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Electrochemical Sensor for Morphine Based on Gold Nanoparticles/ Ferrocene Carboxylic Acid/Poly (3,4-Ethylene-Dioxythiophene) Composite

Nada F. Atta[1,*] *Ahmed Galal*[1], *Samar H. Hassan*[2]

[1] Department of Chemistry, Faculty of Science, Cairo University, 12613, Giza, Egypt
[2] Forensic Chemistry Laboratories, Medico Legal Department, Ministry of Justice, Cairo, Egypt
[*]E-mail: nada_fah1@yahoo.com

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An electrochemical sensors were prepared from different mediators namely ferrocene carboxylic acid (FC1), ferrocene (FC2) and cobaltcene (CC) sandwiched between two layers of poly (3,4-ethylene-dioxythiophene) PEDOT and gold nanoparticles (PEDOT/Mediator/Au_{nano}). The performance of the mediator in these different composites was evaluated for the electrocatalytic oxidation of morphine (MO). The results showed that FC1 mediator possesses higher rate for electron transfer and enhanced electrocatalytic activity due to the inclusion of the ferrocenium ion and the polar substituted–COOH group in the conducting polymer matrix which increases the electronic conduction of the film. Excellent performance of (PEDOT/FC1/ $Au_{nano)}$ modified electrode with low detection limit of 21 nmol L^{-1} in the linear dynamic range 5-300 μmol L^{-1} for the determination of MO in human urine samples with satisfying recovery results was obtained. Furthermore, the PEDOT/FC1/Au_{nano} composite was successfully used for simultaneous determination of tertiary mixture of morphine, ascorbic acid (AA) and uric acid (UA) and binary mixtures of morphine/ascorbic acid (AA) and morphine/dopamine.

Keywords: PEDOT; Morphine; Ferrocene carboxylic acid; Gold nanoparticles; Dopamine.

1. INTRODUCTION

Morphine is the most abundant alkaloid found in opium, which can cause disruption in the central nervous system when used in excess or abused, MO frequently used to relieve severe pain in patients, Furthermore, MO is also one of hydrolysate of heroin in organism and deservedly used as an indicator of heroin usage for forensic cases. It has been reported that almost 10% of the excreted MO remains unmetabolized and 90% of orally administrated MO is excreted in urine within 24 h [1]. There are many techniques for detecting morphine, including the radioimmunoassay (RIA) [2], high performance liquid chromatography (HPLC) [3], gas chromatography–mass spectroscopy (GC–MS) [4], thinlayer chromatography (TLC) [5], surface plasmon resonance based immuno-sensor [6] UV–Vis spectroscopy [7], fluorimetry [8–11], chemiluminescence [12–14], surface plasma resonance (SPR) [15], and electrochemical methods [16–22]. Conducting polymers such as PEDOT poly (3,4-ethylene-dioxythiophene), which is a relatively stable conductive polymer, are attractive sensing materials because they exhibit electronic, magnetic and optical properties of metals and semiconductors while retaining the attractive mechanical properties and processing advantages of polymers [23,24] conducting polymers have the advantages of high sensitivities, short response time, easy fabrication, good mechanical properties [25–29]. Electric contact is maintained usually by mediators–molecules carrying electrons between the sensor active site and the electrode surface. The mediator moieties are very effective as they add a faradic component to the process of charge transfer resulting in enhancement of the charge transfer rate at the electrode-electrolyte interface [30-34]. Mediators can be either dissolved in the solution, or co-immobilized together with the sensor. The co-immobilized mediator systems have the advantage of enhanced electrocatalytic current values as the redox rate is not limited by mediator diffusion rate. Thus, in some cases as in the present study, the co-immobilization of ferrocene in conducting films increases the electronic conduction and hence the rates of charge transfer. Furthermore, the appropriate choice of the mediator can tune the potential of the electrocatalytic reaction to the needed value. Ferrocene-derived compounds are very important electrons mediators highly used in electrochemical systems, enzymatic reactions, ascorbate determination, dopamine, pH and oxygen sensing [34]. They have been used as electron mediators in modification of carbon paste electrodes, glassy carbon and also in the preparation of films on gold surfaces for determination of DA [34]. Polypyrrole modified with ferrocene was utilized as CO sensor and glucose biosensor [35-36]. In addition, ferrocenium incorporated into poly(3-methylthiophene) mediates the electron transfer reaction of the oxidation of some biological molecules [31]. Moreover, carbon paste electrode modified with ferrocene/gold nanoparticles is utilized for selective determination of morphine in presence of interference compounds [30]. Ferrocene-modified carbon nanotube paste electrode was proposed for the electrochemical detection of carbidopa [32] and levodopa [33]. On the other hand, cobaltocene, a readily-available metal sandwich complex of moderate redox strength, was also used for inducing one-electron changes [37]. This black compound has a number of attractive properties, including solubility in a range of non-polar and polar solvents [38]. Gold nanoparticles Au_{nano} have been studied extensively because they have high biocompatibility, good conductivity and satisfactory catalytic activity; this range of properties is particularly useful for the application of Au_{nano} in the construction of electrochemical biosensors [39–42]. A common method for making Au_{nano} is electrodeposition, which enables fine-tuning and can facilitate the transfer of electrons [43-45]. At the same time the procedure is simple and rapid [46–48]. Moreover, no papers reported the incorporation of mediator film between poly(3,4-ethylenedioxythiophene) and gold nanoparticles and the sensing applications of the resulted composite.

This paper shows the performance and synergism of the mediators namely ferrocene carboxylic acid, ferrocene and cobaltcene and their evaluation for the electrocatalytic oxidation and determination of morphine. Furthermore, the application of the proposed composite PEDOT/FC1/Au_{nano} for simultaneous determination of the morphine with ascorbic acid (AA), and uric acid (UA) and with dopamine is examined. In addition, the validity of using PEDOT/FC1/Au_{nano} modified electrode in the determination of MO in spiked urine samples is demonstrated as real sample applications.

