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Article

A novel biosensor based on horseradish peroxidase trapped in silica Sol-Gel/MWCNTs matrix for methyldopa determination in medical and pharmaceutical samples

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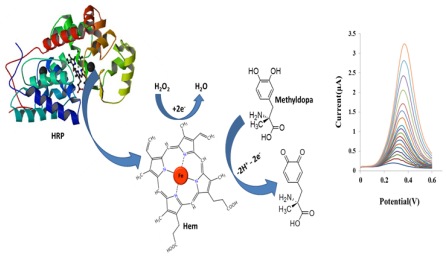
Abstract

A modified electrode composed of horseradish peroxidase (HRP) trapped onto silica Sol-Gel/multi-walled carbon nanotubes (MWCNTs) matrix on carbon paste electrode (CPE) developed and applied for determination of methyldopa by electrocatalytic oxidation of methyldopa with HRP for the first time. The proposed biosensor showed linear response in two ranges of 1 μmol L^{-1} to 3.5 mmol L^{-1} and 3.5 mmol L^{-1} to 12 mmol L^{-1}, and detection limit was obtained about 25.3 nmol L^{-1}. The biosensor performance was much better than a lot of previously reported sensors and biosensors for methyldopa determination. This modified electrode was successfully used for accurate determination of methyldopa in urine and pharmaceutical samples. Using the developed biosensor provided an accurate, sensitive and repeatable method for methyldopa measurement in pharmaceutical and urine samples.



Keywords: *Biosensors, Biotransformations, Horseradish peroxidase, Methyldopa, Enzyme immobilization.*

Introduction

2-amino-3-(3, 4-dihydroxyphenyl)-2-methylpropanoic acid, also known as methyldopa" or "Methyldopa (2-amino-3-(3, 4-dihydroxyphenyl)-2-methylpropanoic acid), is an important member of catecholamines which has been used as an anti-hypertensive for many years. It is converted to α-methyl norepinephrine in adrenergic nerve terminals and then α-methyl norepinephrine leaved its antihypertensive effect by stimulation of central α-adrenoreceptors.[1] Methyldopa concentration in plasma of the patients taking it is usually in the range of 0.1–0.5 mg L^{-1} and average half-time for its elimination is about 2h.[2,3]

This is completely clear that detection and determination of this drug in urine or plasma sample of patients or pharmaceutical products is very important.[4-6] several analytical procedures have been reported for determination of methyldopa in pharmaceutical or biological samples. These procedures include high-performance liquid chromatography,[7] titrimetry,[8] nuclear magnetic resonance spectroscopy,[9] kinetic methods,[6] fluorometry,[10] spectrophotometry,[5] potentiometry,[1] reflectance spectroscopy,[1] mass spectrometry[11] and electrochemical methods.[12]

Although most of these methods have disadvantages such as long analysis times, high costs, difficult control of the experimental conditions or suffering from the tablet matrix interference, complex and tedious sample pre-treatments, and in some cases, a low selectivity and sensitivity which makes theme unsuitable for a routine analysis. On the other hand, electrochemical methods have attracted more attention within the last two decades for environmental, medical and biological samples analyses due to their high dynamic range, high sensitivity, accuracy, rapidness, lower cost and not requiring complex procedure in detection of methyldopa and other analytes.[13–16]

For the first time Adams in 1958 reported the use of carbon paste as electrode. After that many researchers used so many varieties of modifiers such as polymers, enzymes, nanomaterials and ionic liquids with carbon paste electrode. The use of these electrodes in electrochemical studies have become increasingly common because of many advantages such as low cost, facile preparation, wide anodic and cathodic potential ranges, rapid and simple surface renewal, low cost, low residual currents and noises and so on.[17-19]

It is well-known that, the performance of the sensors and biosensors can be significantly improved by using nanomaterials.[20-22] Carbon nanotubes (CNTs) are one of the most common and effective modifiers which has been used to modify carbon paste electrodes.[23,24] CNTs are gaining popularity in electrochemistry as a viable nanomaterial due to their extraordinary electronic properties, large surface area, significant mechanical strength, mass transfer capabilities, high catalytic capability and chemical and structural characteristics.[25,26]

Among the electrochemical methods for phenolic compounds measurement (like catecholamines), using electrodes modified with peroxidase enzymes such as laccase,[27] tyrosinase,[28] and polyphenol oxidase[29] has created promising methods because of high sensitivity and selectivity.

