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Full Paper

Overoxidized Polypyrrole/ Gold Nanoparticles Composite Modified Screen-Printed Voltammetric Sensor for Quantitative Analysis of Methadone in Biological Fluids

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Abstract- A highly sensitive voltammetric sensor for quantitative analysis of methadone in biofluids is introduced. The proposed sensor is based on screen-printed electrode (SPE) modification with overoxidized polypyrrole and gold nanoparticles composite (AuNPs/PPyox/SPE). Electrochemical measurements were carried out using square wave voltammetry (SWV). Morphological and electrochemical characterization of the proposed composite were studied using field emission scanning electron microscopy (FE-SEM), electrochemical impedance spectroscopy (EIS), and cyclic voltammetry. A wide linear range of 1 to 120 µmol L^{-1} and the limit of detection of 0.45 µmol L^{-1} in methadone analysis obtained by the presented sensor. The proposed sensor shows proper stability and high sensitivity in quantitative analysis of standard samples and can be successfully used for methadone determination in biological fluids.

Keywords- Methadone; Overoxidized polypyrrole; Gold nanoparticles; Square wave voltammetry; Biological fluids

1. INTRODUCTION

Methadone.HCl, (6-(dimethylamino)-4,4-diphenyl-3-hepatanone hydrochloride or Dolphin, MET) is an opioid medicine which commonly used to treat dependency in addicts on heroin or other opiate drugs. The drug increases addicts' tolerance to opioids and also decreases their need for them. Methadone.HCl is also used for other purposes such as relief moderate to

severe pains. In spite of the fact that methadone is principally utilized for treating opioid addiction, there are side effects that threaten users; including dizziness, sleepiness, vomiting, severe sweating, weak or shallow breathing, severe constipation, lung problems, and death [1-5]. Due to these mentioned side effects, developing a rapid and accurate method for the quantitative determination of methadone in real samples is necessary. Until now, different methods, such as high-performance liquid chromatography (HPLC) [6], ultra-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS) [7], capillary zone electrophoresis [8], liquid chromatography-UV-visible spectroscopy (LC-UV) [9], gas chromatography-mass spectrometry (GC-MS) [10], thin layer chromatography (TLC) [11] and electrochemical methods [12-14] have been applied for determining the low levels of methadone concentration. Besides their high precision and accuracy, these methods are time-consuming and require expensive apparatus and reagents. Electrochemical detection methods on the contrary offer several advantages in methadone recognition such as simplicity of operation, low cost, direct detection, scaling down, and quick response times [15-24].

Conjugated conducting polymers which were discovered in the mid-70s are a class of conducting polymers with numerous attributes in sensing, particularly high sensitivity and short response time [25]. Conducting polymers have been widely used by researchers to fabricate electrochemical sensors for analyzing the trace levels in different fields of medicine, food, and the environment [26].

Among conducting polymers, PPy with superior characteristics, including high electrical conductivity, good stability, simplicity in synthesis and derivatization, and biocompatibility has been most commonly used in the manufacturing of sensors and electrochromic devices [27-29].

At high positive potentials, under removing dopant ions and decreasing conductivity, PPy can be overoxidized. The overoxidized-polypyrrole (PPyox) has the properties of cation exchanging and molecular sieving toward various species [30-32].

Hybrids of conducting polymers have great importance in sensor fabrication. These hybrid materials are composites consisting of conducting polymers and of other materials such as metallic nanoparticles, carbon nanomaterials, and metal oxides. Hybrids of conducting polymers provide a large surface area which causes an increase in chemical interactions with the surface and consequently facilitates the electron transfer process. Additionally, they show specific physical, electrical, and electrochemical properties which make them popular among researchers in sensor and biosensor fabrication [33-35]. Due to the high conductivity and special structural characteristics, conducting polymers can be used as a carrier or a substrate for metallic nanoparticles [36]. Metallic nanoparticles in conducting polymers provide catalytic sites to accelerate the electron transferring process. In these systems, the reduction of metal salts to metallic micro or nanoparticles can be mediated by the redox properties of conducting polymers [37-40]. Composites of conducting polymers and metallic nanoparticles improve the

sensitivity, stability, reproducibility and limit of detection in the operation of sensors and biosensors .