2. EXPERIMENTAL

2.1. Materials and reagents

All chemicals were used without further purification. 3,4-ethylenedioxythiophene (EDOT), tetabutylammonium hexafluorophosphate ($TBAPF_6$), acetonitrile, uric acid (UA), dopamine (DA), ascorbic acid (AA), ferrocene carboxylic acid (FC1), ferrocene (FC2), cobaltocene (CC), hydrogen tetrachloroaurate ($HAuCl_4$), potassium phosphate (mono, di-basic salt), potassium hydroxide (KOH) were supplied by Aldrich Chem. Co. (Milwaukee, Wl. USA). Morphine sulfate (MO) supplied from Forensic Chemistry Laboratory, Medico Legal Department, Ministry of Justice, Cairo Egypt. Phosphate buffer solution PBS (1.0 mol L^{-1} K_2HPO_4 and 1.0 mol L^{-1} KH_2PO_4) of pH 2-11 was used as the supporting electrolyte. The pH was adjusted using suitable amounts of 0.1 mol L^{-1} H_3PO_4 and 0.1 mol L^{-1} KOH.

2.2. Electrochemical cell and equipments:

Electrochemical polymerization and deposition were carried out with a three-electrode/one compartment glass cell. The working electrode was platinum disc (diameter: 1.5 mm). The auxiliary electrode was a 10 cm long/2.0 mm diameter Pt wire. All the potentials in the electrochemical studies were referenced to Ag/AgCl (4 mol L^{-1} KCl saturated with AgCl) electrode. Working electrode was polished using alumina (2 μm)/water slurry until no visible scratches were observed. Prior to immersion in the cell, the electrode surface was thoroughly rinsed with distilled water and dried. All experiments were performed at 25 °C ± 0.2 °C. The electrosynthesis of the polymer and its electrochemical characterization were performed using a BAS-100B electrochemical analyzer (Bioanalytical Systems, BAS, West Lafayette, USA). The differential pulse voltammetry conditions are as follows: step potential 5 mV; modulation amplitude 25 mV; modulation time 0.05 s; and interval time 0.5 s. Quanta FEG 250 instrument was used to obtain the scanning electron micrographs of the different films (accelerating voltage was 20 keV).

2.3. Construction of PEDOT/mediator/gold nanoparticles modified Pt electrode

Briefly, an inner film of PEDOT was first prepared on Pt electrode by electrochemical polymerization from 0.05 mol L^{-1} EDOT/0.05 mol L^{-1} $TBAPF_6$/AcN at 1.4 V for 5 s. This was followed by the electrochemical deposition of ferrocene carboxylic (FC1) or ferrocene (FC2) from a solution containing 0.01 mol L^{-1} FC1 or FC2 in 0.01 mol L^{-1} $TBAPF_6$/actonitrile by applying 75 repeated cycles in a potential range (-200 to 1200 mV). To deposit cobaltocene (CC), 0.01 mol L^{-1} cobaltocene/0.01 mol L^{-1} $TBAPF_6$/acetonitrile was used and 75 repeated cycles were applied in the potential range (-200 to 600 mV). An outer layer of gold nanoparticles was electrodeposited from a solution containing 6 mmol L^{-1} $HAuCl_4$ prepared in 0.1 mol L^{-1} KNO_3 (prepared in doubly distilled water and deaerated by bubbling with nitrogen). The potential applied between the working electrode and the reference Ag/AgCl (4 mol L^{-1} KCl saturated with AgCl) electrode is held constant at -400 mV (Bulk electrolysis) for 400 s (optimized conditions). The final composites can be represented as PEDOT/FC1/Au, PEDOT/FC2/Au and PEDOT/CC/Au.

2.4. Analysis of urine

The utilization of the proposed method in real sample analysis was also investigated by direct analysis of human urine. Urine sample used for detection was diluted 400 times with 0.1 mol L^{-1} PBS/pH 7.4 to reduce the matrix effect of real samples. MO was dissolved in 0.1 mol L^{-1} PBS/pH 7.40 to make a stock solution with 5×10^{-4} mol L^{-1} concentration. Standard additions were carried out from the MO stock solution in 15 mL of diluted urine.

3. RESULTS AND DISCUSSION

3.1. Electrochemistry of morphine at different mediators modified composites

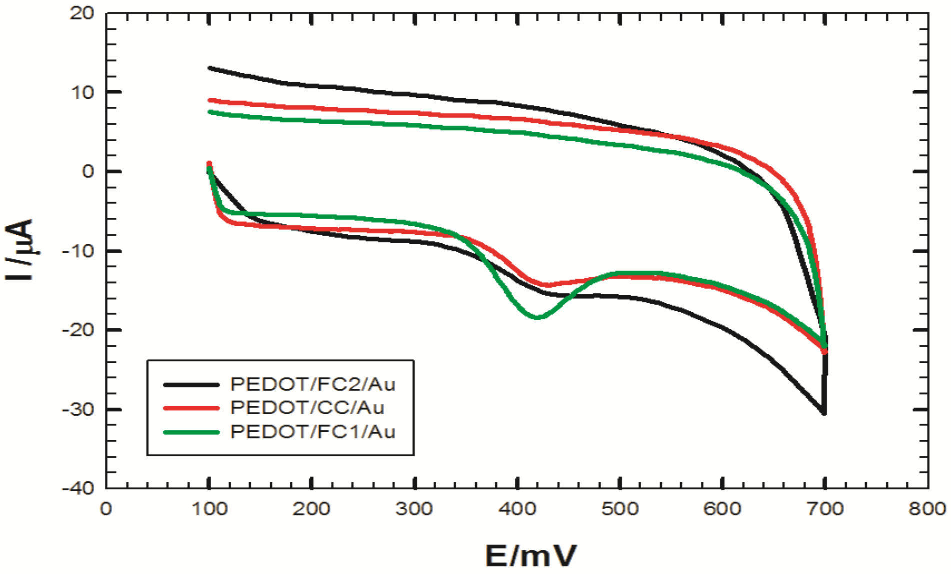

Figure 1. CVs of 5×10^{-4} mol L^{-1} MO /0.1 mol L^{-1} PBS/pH 7.40 at PEDOT/FC2/Au (black line) PEDOT/CC/Au (red line), and PEDOT/FC1/Au (green line), scan rate 50 mV s^{-1}.