The immobilization of enzymes is an indispensable part for the development of enzyme-modified electrodes.[30] electrochemistry of HRP on carbon nanotube platform has been studied in several works and its interesting and useful properties in biosensor applications was verified. [31-35] Applying a silica sol-gel to form a robust and permeable film has been reported as a suitable cover for enzyme immobilization on electrodes in previous studies.[36, 37] Despite many reports about determination of catecholamines with peroxidase enzyme-modified electrodes, there are no reports on using HRP modified electrode for methyldopa determination. In this study HRP was trapped in

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MWCNTs/Silica-Sol-Gel matrix on carbon paste electrode to achieve a sensitive electrode with low detection limit and high linear concentration range for determination of methyldopa.

Experimental

Reagents

Peroxidase from horseradish (EC.1.11.1.7, 250 U mg^{-1}) was purchased from Sigma-Aldrich and was used as received. Tetraethoxysilane (TEOS) was obtained from Merck. MWCNTs with the average diameter of 20–60 nm were purchased from Neutrino Co. (Iran). Methyldopa was from Darou Pakhsh Co. (Iran). Graphite powder and mineral oil were from Sigma-Aldrich. All other reagents were of analytical grade. Phosphate buffers (PB) (50 mmol L^{-1}) were prepared from H_3PO_4, NaH_2PO_4 and Na_2HPO_4 and pH values were adjusted by HCl and NaOH solutions. All phosphate buffers used in the experiments were in pH 7. The solutions were prepared using deionized water and deoxygenated by bubbling high purity (99.99%) nitrogen gas through theme for 15 min prior to the experiments. All experiments were carried out at room temperature. Urine samples as real samples were freshly prepared and used. Samples were diluted to 20 times for methyldopa measurements. To tablet sample preparation, a 250 mg methyldopa tablet was milled and dissolved in 1mL of phosphate buffer and resulted in the stock solution with a concentration of 1.2 mmol L^{-1}.

Apparatus

Voltammetric measurements were performed using a three-electrode system, including the CPE/MWCNTs/HRP/Sol-Gel as working electrode, an Ag/AgCl (3.0 M KCl) as reference electrode, and a platinum foil as counter electrode. Voltammetric measurements were carried out using a computer-controlled μ-Autolab modular electrochemical system (PGSTAT101, the Netherlands), driven with NOVA Software (upgrade 1.10).

Preparation of the CPE/MWCNTs/HRP/Sol-Gel

20 mg of native HRP was dissolved in 2.0 mL of 50 mmol L^{-1} of PB buffer. 15 mg of MWNTs were dispersed in 10 mL of acetone by ultrasonication for 5 min. Sol–gel solution was prepared by mixing of 5 mL of TEOS, 1.5 mL of double distilled water and 100 μL of 0.1 mmol L^{-1} HCl.[38] Using HCl in sol-gel preparation process can protect HRP from denaturization and leads to faster formation of the sol-gel film. The CPE was initially prepared by homogenizing of 85 mg of graphite powder and 25 mg of mineral oil in a small glass oven and completely mixed for several minutes to produce the final paste. The obtained paste was packed into a tip of 1 mL insulin plastic syringe and a copper wire was inserted to obtain the external electric contact. Finally, electrode surface got polished with piece of soft paper for immobilization of MWCNTs/HRP on it. 10 μL of MWCNTs solution in acetone (1.5 mg mL^{-1}) were cast on the surface of the CPE and dried in air to form a layer of MWCNTs on electrode surface (CPE/MWCNTs) then 5 μL of HRP solution was dropped on CPE/MWCNTs surface. Finally for physical immobilization of MWCNTs/HRP composite on CPE, 10 μL of sol-gel was dropped on CPE/MWCNTs/HRP electrode. The obtained electrode was left for 15 min at room temperature to form sol-gel film and then washed with buffer for several times to release the unimmobilized enzymes.

Results and discussion

Figure 1 shows the SEM images of developed electrode in all steps of fabrication. Figure 1a shows the soft and uniform surface of bare CPE and in Figure 1b, high amount of MWCNTs uniformly distributed on CPE is clearly seen and nano-dimension of some carbon tube is observable. Also, Figure 1c displays the surface of the CPE/MWCNTs/HRP electrode which shows the carbon nanotubes with aggregations of HRP on the electrode surface. Figure 1d shows the dried and layered sol-gel film on final electrode which has completely covered the HRP/MWCNTs content and provides appropriate cover to prevent direct contact of HRP with solution.