In this study, we aim to develop a conducting polymer composite modified screen-printed electrode for methadone determination in biological fluids. The composite of gold nanoparticles and overoxidized polypyrrole is prepared by sequential or in situ synthesis method on the surface of the carbon working electrode. The AuNPs/ PPyox composite has greatly increased the sensor response toward methadone. Additionally, a significantly negative shift was observed in the methadone peak potential. The proposed sensor shows high stability and low limit of detection in voltammetric detection of methadone hydrochloride in biological fluids.

2. Experimental

2.1. Reagents

Pyrrole (Merck, > 98%) after purification by double distillation, protected from light and stored at low temperature. Potassium iodide (KI) and tetrachloroauric (III) acid trihydrate ($HAuCl_4.3H_2O$) were obtained from Merck (Darmstadt, Germany). All other chemicals including sodium hydroxide, potassium nitrate, potassium chloride, nitric acid, potassium hexacyanoferrate, ascorbic acid, uric acid, and glucose were of analytical reagent grade and purchased from Merck (Darmstadt, Germany). Methadone hydrochloride was obtained from Darou Pakhsh Pharmaceutical Company (Tehran, Iran). A stock solution of methadone hydrochloride was prepared in 0.1 M phosphate buffer solution (pH 7) and diluted to prepare other concentrations.

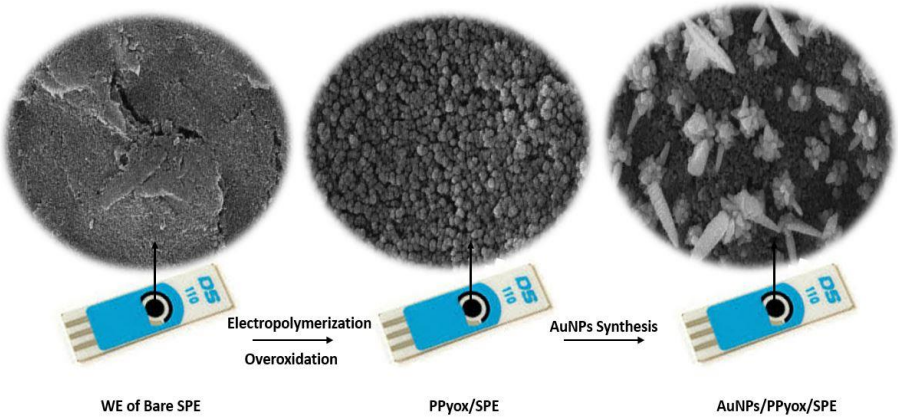
2.2. Apparatus

Electrochemical polymerization of pyrrole and other voltammetric measurements including cyclic voltammetry and square wave voltammetry were performed using a DropSens μStat400 Bipotentiostat/Galvanostat (Asturias, Spain). Screen-printed electrodes which contain silver reference electrodes and carbon counter and working electrodes were purchased from DropSens, (Asturias, Spain). An AUTOLAB PGSTAT 30 was used to perform electrochemical impedance spectroscopy (EIS) measurements. In order to characterize the morphology of the modified surfaces, field emission scanning electron microscopy (FE-SEM) was carried out using a Hitachi S4160 (Cold Field Emission) microscope.

2.3. Fabrication of the AuNPs/PPyox composite modified screen-printed electrode

In order to fabricate the composite modified screen-printed electrode, electro-polymerization of polypyrrole on the screen-printed electrode was firstly performed with 50 μL of a solution containing 0.1 M pyrrole and 0.1 M KI in the potential range of 0.0 to 0.8 V

for 3 cycles with the scan rate of 20 mVs^{-1}. The obtained electrode indicated as PPy/SPE. The electrodeposited PPy was overoxidized by 50 µL NaOH 0.1M at +1V for 700s. The resulting electrode is indicated as PPyox/SPE. Gold nanoparticles were synthesized electrochemically on the surface of overoxidized polypyrrole with 50 µL of 0.6 mM $HAuCl_4.3H_2O$ containing 0.1M KNO_3 solution in the potential range of -1 to 0.2 V for 10 cycles with the scan rate of 50 mVs^{-1}. This electrode is indicated as AuNPs/ PPyox/SPE and Scheme1 represents the required procedures for its preparation.