In order to investigate the effect of the mediator on the electrocatalytic activity of the resulting composite, the electrochemistry of 5×10^{-4} mol L^{-1} MO /0.1 mol L^{-1} PBS/pH 7.4 was compared at different mediators modified composites; namely PEDOT/FC1/Au PEDOT/FC2/Au and PEDOT/CC/Au (Fig.1), respectively. The oxidation current values of MO were 18 µA, 15 µA and 13.2 µA at the three modified composites, respectively (Fig.1) & (Table1). As obvious from the previous results, ferrocene carboxylic acid modified composite exhibited higher mediation and faster charge transfer rate compared to the other mediators. The presence of the substituted polar carboxylic group of ferrocene in the moiety of the conducting polymer as well as the incorporation of the ferrocenium ion resulted in the improvement of the electronic conduction of the polymer and enhancement of the charge transfer rate through the film. Moreover, there is a little difference in the reactivity of pure ferrocene and cobaltocene which is attributed to the relative difference in the atomic size of the mediator metals [37]. We concluded that PEDOT/FC1/PEDOT composite presented higher catalytic activity in terms of higher current response and faster electron transfer rate.

Table 1. Summary of CV results obtained at PEDOT, PEDOT/Au, PEDOT/FC1/Au PEDOT/FC2/Au and PEDOT/CC/Au for 5 × 10^{-4} mol L^{-1} of MO in 0.1 mol L^{-1} PBS/pH (7.40), scan rate 50 mVs^{-1}

Electrode	E_{pa} (mV)	I_{pa} (μA)	$D_{app} \times 10^{-4}$ (cm^2/s)
PEDOT	420	4	0.07
PEDOT/Au	422	7.2	0.23
PEDOT/FC1/Au	413	18	1.429
PEDOT/FC2/ Au	439	15	0. 993
PEDOT/CC/Au	429	13.2	0.77

E_{pa}; the anodic peak potential, I_{pa}; the anodic peak current, D_{app}; the apparent diffusion coefficient.

3.2. Electrochemistry of morphine at different modified electrodes

The electrochemistry of 5 × 10^{-4} mol L^{-1} MO /0.1 mol L^{-1} PBS/pH 7.4 was investigated at different modified Pt electrodes; PEDOT, PEDOT/ Au and PEDOT/FC1/ Au (Fig. 2). No peak was observed for MO at the modified electrode PEDOT/FC1/ Au in 0.1 mol L^{-1} PBS/pH 7.4 buffer (Fig. 2). The results illustrated that the oxidation current increases form 4 μA at PEDOT to 7.2 μA at PEDOT/Au and 18 μA at PEDOT/FC1/Au. The higher electrocatalytic activity was obtained for PEDOT/FC1/Au which can be explained as follows: the modification of PEDOT film with gold nanoparticles enhances the electrocatalytic activity of the resulting film as gold nanoparticles act as a promoter to increase the electrochemical oxidation reaction and accelerate the electron transfer rate by increasing the surface area [49]. Moreover, the incorporation of the ferrocenium moiety and the polar substituted carboxylic group to the conducting polymer PEDOT increases the electronic conduction of the polymer and consequently enhances the rate of the charge transfer at the polymer-electrolyte interface [49]. Therefore, an enhanced electrocatalytic activity toward MO oxidation was observed at PEDOT/FC1/Au. The new composite shows the synergism between the PEDOT, ferrocene carboxylic acid and gold nanoparticles resulting in excellent mediation and promotion of a faster electron transfer reaction [49].

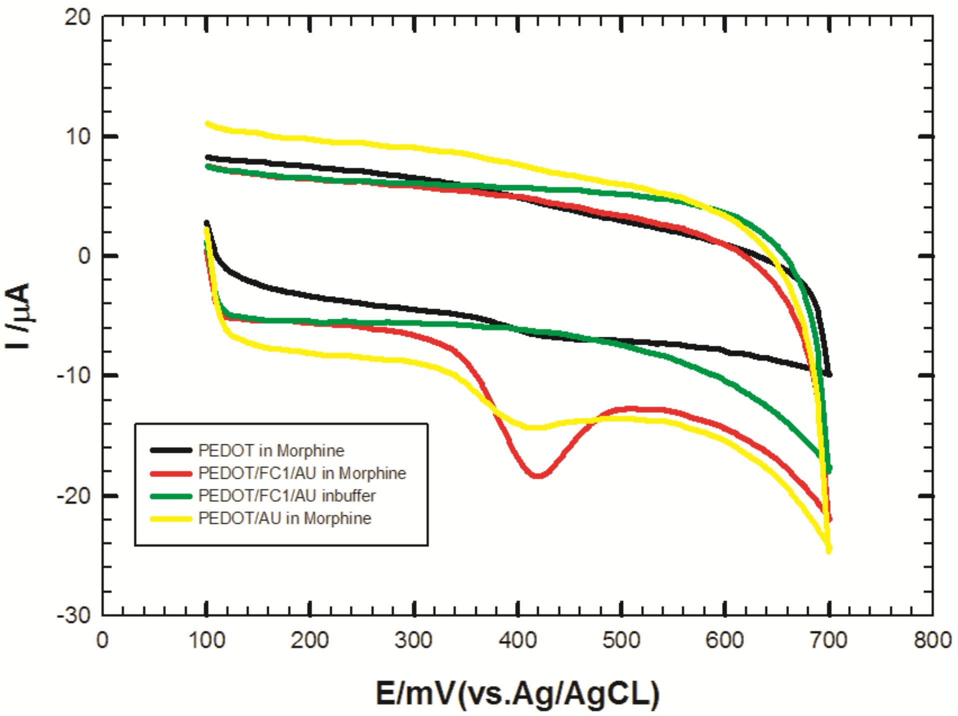

Figure 2. CVs 5 × 10^{-4} mol L^{-1} MO /0.1 mol L^{-1} PBS/pH 7.40 at PEDOT (black line), PEDOT/Au (Yellow line) and PEDOT/FC1/Au (Red line). Green line represents the CV of PEDOT/FC1/AU in the absence of MO, scan rate 50 mV s^{-1}.