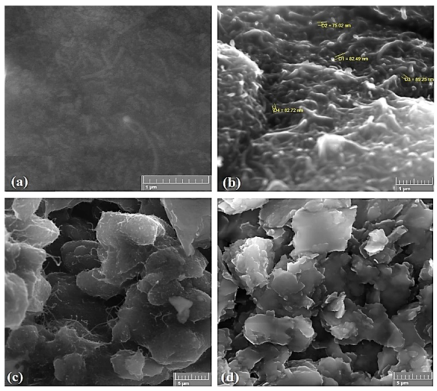
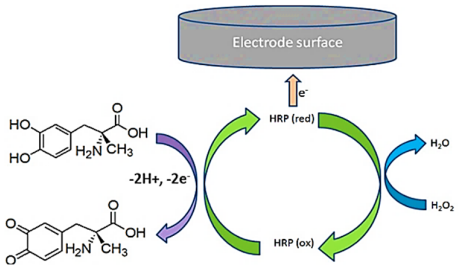


Figure. 1. *SEM images of a) bare CPE b) CPE/MWCNTs c) CPE/MWCNTs/HRP and d) CPE/MWCNTs/HRP/Sol-Gel surfaces.*

Phenolic compounds electrodes modified with peroxidase enzymes, work on the basis of electrocatalytic oxidation of phenolic compound by enzymes in presence of H_2O_2. Oxidation peak current of methyldopa was used to determine methyldopa concentration. As it shown in the following, oxidation peak for methyldopa using enzyme modified CPE in presence of H_2O_2 is too stronger in comparison with the other electrodes. It means that enzyme plays the key role in oxidation of methyldopa in presence of H_2O_2 on electrode surface. The proposed mechanism as follows: heme group of HRP get oxidized by H_2O_2 present in solution and then oxidized heme can easily oxidize phenolic compound (methyldopa here). Resulted oxidized form of phenolic compounds (known as quinones) can directly reduce on the electrode surface (by applying reduction potentials) and cause the reduction current proportional to phenolic compound concentration. On the other hand, reduced heme can get oxidized again on electrode surface and causes the oxidation current. Considering that H_2O_2 amount is a fixed amount, (for all methyldopa concentrations will be measured) this oxidation current is directly proportional to phenolic compound concentration. Indeed enzyme on the electrode surface acts as mediator for electron transfer from phenolic compound to electrode surface. The proposed mechanism for electrodic reaction is represented in Scheme 1. In this work the oxidation peak currents were used for methyldopa determination.



Scheme 1. *Schematic representation of proposed mechanism for oxidation of methyldopa on the modified electrode surface in the presence of H_2O_2.*

Figure 2a shows the differential pulse voltammetry (DPV) of a) phosphate buffer (blank), b) 150 µmol L^{-1} of H_2O_2 in phosphate buffer, c) 1 mmol L^{-1} of methyldopa in phosphate buffer and d) 1 mmol L^{-1} of methyldopa with 150 µmol L^{-1} of H_2O_2 in PB (pH of all buffers is 7.0) by CPE/MWCNTs/HRP/Sol-Gel as working electrode. The potential scanned was adjusted 0.0 to 0.6 V *vs.* Ag/AgCl (potential scan rate is 50 mV s^{-1}).