Scheme 1. Schematic representation of the procedure for AuNPs/ PPyox/SPE preparation

2.4. Electrochemical impedance spectroscopy (EIS) and square wave voltammetry (SWV) measurements

Electrochemical impedance spectroscopy (EIS) measurements were performed using an AUTOLAB PGSTAT 30 on the bare and modified screen-printed electrodes with 50 µL of the aqueous solution containing 10 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ and 0.1 mol L^{-1} KCl. The measurements were performed in the frequency range from 1000 kHz to 0.1Hz with the applied potential of 0.2 V and the AC voltage amplitude of 10 mV. The square wave voltammetry measurements were carried out with 50 µL of the solution on the bare and modified surfaces in the potential range from 0.55 to 0.95 V with a frequency of 10 Hz and a pulse amplitude of 0.05 V.

2.5. Interference studies

In order to investigate the effect of the interferences on the sensor response, different solutions containing methadone and other species including ascorbic acid, uric acid, glucose, calcium, and potassium with the ratio of 1:100 (methadone: interfering species) were prepared in phosphate buffer and analyzed by the composite modified screen-printed electrode in the optimized conditions with square wave voltammetry.

2.6. Real sample analysis

Urine and blood samples were taken from healthy volunteers. In the case of urine samples, 5 ml of each sample, after filtering, was adjusted to the optimum pH by adding 5 ml of 0.1 M phosphate buffer solution (pH 7), and subsequently urine samples were spiked with 100 µL, 250 µL, and 500 µL of 1mM methadone solution. After that 50 µL of the prepared solutions were dropped on the AuNPs/PPyox modified SPE and recoveries in methadone determination were obtained by SWV. For blood samples, firstly the plasma was separated by centrifugation. To remove the proteins, concentrated acids were added to the plasma samples and centrifuged subsequently. Proper amounts of supernatant were diluted 10 times and spiked with 100 µL, 250 µL, and 500 µL of 1mM methadone. The result solutions were analyzed by the AuNPs/PPyox modified screen-printed electrodes.

3. Results and Discussion

3.1. Structure and Morphology Characterization of the AuNPs/PPyox composite

Characterization of the bare and modified screen-printed surfaces was carried out using a scanning electron microscope. The FE-SEM images of these surfaces are presented in Figure 1. For the bare screen-printed electrode (Figure 1a), only the carbon layer used in the working electrode construction is observed while in the PPyox/SPE images (Figure 1b), the spherical overoxidized polypyrrole particles with an average diameter of 80 nm are distributed uniformly on the electrode surface. In the FE-SEM images of the AuNPs/PPyox/SPE (Figure 1c), the uniform distribution of the flower-shaped gold nanoparticles on the overoxidized polypyrrole is clearly observed. The porous structure of the composite and the uniform distribution of the synthesized particles provides a large electroactive surface which facilitates the electron transfers on the composite modified electrodes compared to the bare electrodes.