3.3. Effect of scan rate

The dependence of the electrocatalytic activity of the resulting composites upon changing the mediator type (structure & metal) was revealed by studying the scan rate effect and calculating the diffusion coefficient values of MO at different modified electrodes; namely PEDOT/FC1/Au, PEDOT/FC2/Au and PEDOT/CC/Au. Figure 3 illustrates the cyclic voltammetric studies of 5 × 10^{-4} mol L^{-1} MO/0.1 mol L^{-1} PBS/pH 7.4 at PEDOT/FC1/Au at different scan rates from 20 to 200 mV s^{-1}. By increasing the scan rate, the oxidation peak current of MO increased and the oxidation potential was shifted to more positive value. Moreover, the inset of (Fig. 3) showed the linear relation between the anodic peak current (I_{pa}, A) and the square root of the scan rate proving that the electrooxidation of MO in the studied scan rate range was diffusion-controlled process. The relation between the anodic peak current (I_{pa}, A) and the scan rate has been used for the estimation of the “apparent” diffusion coefficient (D_{app}, cm^2 s^{-1}) of MO. This relation was represented by Randles Sevcik equation (1):

$$I_{pa} = (2.69 \times 10^5)\, n^{3/2}\, A\, C^o\, D^{1/2}\, \upsilon^{1/2} \qquad (1)$$

Where n is the number of electrons exchanged in oxidation at T = 298 K, A is the electroactive area = 0.0176 cm^2, C^o is the analyte concentration (5×10^{-7} mol cm^{-3}) and υ is the scan rate V s^{-1}. A plot of the anodic peak current values versus the square root of the scan rate results in a straight-line relationship indicating a diffusion-controlled process. Higher current values and slope were achieved in case of FC1. The calculated value of the diffusion coefficient of MO at the PEDOT/FC_1/Au illustrated greater mediation and faster electron transfer rate, which confirms the above results where FC1 mediates the electron transfer more effectively.

D_{app} values were 1.429×10^{-4}, 0.993×10^{-4} and 0.77×10^{-4} cm^2s^{-1} at PEDOT/FC1/Au, PEDOT/FC2/Au and PEDOT/CC/Au, respectively. It was revealed that FC1 affects remarkably the diffusion component of the charge transfer at the electrode surface as indicated from the D_{app} values. The relatively higher value of D_{app} for PEDOT/FC1/Au compared with PEDOT/FC2/Au and PEDOT/CC/Au indicates that the electron transfer step of the electrocatalytic reaction takes place faster at the former electrode surface. In other words, PEDOT/FC1/Au composite exhibited higher catalytic activity and faster charge transfer rate compared to the other composites.

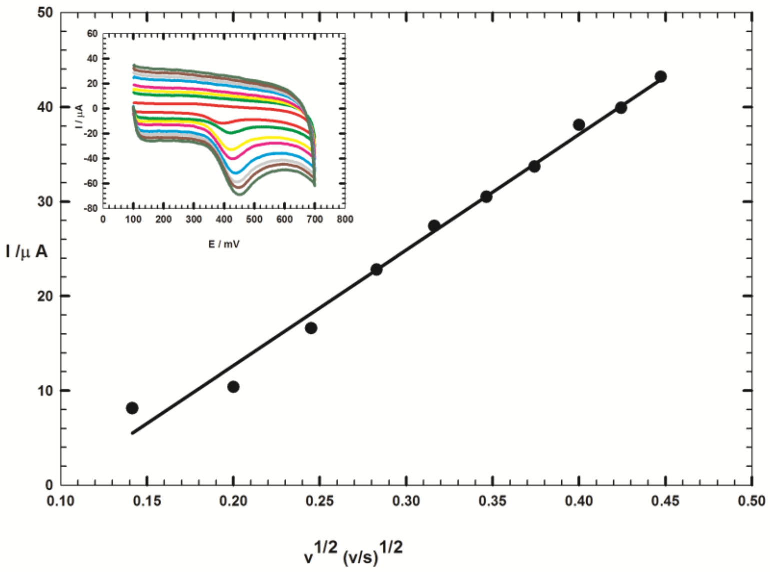

Figure 3. The linear relation between the anodic peak current of MO (μA) and the square root of the scan rate (V s^{-1})$^{1/2}$. The inset; CVs of 5×10^{-4} mol L^{-1} MO /0.1 mol L^{-1} PBS/pH 7.40 at PEDOT/FC1/Au at different scan rates (20 – 200 mV s^{-1}).

3.4. pH effect

It is clear that changing the pH of the supporting electrolyte affected remarkably the redox reaction parameters of MO. The anodic peak potential shifted negatively with increasing the solution pH indicating that electrocatalytic oxidation of MO at the modified electrode is a pH-dependent reaction and protonation/deprotonation is taking part in the charge transfer process. Figure 4 illustrates the CVs of 5×10^{-4} mol L^{-1} MO /0.1 mol L^{-1} PBS with different pH values (2- 11) at PEDOT/FC1/Au and the inset of (Fig. 4) shows the relationship between the anodic peak potential and the solution pH value (over the pH range of 2-11). This relationship could be fit to the linear regression equation (2).

$$E_{pa}\ (V) = 0.844 - 0.058\ pH \qquad (2)$$

With a correlation coefficient of r^2 = 0.991and the slope was found to be -58 mV/pH units at the PEDOT/FC1/Au over the pH range which is close to the theoretical value of -59 mV. This indicated that the number of protons and transferred electrons involved in the oxidation mechanism is equal $1e^-/1H^+$ process.