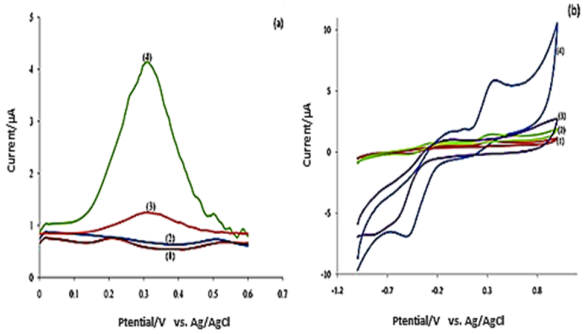


Figure 2. *a) DPV voltammograms of 1) phosphate buffer (blank), 2) 150 µmol L^{-1} of H_2O_2, 3) 1 mmol L^{-1} of methyldopa and 4) 1 mmol L^{-1} of methyldopa with 150 µmol L^{-1} of H_2O_2 in phosphate buffer by CPE/MWCNTs/HRP/Sol-Gel as working electrode. b) CV of 1) 1 mmol L^{-1} of methyldopa measured by unmodified CPE 2) 1 mmol L^{-1} of methyldopa with 150 µmol L^{-1} of H_2O_2 measured by unmodified CPE 3) 1 mmol L^{-1} of methyldopa measured by CPE/MWCNTs/HRP/Sol-Gel, 4) 1 mmol L^{-1} of methyldopa with 150 µmol L^{-1} of H_2O_2 measured by CPE/MWCNTs/HRP/Sol-Gel. For all voltammograms pulse amplitude 50 mV, pulse width 50 ms and scan rate 100 mV s^{-1}.*

As can be seen in Figure 2a, there is no significant oxidation current for PB or H_2O_2 but DPV related to methyldopa (4) shows an intensive oxidation peak that strongly suggests heme (firstly was oxidized by H_2O_2 and then reduced by phenolic compound) oxidized again by applying oxidation potential on CPE surface. DPV corresponded to methyldopa without H_2O_2 (3) also shows oxidation peak for methyldopa but as seen its sensitivity is much lower than DPV (4) that refers to mentioned mechanism for methyldopa electrocatalytic oxidation by heme in presence of H_2O_2. Fig 2b shows the CV voltammograms of 1) 1 mmol L^{-1} of methyldopa measured by unmodified CPE, 2) 1 mmol L^{-1} of methyldopa with 150 µmol L^{-1} of H_2O_2 measured by unmodified CPE 3) 1 mmol L^{-1} of methyldopa measured by CPE/MWCNTs/HRP/Sol-Gel and 4) 1 mmol L^{-1} of methyldopa with 150 µmol L^{-1} of H_2O_2 measured by CPE/MWCNTs/HRP/Sol-Gel. As can be seen, developed biosensor shows well defined redox peak for methyldopa in presence of H_2O_2 (voltammogram 4) and no any distinct oxidation peak is seen for methyldopa in absence of H_2O_2 which strongly supports the suggested mechanism for methyldopa oxidation by HRP heme. Moreover unmodified CPE as shown in Figure 2a (1, 2), are not significantly sensitive to this amount of methyldopa in presence or absence of H_2O_2.

A critical parameter that must be optimized for peroxidase catalytically determination of phenolic compounds is H_2O_2 concentration. In the previous works, it was observed that the electrode sensitivity is increased with increasing of H_2O_2 concentration and then reaches a maximum value and finally decreases a little with more increase of the H_2O_2 concentration. This occurs because the high concentrations of hydrogen peroxide can avoid from enzyme activity and creating inactive enzymes decrease the electrode sensitivity.[39] Figure 3 shows the electrode response to 1 mmol L^{-1} of methyldopa in PB in various concentrations of H_2O_2. As can be seen, sensitivity increases with concentration increase of H_2O_2 to 150 µM and then decreases with more increasing of H_2O_2 concentration. This test repeated with some different amounts of methyldopa and showed the same optimized amount for H_2O_2. This result highly suggests that the optimized amount of H_2O_2 is independent of methyldopa concentration and probably depends only on trapped HRP amount on CPE.

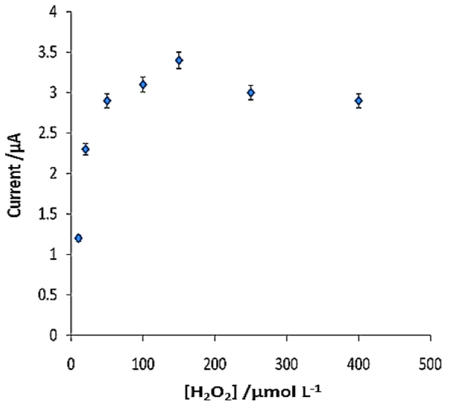


Figure 3. *Electrode response (DPV oxidation peak current) to 1 mmol L^{-1} of methyldopa in phosphate buffer in various concentrations of H_2O_2. Pulse amplitude 50 mV, pulse width 50 ms, scan rate 100 mV s^{-1}.*

For investigation of electrode performance, sensitivity of developed electrode was compared with unmodified and partially modified CPEs. Figure 4 shows DPVs obtained for 2.3 mmol L^{-1} of methyldopa and 0.15 mmol L^{-1} of H_2O_2 (optimized amount of H_2O_2) in PB in pH=7 by a) CPE, b) CPE/MWCNTs/Sol-Gel, c) CPE/HRP/Sol-Gel and d) CPE/MWCNTs/HRP/Sol-Gel as working electrodes. As can be seen, sensitivity of CPE/MWCNTs/HRP/Sol-Gel is higher than other electrodes. Also, the sensitivities of electrodes c and d (with HRP) are significantly higher than two other electrodes (without HRP), which verifies that HRP plays the key role in electrode response to methyldopa.