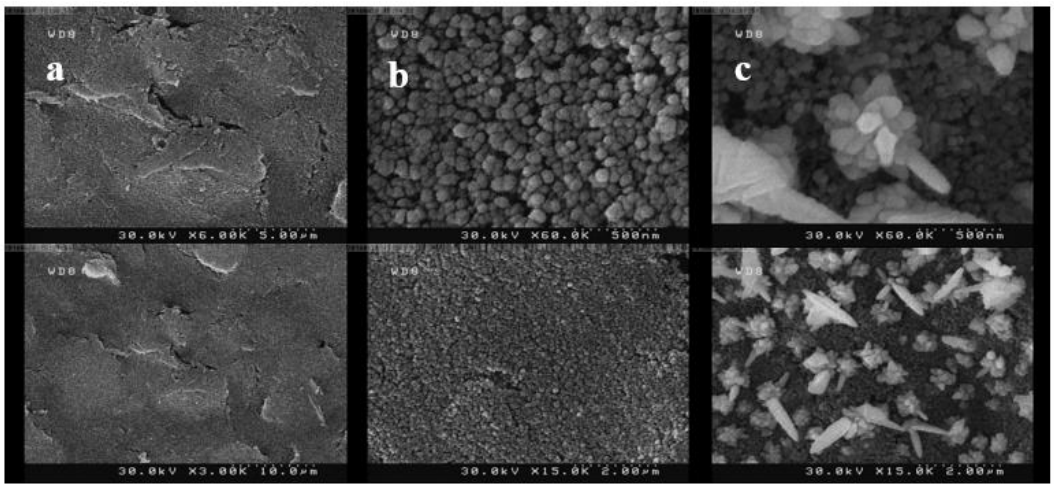


Figure 1. SEM images of (a) Bare SPE, (b) PPyox/SPE and (c) AuNPs/PPyox/SPE

3.2. Electrochemical characterization of AuNPs/PPyox composite

3.2.1. Cyclic voltammetry and electrochemical impedance spectroscopy studies of electrochemical marker on the surface of the AuNPs/PPyox/SPE

In these studies, $[Fe(CN)_6]^{3-/4-}$ couple was used as a marker for electrochemical characterization of the unmodified and AuNPs/PPyox composite modified surfaces. Cyclic voltammograms on the surface of the bare SPE, PPyox/SPE, and AuNPs/PPyox/SPE in 10 mmol L^{-1} solution of $[Fe(CN)_6]^{3-/4-}$ containing 100 mmol L^{-1} KCl are shown in Figure 2. The peak current on the AuNPs/PPyox/SPE surface is significantly higher than the peak current in the case of bare SPE. Additionally, there is about a 200 mV negative shift in the redox potential of the electrochemical marker at the AuNPs/PPyox/SPE surface in comparison with the bare SPE. These results indicate the increase in surface area and electrical conductivity in the presence of the overoxidized polypyrrole and gold nanoparticles composite.

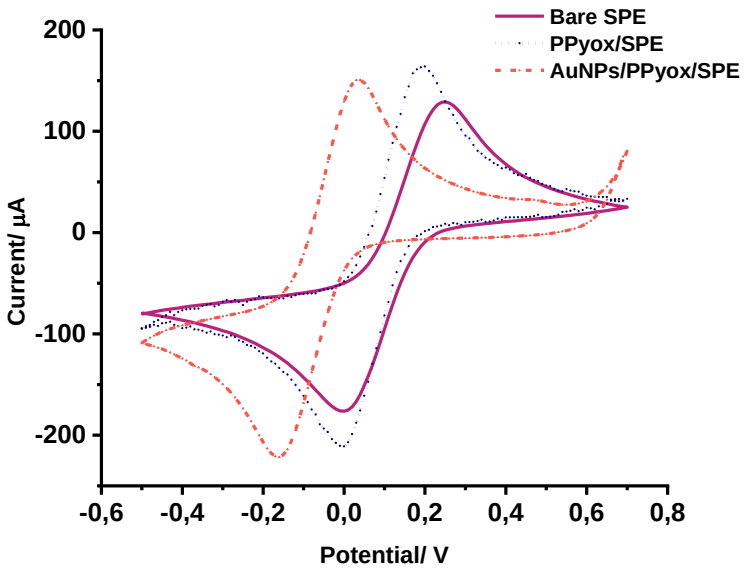


Figure 2. Cyclic voltammograms in the aqueous solution of $[Fe(CN)_6]^{3-/4-}$ 0.01 mol L^{-1} containing 0.1 mol L^{-1} KCl on the surface of bare SPE, PPyox/SPE, and AuNPs/PPyox/SPE

Electrochemical impedance spectroscopy (EIS) is an appropriate technique to study the interfacial characteristics of the modified electrode surfaces. The magnitude of charge-transfer resistance (R_{CT}) as an important characteristic of the modified electrode surfaces can be represented by the diameter of the semicircle part of the Nyquist plot. As can be seen in Figure 3, the R_{CT} value for bare SPE is about 253 Ω while after modifying the electrode surface with polypyrrole, the R_{CT} significantly decreased, indicating that PPy accelerates the process of the interfacial electron transfer. After overoxidation of the PPy film, the conductivity will decrease and R_{CT} slightly increase, however, overoxidation is an essential step for accumulation and adsorption of methadone on the electrode surface. By electrodeposition of the gold

nanoparticles on the PPyox film, the R_{CT} will slightly decrease, suggesting that the AuNPs/PPyox/SPE with large surface area and high electrical conductivity can facilitate the electron transferring process in methadone determination.