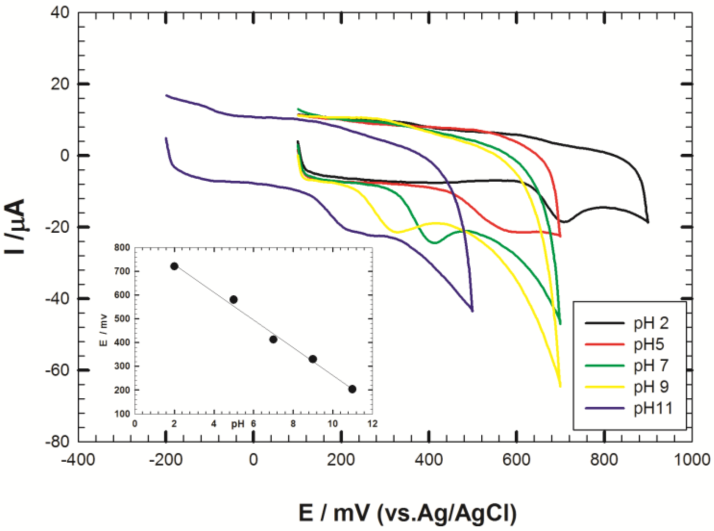

Figure 4. CVs of 5 × 10^{-4} mol L^{-1} MO /0.1 mol L^{-1} PBS with different pH values (2- 11) at PEDOT/FC1/Au, the inset; the linear relationship between the anodic peak potential of MO (mV) and the pH value, scan rate 50 mV s^{-1}.

3.5. Stability of the proposed composite

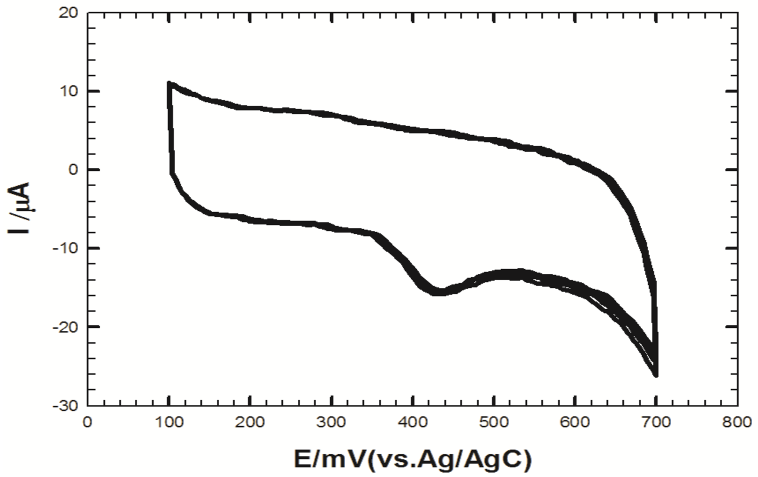

Figure 5. Repeated cycle stability of 5 × 10^{-4} mol L^{-1} MO/0.1 mol L^{-1} PBS/pH 7.40 at PEDOT/FC1/Au, 25 repeated cycles, scan rate 50 mV s^{-1}.

The stability of the modified electrode; PEDOT/FC1/AU was studied via repeated cycles up to 25cycles in 5×10^{-4}mol L^{-1} MO/1.0 mol L^{-1} PBS/pH 7.4 (Fig. 5). Excellent stability without any noticeable decrease in the current response and no change in the potential peak indicating that this modified electrode has a good stability and does not suffer from surface fouling during the repetitive voltammetric measurement. Thus, the proposed sensor exhibited enhanced sensitivity, low detection limit and good stability.

3.6. Determination of MO in real samples at PEDOT/FC1/AU

It is very important to check the validity of the sensor in the real sample analysis. In addition, the determination of MO in urine samples is of medical importance therefore, the direct analysis of MO in human urine was investigated at PEDOT/FC1/Au (Fig. 6). The inset of (Fig. 6) showed DPVs of standard additions of $5x10^{-4}$ mol L^{-1} MO in 1.0 mol L^{-1} PBS/pH 7.40 to 15 mL of diluted urine at PEDOT/FC1/Au indicating that by increasing the concentration of MO, the anodic peak current increases.

Figure 6 shows the calibration curve of the anodic peak current values of MO in urine in the linear range of 5 μmol L^{-1} to 300 μmol L^{-1} with correlation coefficient (r^2) = 0.995 and detection limit = 21 nmol L^{-1}.

The detection limit (DL) was calculated from the following equation (3):

$$DL= 3 (s/b) \qquad (3)$$

Where, s is the standard deviation and b is the slope of the calibration curve. The results indicated that the present procedures are free from interferences of the urine sample matrix and strongly proved that MO can be sensitively determined at PEDOT/FC_1/Au in urine sample with low detection limit. Table 2 shows the comparison for the determination of MO at the different proposed composites with various modified electrodes based in literature reports.

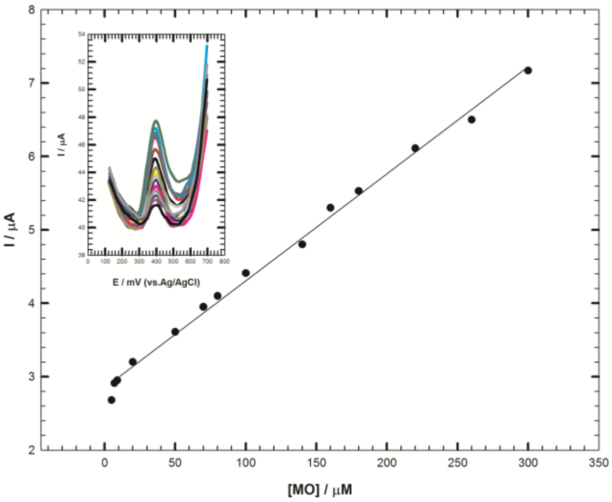

Figure 6. Calibration curve for MO in the linear range (5 μmol L^{-1} – 300 μmol L^{-1}) at PEDOT/FC1/Au. Inset DPVs of MO in urine at PEDOT/FC1/Au for concentrations from (5 μmol L^{-1} to 300 μmol L^{-1}).