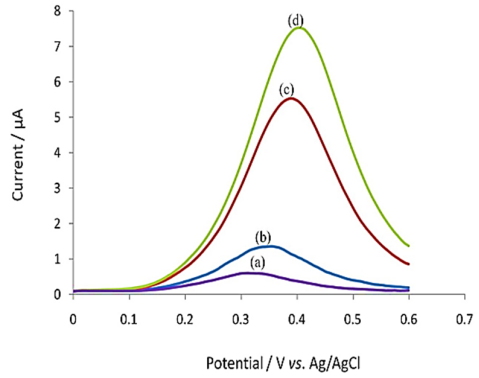


Figure 4. *Differential pulse voltammograms for 2.3 mmol L^{-1} of methyldopa and 0.15 mmol L^{-1} of H_2O_2 (optimized amount of H_2O_2) in phosphate buffer at pH=7 by a) CPE, b) CPE/MWCNTs/Sol-Gel, c) CPE/HRP/Sol-Gel and d) CPE/MWCNTs/HRP/Sol-Gel as working electrodes. Pulse amplitude 50 mV, pulse width 50 ms and scan rate 100 mV s^{-1}.*

Our developed biosensor showed two linear concentration ranges for methyldopa. These ranges were 1 µmol L^{-1} to 3.5 mmol L^{-1} and 3.5 mmol L^{-1} to 12 mmol L^{-1}. Figures 5a and 5b show the methyldopa DPVs obtained by successive additions of methyldopa to PB contains optimized amount of H_2O_2 for regions of 1 µmol L^{-1} to 1.1 mmol L^{-1} and 1.1 mmol L^{-1} to 12 mmol L^{-1} respectively. Figure 5c shows calibration curves resulted from these DPV voltammograms in two above mentioned linear ranges.

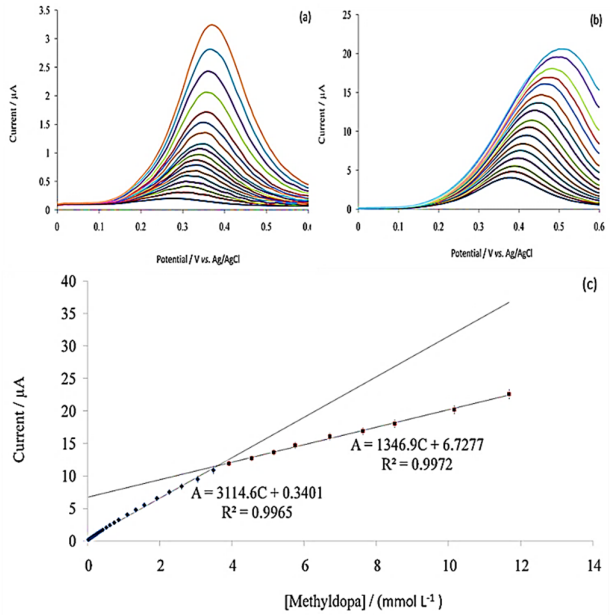


Figure 5. *a) and b) show DPV voltammograms of methyldopa solution with concentrations of 1 µmol L^{-1} to 1.1 mmol L^{-1} and 1.1 mmol L^{-1} to 12 mmol L^{-1} in the presence of 0.15 mmol L^{-1} of H_2O_2, successively. c) Methyldopa calibration curves consist of two linear regions. Regression equations and correlation coefficients are shown for both linear regions. Pulse amplitude 50 mV, pulse width 50 ms and scan rate 100 mV s^{-1}.*

