphite electrode	DPV	87	0.1 – 15	[19]
(Graphene /Ag NPs)$_2$ /GCE	DPV	180	1.0–100	[20]
Functionalized MWCNT/GCE	DPV	280	0.5 – 15	[21]
Poly (diallyldimethylammonium chloride)/MWCNT/functionalized carbon quantum dots	DPV	30	0.1–225	[23]
MWCNT/CPE	SWV	300	0.5 – 300	[22]
Au NPs/MWCNT/GCE	SWV	5	0.1–500	[21]
Fe_3O_4@SiO_2/CPE	SWV	3	0.01 – 8	[24]

SWV: square wave voltammetry

The hindered electron transfer kinetics may result in reduced sensitivity at high concentrations of methadone (≥ 600 μM) [43, 52]. The sensitivity of P-L-Arg/CNTs/CPE is obtained to be 0.02961 μA/μM, and limit of detection (S/N = 3) is be determined to be 22 nM. The obtained sensing parameter of P-L-Arg/CNTs/CPE is compared with the other reported methadone sensors in litereture in Table 1. As found, the proposed sensor in this study reveals the broad linear range to determination of methadone and an appropriate limit of detection value toward the other reported methadone sensors that can be related to the introduction of -NH_2 and -COOH groups of amino acids of L-Arg into the CNTs and aminofunctionalized CNTs acts as useful precursor for reacting with analyte molecules [29].

Possible interfering effects of external species were also investigated by amperometric measurements using P-L-Arg/CNTs/CPE upon consecutive additions of a solution containing 10 μM methadone and 50 μM external species solutions in 0.1M PBS at applied potential of 0.82 V. The results tabulated in Table 2 demonstrate that after adding methadone solution in electrochemical cell, a considerable amperometric signal is formed, and addition interference species in electrolyte solution show no significant impact on the methadone signal, indicating the proper selectivity of the proposed methadone electrochemical sensor.

Table 2. The results of amperometric measurements using P-L-Arg/CNTs/CPE upon consecutive additions of a solution containing 10 μM methadone and 50 μM external species solutions in 0.1 M PBS

Substance	**Added(μM)**	**Amperometric signal(μA) at 0.82 V**	**RSD**
Methadone	10	0.2977	±0.0045
ascorbic acid	50	0.0178	±0.0019
Glucose	50	0.0126	±0.0013
uric acid	50	0.0121	±0.0011
Morphine	50	0.0361	±0.0010
citric acid	50	0.0106	±0.0017
Acetaminophene	50	0.0198	±0.0012
oxalic acid	50	0.0097	±0.0008
Caffeine	50	0.0110	±0.0010
Ca^{2+}	50	0.0151	±0.0013
Na^{+}	50	0.0072	±0.0010
Al^{3+}	50	0.0084	±0.0011
Ag^{+}	50	0.0087	±0.0011
Fe^{2+}	50	0.0086	±0.0012
Mg^{2+}	50	0.0067	±0.0012
NH_4^{+}	50	0.0065	±0.0012
NO_3^{-}	50	0.0074	±0.0010

The P-L-Arg/CNTs/CPE as a proposed sensing system for analysis of methadone in real samples prepared from urine of four athletes was evaluated. Figure 6 depicts the obtained data from

amperometric measurements of P-L-Arg/CNTs/CPE through consecutive additions of a solution containing 30 µM methadone in 0.1 M PBS (pH 7.0) prepared from urine sample at an applied potential of 0.82 V. The related calibration graph in Figure 6b displays that the methadone level in prepared sample is 72.87 µM. Additionally, the findings of analytical studies in Table 3 reflect that the RSD values (3.56% to 4.41%) and recovery (98.86% to 99.91%) satisfy appropriate validity and good potential of proposed sensor in the practical sample analysis.

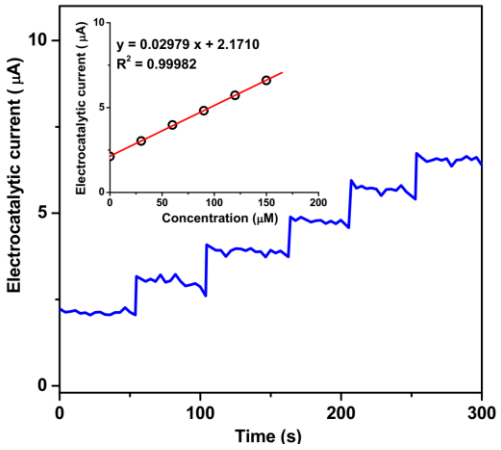

Figure 6. (a) obtained data from amperometric measurements of P-L-Arg/CNTs/CPE through consecutive additions of a solution containing 30 µM methadone in 0.1 M PBS (pH 7.0) prepared from urine sample at an applied potential of 0.82 V; (b) the corresponding calibration graph.

Table 3. Analytical applicability of P-L-Arg/CNTs/CPE to determine methadone in real specimens.

Adding (µM)	Detected (µM)	Recovery (%)	RSD (%)
30.00	29.82	99.40	4.41
60.00	59.95	99.91	3.56
90.00	88.98	98.86	4.18
120.00	119.90	99.91	4.14

4. CONCLUSION

Summary, this work was carried out for the synthesis of P-L-Arg/CNTs/CPE based on electropolymerization technique as a wide range electrochemical sensor for the determination of methadone in biological samples for doping monitoring between athletes. Structural analyses indicated that the p-L-Arg/CNTs/CPE surface had been covered with a homogenous structure of rod-like snowflakes. The nano-sized twisted bundled CNTs network with the great surface area served as nucleation sites for the electropolymerization of L-Arg monomers. Results of electrochemical analyses of P-L-Arg/CNTs/CPE illustrated considerable improvement in sensitivity and selectivity of P-L-

Arg/CNTs/CPE due to a synergistic effect of P-L-Arg and CNTs. results showed that linear response was from 0 to 600 μM with a correlation coefficient of 0.99990. The sensitivity and limit of detection of P-L-Arg/CNTs/CPE were obtained to be 0.02961 μA/μM and 22 nM, respectively. The comparison between the obtained sensing parameter of P-L-Arg/CNTs/CPE was compared with the other reported methadone sensors in the litereture revealed the broad linear range to the determination of methadone and an appropriate limit of the detection value of P-L-Arg/CNTs/CPE toward the other reported methadone sensors that it can be related to the introduction of -NH_2 and -COOH groups of amino acids of L-Arg into the CNTs and aminofunctionalized CNTs acts as a useful precursor for reacting with analyte molecules. The P-L-Arg/CNTs/CPE as a proposed sensing system for analysis of methadone in real samples prepared from the urine of four athletes was evaluated and results exhibited that the RSD and recovery values satisfied appropriate validity and promising potential of the proposed sensor in the practical sample analysis.

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