Table 2. Comparison for determination of MO at various modified electrodes-based literature reports.

Electrode	LDR (µM)	LOD (nM)	Reference
GNFMCPE	1.0 – 1800	3.51	[49]
(HTP-MWCNT-CPE)	1.0 – 950	66	[50]
(OMC/GCE)	0.1 – 20	10	[51]
(MWCNTs–Nafion/GCE)	0.1– 4	32	[52]
GNP- Nafion /CPE	0.2 – 260	1.33	[53]
Pt/PEDOT/FC/Au	5 – 300	21	This work

LDR; Linear dynamic range, LOD; limit of detection, GNFMCPE; carbon paste (CP) electrode with ferrocene /gold nanoparticles, (HTP-MWCNT-CPE); carbon paste electrode spiked with 4-hydroxy-2-(triphenylphosphonio)phenolate (HTP) and multi-wall carbon nanotubes, (OMC/GCE); mesoporous carbon modified glassy carbon electrode, (MWCNTs–Nafion/GCE); the multi-walled carbon nanotubes/Nafion polymer composite modified glassy carbon electrode, GNP- Nafion /CPE; gold nanoparticles/Nafion carbon paste electrode.

Four different concentrations on the calibration curve are chosen to be repeated five times to evaluate the accuracy and precision of the proposed method which is represented in (Table 3).

Table 3. Evaluation of the accuracy and precision of the proposed method for the determination of MO in urine sample.

Sample	[MO] added (µmol L^{-1})	[MO] found (µmol L^{-1})	Recovery (%)	Standard deviation $\times 10^{-8}$	Standard error $\times 10^{-8}$
1	5	4.99	99.90	1.30	0.58
2	70	69.8	99.75	1.14	0.51
3	180	179.8	99.89	1.14	.051
4	280	297	99.69	10.95	1.45

[a] Average of five determinations.

3.7. Morphology study

The analysis of the morphology of the modified electrodes can be accounted for the variation in their electrocatalytic behavior (Fig. 7). Figure 7(A-B) shows the SEM images of PEDOT and PEDOT/FC1/Au electrodes, respectively. The morphology of PEDOT film (Fig. A) has globular morphology and the surface looks rough due to the Pt substrate. The SEM image of PEDOT/FC1/Au (Fig. B) shows that metallic nanoparticles are located at different elevations over the PEDOT/FC1 film exhibiting a large surface area. Gold nanoparticles are smaller and homogenously distributed at the film and this affects the conductivity level and the electrocatalytic activity of the resulting composite.