The response time for the biosensor was short, reaching about 95% of its maximum response in about 5 s. Response time in here is a period of time for electrode placing in analyte solution until potential scanning start. As referred obtained calibration curves for methyldopa show linear ranges of 1 µmol L^{-1} to 3.5 mmol L^{-1} and 3.5 mmol L^{-1} to 12 mmol L^{-1} and correlation coefficients were 0.9965 (for n=26) and 0.9970 (for n=9) successively. Its detection limit was calculated about 25.3 nmol L^{-1}. The equations of the regression line obtained for methyldopa in these two different linear ranges were calculated to be: I=3115C+0.3399 and I = 1346.4C+6.7173 respectively. Where I is the current in µA and C is the concentration of methyldopa in mmol L^{-1}. To study some electrochemical characteristics of developed electrode by scan rate effect, several investigations were performed as follows:
The active surface area of the electrodes investigated according to the slope of the i_{Pa} versus $v^{1/2}$ plot for a known concentration of $K_4Fe(CN)_6$, based on the Randles–Sevcik equation:

$$i_{pa} = (2.69\times10^5)n^{3/2}AD_R^{1/2}C_0v^{1/2} \quad (1)$$

where i_{pa} refers to the anodic peak current, n the electron transfer number, A the surface area of the electrode, D_R the diffusion coefficient, C_0 the concentration of $K_4Fe(CN)_6$, and v is the potential scan rate. For 1.0 mmol L^{-1} $K_4Fe(CN)_6$ in 0.10 M of KCl electrolyte with n=1 and D_R=7.6×10^{-6} cm s^{-1},[35] the microscopic areas of electrodes were calculated by the slope of the i_{pa}–$v^{1/2}$ curves. They were 0.09, 0.19, 0.12, and 0.21 cm^2 for a) CPE, b) CPE/MWCNTs/Sol-Gel, c) CPE/HRP/Sol-Gel and d) CPE/MWCNTs/HRP/Sol-Gel, respectively. These results show that the most active surface area of the compared electrodes is related to the complete modified electrode (CPE/MWCNTs/HRP/Sol-Gel). Close amounts of the surface area values for the electrodes of b and d shows that increasing of surface area is mainly related to MWCNTs.

With knowing anodic currents and surface area for above-mentioned electrodes (anodic currents related to the distinct amount of methyldopa for these electrodes are shown in Figure 4), the current density was easily calculated by dividing of anodic current to surface area. Calculated current densities were 6.66, 7.16, 46.08 and 35.85 mm^2 for CPE, CPE/MWCNTs/Sol-Gel, CPE/HRP/Sol-Gel and CPE/MWCNTs/HRP/Sol-Gel respectively. The effect of scan rate (v) on the oxidation and reduction peak currents of methyldopa was also investigated by cyclic voltammetry (CV) in potential scan rate of 10 to 200 mV s^{-1} for PB containing 3 mmol L^{-1} of methyldopa. These CVs are shown in Figure S1 in supplementary material section. As it is observed, the anodic and catholic peak currents linearly increase with an increase in the square root of scan rate according to regression equations of A = 46.799$\upsilon^{1/2}$ -0.7483 and A = -36.132$\upsilon^{1/2}$ - 2.4867 for oxidation and reduction currents, respectively. These results are summarized in Figure S2 in supplementary material section. The linear dependence between anodic and cathodic currents with potential scan rate shows that the electrochemical process is controlled by the diffusion step.

On the other hand, CVs in Figure S1 of supplementary material section shows that increasing of scan rate is accompanied by negative and positive shifts of the oxidation and reduction peak current potentials, respectively, which are linearly dependent on the log υ based on the Laviron theory, plotting of reduction and oxidation peak current potentials *vs.* Log υ yield straight lines with slopes of $-2.3RT/\alpha nF$ and $2.3RT/(1-\alpha)nF$, respectively. According to these linear relationships, the electron transfer number in the rate-determining step (n) and the charge transfer coefficient (α) can be calculated as 1.42 And 0.572 using the following equation:[35]

$$\log\frac{k_a}{k_c} = \log\frac{\alpha}{1-\alpha} \quad (2)$$

Where k_a and k_c refer to the slopes of the straight lines for E_{pa} (anodic peak potential) and E_{pc} (cathodic peak potential) vs. log υ, respectively. Figure S3 in supplementary material section shows the plot for E_{pa} and E_{pc} vs. log υ with their regression lines. The performance of the proposed biosensor was compared with some other previously reported electrodes for methyldopa determination. As in shown in Table 1, our modified electrode has the acceptable linear range and detection limit compared to other electrodes.