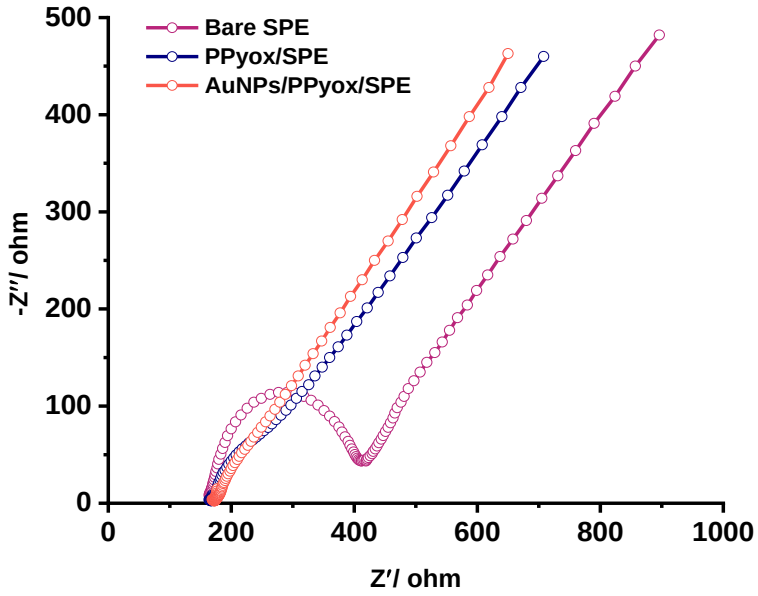


Figure 3. Nyquist plots in the aqueous solution of $[Fe(CN)_6]^{3-/4-}$ 0.01 mol L^{-1} containing 0.1 mol L^{-1} KCl on the surface of bare SPE, PPyox/SPE, and AuNPs/PPyox/SPE

3.2.2. Cyclic voltammetry and square wave voltammetry studies of methadone on the surface of the AuNPs/PPyox/SPE

Cyclic voltammetry (CV) is the most important and extensively used technique in analytical electrochemistry to study the redox processes and the kinetics of electron transfer. In order to investigate the redox properties of the proposed conducting polymer composite, the electrochemical behavior of methadone was studied on the surface of the bare and composite modified screen-printed electrodes by cyclic voltammetry. Figure 4 (Left) shows the cyclic voltammograms of methadone in the optimized conditions on the surface of bare and modified electrodes. As presented in this figure, the electrochemical oxidation of methadone on the bare SPE shows an irreversible peak with an oxidation potential of 832 mV. On the surface of the PPyox/SPE, the voltammogram shows a notable enhancement in the peak current and a negative shift to the oxidation potential of 762 mV. The current response on the surface of the AuNPs/PPyox/SPE increased significantly and a large negative shift to the oxidation potential of 631 mV was observed. This phenomenon is related to the high surface area and the facility of the electron transferring process in the presence of the proposed conducting polymer composite.