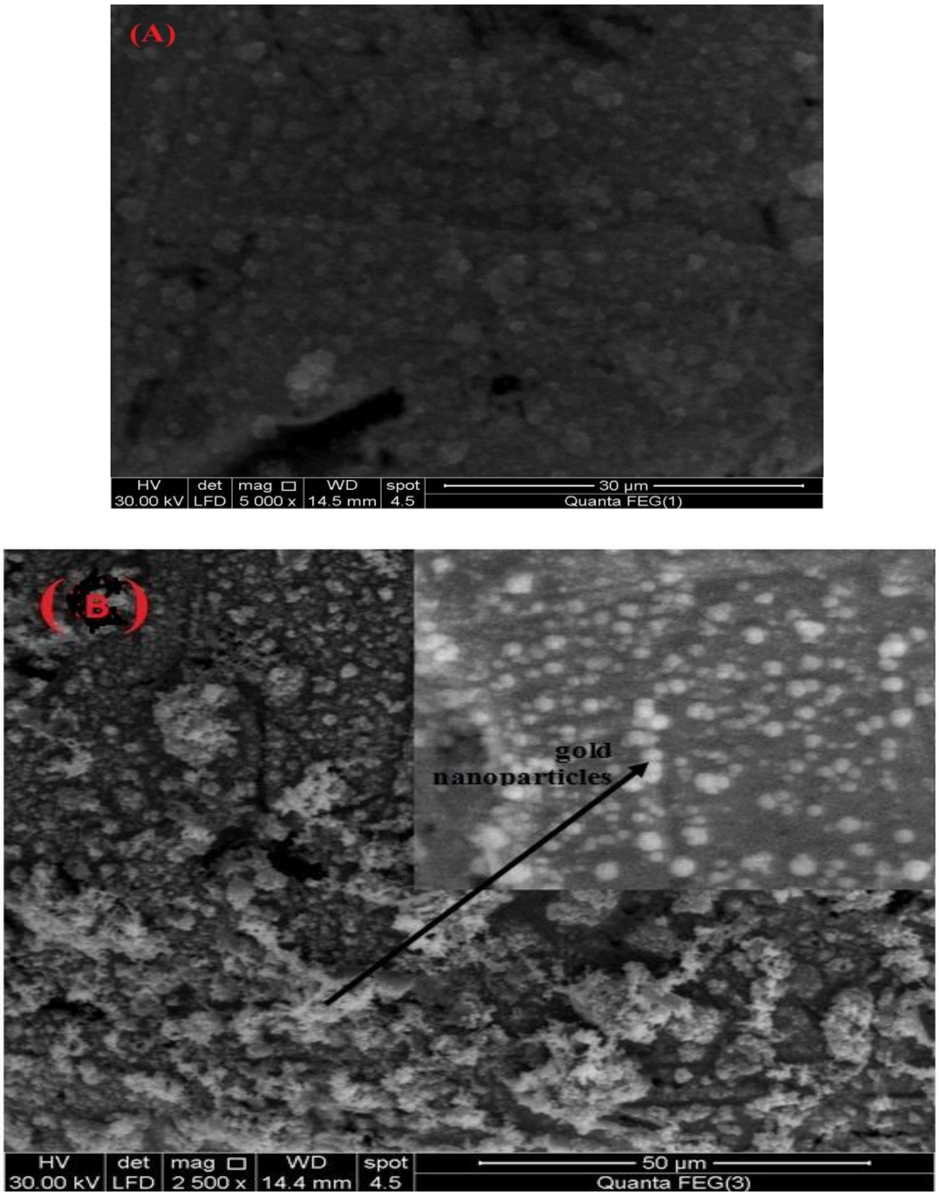

Figure 7. SEM micrographs of **(A)** PEDOT**,** **(B)** PEDOT/FC1/Au, inset; greater magnification (100000×).

3.8. Interference study

Simultaneous determination of MO in the presence of DA was examined as DA usually interferes with MO analysis in urine or blood. Good selectivity and high sensitivity are two most important requirements for the detection of MO in practical clinical applications. Figure 8 (A) and the inset show the DPVs for a mixture of 5 × 10^{-4} mol L^{-1} MO with 5 × 10^{-4} mol L^{-1} DA in the mixture solution 1.0 mol L^{-1} PBS/pH 7.40 at PEDOT/FC1/AU and PEDOT modified electrodes. At PEDOT/FC1/AU two resolved peaks at 160 mV and 396mV are observed with higher current response compared to PEDOT modified electrode, where the peaks observed at 168 mV and 424 mV.

Furthermore large doses of AA have been reported to suppress withdrawal symptoms in opiate addicts and to prevent the development of tolerance to and physical dependence on MO. In addition, MO increases UA levels and AA oxidation. Figure 8 (B) and the inset(1) show the DPVs of 1 mmol L^{1} MO, 1mmol L^{-1} UA and 5mmol L^{-1}AA in the mixture solution (1.0 mol L^{-1} PBS/pH 7.40) at PEDOT/FC1/AU and PEDOT modified electrodes respectively. Three peaks are obtained at PEDOT/FC1/AU at 54mV, 381mV and 483mV and peaks at 43 mV, 361 mV and 504 mV are observed at PEDOT modified electrode for ascorbic acid, uric acid and morphine respectively. Furthermore the inset(2) shows the DPV for a mixture of 5 × 10^{-4} mol L^{-1} MO with 5 × 10^{-4} mol L^{-1} AA in the mixture solution 1.0 mol L^{-1} PBS/pH 7.40 at PEDOT/FC1/AU, two resolved peaks at 80 mV and 404mV are observed Fig. 8 (B). The previous results revealed the validity of the proposed sensors for the simultaneous determination of MO in presence of interfering species with large peak separation and high sensitivity.

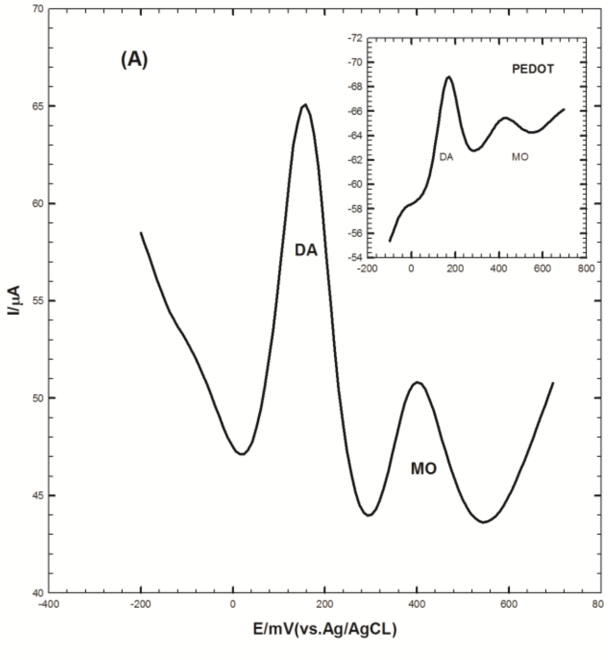

Figure 8. **(A)** DPV of 5 × 10^{-4} mol L^{-1} MO in the presence of 5 × 10^{-4} mol L^{-1} DA at PEDOT/FC1/Au, the inset; the DPV of 5 × 10^{-4} mol L^{-1} MO in the presence of 5x10^{-5} mol L^{-1} DA at PEDOT.

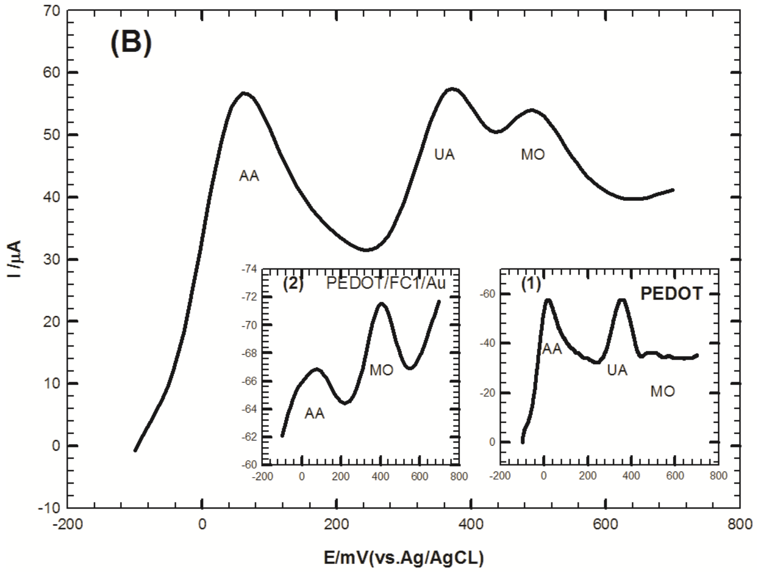

Figure 8. **(B)** DPV of 1 mol L^{-1} MO in the presence of 1 mmol L^{-1} UA and 5 mmol L^{-1} AA at PEDOT/FC1/Au, inset (1); the DPV of 1 mmol L^{-1}MO in the presence of 1 mmol L^{-1} UA and 5 mmol L^{-1} AA at PEDOT, inset (2) DPV of 5 × 10^{-4} mol L^{-1} MO in the presence of 5 × 10^{-4} mol L^{-1} AA at PEDOT/FC1/Au.