To evaluate the applicability of our developed biosensor in methyldopa determination in real samples, methyldopa concentration was determined in one and two samples of tablet and urine respectively for 3 times by standard addition method. A RSD of 4.2% was obtained, demonstrating a good reproducibility. Results are summarized in Table 2. As seen biosensor has good performance in methyldopa determination even in urine from a person who takes acetaminophen and amoxicillin which clearly shows the selectivity of developed biosensor.

Recovery is between 92.2 and 106.8% which is very satisfactory for accurate drug determination in real samples. Finally, stability of developed biosensor was examined and results showed biosensor maintains 92% of its initiate response after three weeks if stored in 4 ºC. Also, 20 successive times for methyldopa determination in a fixed solution showed RSD of 3.9%. Reproducibility of the electrode fabrication procedure was examined by fabrication of 5 mentioned biosensor and their response to a fixed methyldopa solution showed good RSD of 2.5%.

Table 1. *Comparison of our developed biosensor performance in the determination of methyldopa with some electrochemical methods recently reported.*

Method	Linear range	Detection limit (μ mol L^{-1})	Oxidation potential (V)	Sensibility (μA/μM)	Electrode	Ref.
DPV	50 n mol L^{-1} - 40 μmol L^{-1}	10.0	0.44	5.47&0.57	Pt–RuNPs[1]/MWCNTs/GCE[2]	40
SWV[3]	0.08 μmol L^{-1} –380 μ mol L^{-1}	0.03	0.46	0.441	MgONPs/IL[4]/CPE	41
SWV	34.8 μmol L^{-1} - 370.3 μ molL^{-1}	5.5	-	0.06	CA[5]/IL/Lac[6]/CPE	30
ASV[7]	0.1–30 μmol L^{-1} & 30.0 –300 μ mol L^{-1}	0.08	0.25-0.35	0.622	CMWCNTs[8]/GCE	42
SWV	90 nmol L^{-1} – 500 μmol L^{-1}	50.0	0.33	0.657&0.024	GN[9]/CPE	43
DPV	0.2 μmol L^{-1} - 100 μmol L^{-1}	80.0	0.38	0.818&0.052	FMA[10]/CPE	44
SWV	0.1 μmol L^{-1}–210.0 μmol L^{-1}	0.048	0.255	0.434	(5AEB[11])/CNTs/CPE	45
DPV	50 nmol L^{-1} - 20 μmol L^{-1}	0.017	0.44	0.125	OPPY[12]/TY[13]/Au	46
DPV	1- 3500 μmol L^{-1} & 3.9-12 mmol L^{-1}	0.025	0.30-0.51	3.11&1.35	Sol-Gel/HRP/MWCNTs/CPE	Our work

1. platinum–ruthenium nanoparticles, 2. glassy carbon electrode, 3. square wave voltammetry, 4. ionic liquid, 5. cellulose acetate, 6. laccase, 7. anodic stripping voltammetry, 8. carboxylated multi-walled carbon nanotube, 9. graphite nanosheet, 10. ferrocene monocarboxylic acid, 11. 5-amino-2'-ethyl-biphenyl-2-ol, 12. overoxidized polypyrrole, 13. titan yellow.

Table 2. *Determination of methyldopa in tablet and urine samples.*

Sample	Added(μmol L^{-1})	Expected (μmol L^{-1})	Found (μmol L^{-1}) (The mean of three measured values)	Recovery (%)
Tablet [a]	-	5	4.81	96.2
	10	10	10.68	106.8
	15	15	15.5	103.4
Urine 2 [c]	5	5	4.61	92.2
	10	10	10.22	102.2
	15	15	15.31	102.1
Urine 1 [b]	10	10	9.89	98.9
	20	20	20.01	100.5
	30	30	30.66	102.2

a) tablet product of Darou Pakhsh Co. (Iran)
b) urine sample from a 20 years old man with no drug taking
c) urine sample from a 26 years old man with taking acetaminophen and amoxicillin

Conclusion

Using of carbon paste electrode as base electrode gives us the possibility to apply some other modifiers easily which can further improve the HRP modified electrodes performance in future works. It is clear from what has been described that the developed biosensor for methyldopa determination has significant accuracy and repeatability compared with many other electrochemical reported works. This biosensor allows the determination of methyldopa in a wide concentration range with very low detection limit and it has very short response time and long period stability. Moreover, it is possible to use the developed electrode in real samples successfully. Finally, the electrode is easy and fast to fabricate.

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