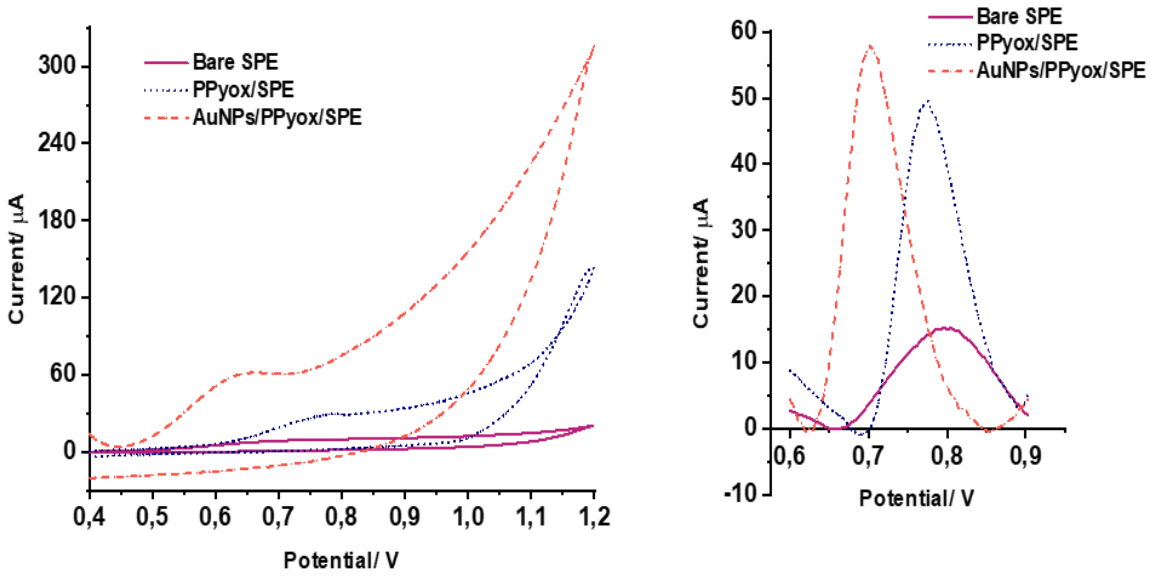


Figure 4. (Left) Cyclic voltammograms in 1000 µmol L^{-1} and (Right) Square wave voltammograms in 100 µmol L^{-1} of methadone solution prepared in phosphate buffer, pH 7, on the surface of bare SPE, PPyox/SPE and AuNPs/PPyox/SPE (SWV frequency: 10 Hz, pulse amplitude: 0.05V)

Figure 4 (Right) shows the square wave voltammograms on the surfaces of bare and modified SPEs in 100 µmol L^{-1} of methadone prepared in phosphate buffer with pH 7. As shown in this figure, the methadone response on the bare SPE is insignificant in comparison with the response on the surface of PPyox/SPE and AuNPs/PPyox/SPE. In the case of PPyox/SPE, the presence of carbonyl and hydroxyl groups on the polypyrrole surface which formed during the overoxidation led to the adsorption of methadone with a positive charge (pk_a 9.12), and consequently, the response at the PPyox/SPE will increase compared to the bare SPE. On the AuNPs/PPyox/SPE surface, not only does the overoxidized PPy act as an adsorption layer but also gold nanoparticles provide a large surface area with electrocatalytic activity toward methadone. As a result, at the AuNPs/PPyox/SPE the current response is about four-fold than that at the bare SPE, additionally around 100mV negative shift in methadone peak potential is observed on the AuNPs/PPyox/SPE in comparison with bare SPE.

3.3. Analytical performance of the sensor

The calibration curve was plotted using the presented AuNPs/PPyox screen printed-electrode at optimized conditions. The square wave voltammograms were recorded after dropping 50 µL of methadone with different concentrations on the surface of composite modified SPE. The equation of I(µA)=0.36C (µmol L^{-1}) +14.11 with the correlation coefficient of 0.98 shows the dependence of the recorded current on the methadone concentration.

According to the obtained results, the linear range and limit of detection of the proposed sensor were obtained to be 1 to 120 µmol L^{-1} and 0.45 µmol L^{-1} respectively (Figure 5).

3.4. Interference studies

Selectivity is one of the most important characteristics in the operation of the sensor. In order to investigate the selectivity of the proposed sensor, as mentioned in the experimental part, the response of the composite modified SPE toward methadone was analyzed in the presence of other species including ascorbic acid, uric acid, glucose, calcium, and potassium. The results showed that the presence of these species even with the ratio of 1:100 (methadone: interfering species), has no significant effect on the sensor response toward methadone. According to the obtained results, the presented sensor modified with overoxidized polypyrrole and gold nanoparticles composites can be successfully used for methadone concentration determination in complex biological samples.