3.9. Electrochemical Impedance Spectroscopy

The EIS measurements for the proposed composite were carried out in 5x10^{-4} mol L^{-1} MO/0.1 mol L^{-1} PBS/pH 7.4 in the region corresponding to the electrolytic oxidation of MO. The typical impedance spectra in the form of Nyquist plot for PEDOT/FC1/Au and pt electrodes were shown in (Fig. 9) and inset. A semicircle portion with a diminished diameter was obtained at the sandwiched composite with small charge transfer resistance. The experimental data was compared to an equivalent circuit that used some of the conventional circuit elements (inset). R_s is the solution resistance, R_p is the charge transfer resistance, C_f and C_c represent the capacitive components of the composite, CPE1 and CPE2 are constant phase elements, n and m are their corresponding exponent and W is the Warburg impedance due to diffusion.

The value of the charge transfer resistance R_p equals 0.12 × 10^{3} Ωcm^{2} in case of PEDOT/FC1/Au and 2.3 × 10^{3} Ωcm^{2} for the pt electrode. As well, the value of Warburg impedance due to diffusion (W) for pt is 1.54 × 10^{4} Ω s$^{-1/2}$ which decreases greatly to 0.43 × 10^{4} Ω s$^{-1/2}$ for the proposed sensor, indicating less electronic resistance and more facilitation of charge transfer. On the other hand, the capacitive component value of PEDOT/FC1/Au is higher compared to that at pt electrode (Table 4). This confirms the great electronic conductivity of the proposed composite and the high charge transfer rate.

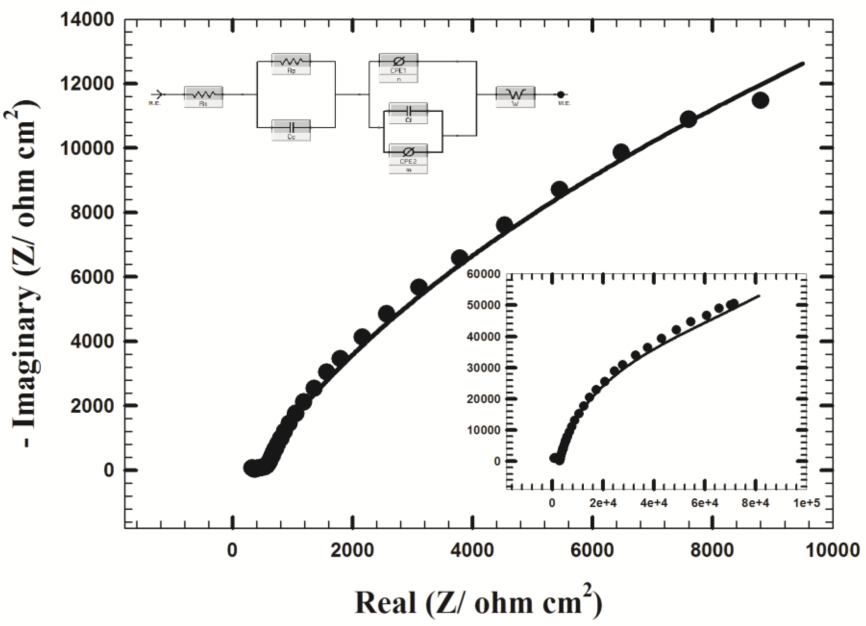

Figure 9. Nyquist plot of Pt/PEDOT/FC1/Au and bare Pt in 5 × 10^{-4} mol L^{-1} of MO /0.1 mol L^{-1} PBS/pH 7.40 at the oxidation potential of MO. (Symbols and solid lines represent the experimental measurements and the computer fitting of impedance spectra, respectively), frequency range: 0.1–100000 Hz. Inset, the equivalent circuit used in the fit procedure of the impedance spectra.

Table 4. EIS fitting data corresponding to Fig. 9 for bare electrode and the proposed sensor.

Electrode	Rs (Ωcm^2)	Cc (Fcm^{-2})	Rp (Ωcm^2)	CPE1 (Fcm^{-2})	n	W ($\Omega\ s^{-1/2}$)	CPE2 (Fcm^{-2})	m	Cf (Fcm^{-2})
Pt	349	1.6×10^{-9}	2.3×10^{3}	6.20×10^{5}	0.93	1.54×10^{4}	7.52×10^{4}	0.26	1.52×10^{-5}
Sensor	260	1.7×10^{-8}	0.12×10^{3}	4.71×10^{5}	0.98	0.43×10^{4}	1.49×10^{4}	0.33	7.96×10^{-5}

4. CONCLUSIONS

Electrochemical sensors for morphine based on gold nanoparticles/mediator/poly (3,4-ethylene-dioxythiophene) composite were fabricated. The mediators were ferrocene carboxylic acid, ferrocene, cobaltcene. The results showed that FC carboxylic acid and gold nanoparticles possess high rate for electron transfer and enhanced electrocatalytic activity due to the inclusion of the ferrocenium ion and the polar substituted–COOH group in the conducting polymer matrix which increases the electronic conduction of the film. In addition, this method was successfully applied for determination of MO in human urine samples with good precision, accuracy, and relatively low detection limit. In this work, we demonstrated the selective determination of MO in presence of AA and simultaneous determination of morphine and dopamine with good separation and resolution.

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