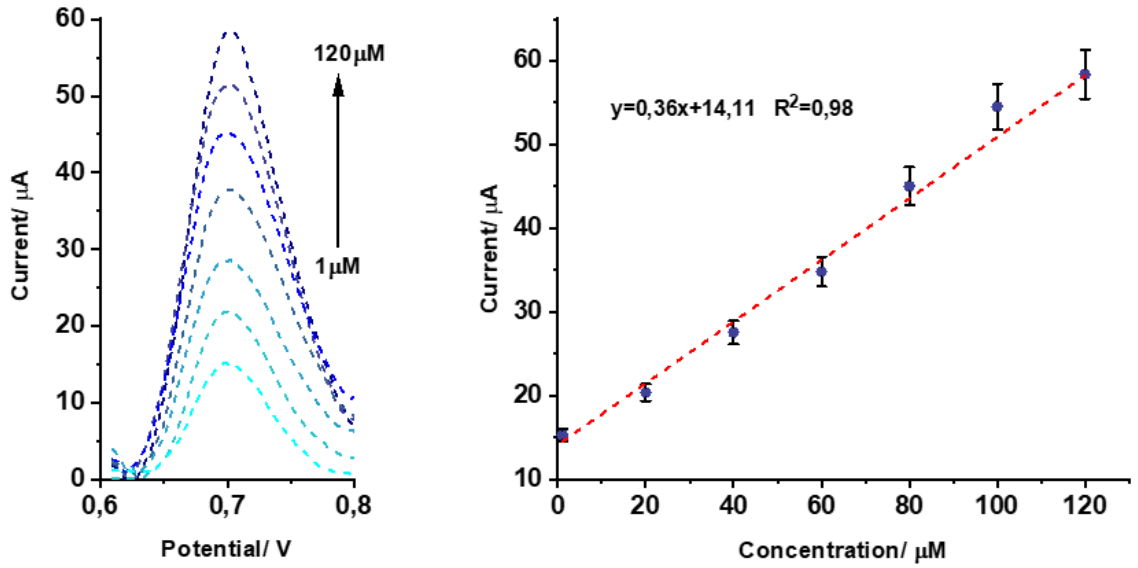


Figure 5. (Left) Square wave voltammograms for solutions containing different concentrations of methadone on the surface of AuNPs/PPyox/SPE, (Right) Dependence of the recorded current by the proposed sensor on the concentration of methadone

3.5. Analytical applicability of the AuNPs/PPyox composite modified SPE for methadone determination in urine and blood samples

To evaluate the applicability of the proposed sensor, methadone contents of the urine and blood samples were analyzed by the procedure explained above. According to the results presented in Tables 1 and 2, the obtained recoveries are close to 100% which confirms the applicability of the AuNPs/PPyox composite modified sensor for determining low levels of methadone concentration in urine and blood samples.

Table 1. Obtained recoveries for methadone determination in a urine sample at AuNPs/PPyox/SPE; RSD values were less than 2.4% for successive determinations in urine (n= 3).

Sample	Amount added (mol L^{-1})	Amount detected (mol L^{-1})	Recovery (%)
Urine	0.0	-	-
	1×10^{-5}	0.98×10^{-5}	98.00
	2.5×10^{-5}	2.58×10^{-5}	103.20
	5×10^{-5}	4.85×10^{-5}	97.00

Table 2. Obtained recoveries for methadone determination in plasma at AuNPs/PPyox/SPE; RSD values were less than 3% for successive determinations in plasma (n = 3).

Sample	Amount added (mol L^{-1})	Amount detected (mol L^{-1})	Recovery (%)
Plasma	0.0	-	-
	1×10^{-5}	1.03×10^{-5}	103.00
	2.5×10^{-5}	2.42×10^{-5}	96.80
	5×10^{-5}	5.06×10^{-5}	101.20

4. Conclusions

In this study, a composite modified screen-printed electrode has been presented for the rapid and inexpensive determination of methadone in biological fluids. The modification layer is composed of electrodeposited gold nanoparticles on overoxidized polypyrrole which is prepared by in situ synthesis method on the surface of the carbon working electrode. Besides a significant negative shift in the oxidation peak potential, the AuNPs/ PPyox composite greatly increased the sensor response in methadone determination. The limit of detection of 0.45 µmol L^{-1} with a wide linear range of 1 to 120 µmol L^{-1} was obtained for the proposed sensor. In addition to the high sensitivity and proper selectivity, the presented composite modified SPE shows simplicity and high stability for methadone determination in biological fluids.

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