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Electrochemical Determination of L-dopa in the Presence of Ascorbic Acid by Gold Nanoparticles Functionalized 8-Hydroxyquinoline Modified Glassy Carbon Electrode

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Abstract- A new modified glassy carbon electrode (GCE) was prepared using gold nanoparticles functionalized by 8-HQ. This modified surface was characterized by cyclic voltammetry, differential pulse voltammetry, scanning electron microscopy and FT-Raman spectroscopy. The modified electrode exhibits strong electrocatalytic activity towards the oxidation of a mixture of L-dopa and ascorbic acid (AA) with reduction of over potentials. Simultaneous analysis of this mixture at the conventional bare electrode surface is not feasible. However, two well-resolved oxidation peaks for AA and L-dopa are observed in the modified electrode and can be used for the estimation of AA and L-dopa simultaneously. Simultaneous analysis of AA and L-dopa using the DPV method, shows that the oxidation current is linear with L-dopa concentration in the range of 3.0–10 µM with a detection limit of about 0.31 µM (S/N=3). The proposed method was applied for the detection of L-dopa in real samples.

Keywords- 8-hydroxyquinoline, Gold Nanoparticle, L-dopa, Ascorbic Acid, Modified Electrode

1. INTRODUCTION

Parkinson's disease is a chronic, progressive neurodegenerative movement disorder that occurs when the substantia nigra of the mid-brain dies and fails to produce enough dopamine [1]. This condition causes tremors, rigidity, poor balance and dyskinesia. Because it cannot penetrate the blood–brain barrier, dopamine cannot be effectively used for the treatment of this serious disease [2]. L-dopa (levodopa, 3,4-dihydroxy-l-phenylalanine) is widely used as a source of dopamine in the treatment of most patients with Parkinson's disease and epilepsy [3]. This drug can be principally metabolized by an enzymatic reaction (dopa-descarboxylase) to dopamine compensating for the deficiency of dopamine in the brain. With its serious side effects with long-term use on human health, e.g., nausea, vomiting, paranoia and dyskinesia [4,5], L-dopa should be analyzed accurately in both pharmaceutical formulations and biological fluids. At present, several methods have been reported for L-dopa analysis such as titration [6], spectrophotometry [7] and high-performance liquid chromatography [8]. These methods often require some complicated and time-consuming pretreatments.

Electrochemical techniques for the determination of these compounds in body fluid samples have attracted great interest as they are less time-consuming and cost-effective, besides possessing low detection limit and high accuracy. However, one of the major problems frequently encountered is due to the unmodified electrode because most of the unmodified solid electrodes show a slow electron transfer for the electrochemical oxidation of L-dopa with a high over potential. The oxidation product of L-dopa easily adsorbs at the bare surface of the electrode, resulting in poor reproducibility and repeatability. The oxidation potential of AA is similar to that of L-dopa. This leads to serious interference in the determination of L-dopa [9]. To overcome these problems, many chemically modified electrodes have been reported for the L-dopa determination. Bergamini et al. applied a gold screen-printing electrode to monitor L-dopa in stationary solution [10]. Yan et al. used single-walled carbon nanotubes to modify glassy carbon electrode (GCE) for the determination of L-dopa [11]. Teixeira et al. fabricated a carbon paste electrode modified with trinuclear ruthenium ammine complex (Ru-red) supported on Y-type zeolite for the determination of L-dopa [12]. Xiang et al. reported a multi-walled carbon nanotube-Nafion to modify GCE for the determination of L-dopa [13]. Sivanesan and John fabricated a GCE modified with tetraaminophthalocyanatonickel (II) film for the selective determination of L-dopa in the presence of AA [14]. Shahrokhian and Asadian reported a GCE modified by a bilayer of multi-walled carbon nanotube and poly-pyrrole doped with tiron for the determination of L-dopa [15]. Mathiyarasu and Nyholm constructed a poly-(3, 4-ethylenedioxythiophene)-single-walled carbon nanotube composite-modified microelectrode and applied it to the electrochemical determination of L-dopa [16]. Hu et al. reported gold nanoparticle self-assembled carbon nanotube-modified pyrolytic graphite electrode for L-dopa determination

[17]. Prabhu et al. reported determination of L-dopa by nickelhexacyanoferrate film-modified gold nanoparticle graphite composite electrode [18]. In the last few decades metal nanoparticles have drawn particular attention due to their high surface area, effective mass transport, catalysis and control over local microenvironment compared with macro-electrodes [19–21]. Among the various nanomaterials gold has special attention because of its high electrocatalytic activity and high stability [22–25]. The electrocatalytic behavior of 8-hydroxyquinoline-5-sulfonic acid over catecholamines has been reported [26,27]. In the present study, the bare electrode was modified by gold nanoparticle functionalized 8-hydroxyquinoline by the electrochemical method. This modified GCE and its performance were characterized by cyclic voltammetry and differential pulse voltammetry. The Raman spectroscopy and SEM analysis were used to demonstrate surface modification and functionalization.

2. EXPERIMENTAL

2.1. Apparatus

Cyclic Voltammetry (CV), Differential Pulse Voltammetry (DPV) experiments were performed using a CHI6041C electrochemical workstation (CH Inc., USA) coupled with a conventional three-electrode cell. The three electrodes namely the working electrode, the auxiliary electrode, and the reference electrode were GCE, Pt wire and Ag/AgCl electrode, respectively. All the potentials in this paper are given against Ag/AgCl. SEM (FEI Quanta FEG 200-High resolution scanning electron microscope) was used to determine the surface morphology. BRUCKER RFS 27: Stand alone FT-Raman spectrometer was used to determine the functionalization. An EI-1L model (34 kHz ultrasonic bath) was used for cleaning the electrodes and to prepare a homogeneous mixture.

2.2. Chemicals and solutions

L-dopa, AA, $HAuCl_4.3H2O$, and 8-hydroxyquinoline, 0.1 M of sodium phosphate buffer, HCl, ethanol and all other chemicals were of analytical grade. The solutions were prepared by using double distilled water. The experiments were carried out at 25 ºC.

2.3. Modifying the bare electrode surface by gold nanoparticles functionalized 8-HQ

Before modifying, the GC electrode was polished with 0.3 and 0.05 μm of alumina slurries for 2 min each, followed by thorough rinsing with double distilled water; the electrode was then sonicated with ethanol and distilled water for 2 min each. After sonicating the electrode, it was rinsed with double distilled water and was examined by cyclic voltammetry using standard 1 mM potassium ferric cyanide solution (by evaluating the oxidation and reduction peak potential). Then, 1 mM 8-hydroxyquinoline in 0.04 M of HCl and 5 mM of gold chloride in 0.1 M of KCl were prepared and mixed. The mixture was then

sonicated to attain homogeneity. The GCE was then scanned up to 10 cycles between the potential of -1.5 and +2.5 V with a scan rate 100 mV s^{-1}. Now this electrode is named as gold functionalized 8-HQ. After this process, the electrode was washed thoroughly with double distilled water to remove the physisorbed 8-HQ and gold. Then the modified electrode was stored by immersing in 0.1 M phosphate buffer solution of pH 7.0 until further use.

3. RESULTS AND DISCUSSION

3.1. Electrochemical reduction and functionalization of gold and 8-hydroxyquinoline

Functionalization and reduction of 8-HQ and gold has been carried out between –1.5 and +2.5 V on the surface of the GCE at a sweep rate of 100 mV s^{-1} for 4 cycles. (Fig. 1) shows the growth of the film during this process of multiple cycles, the current gradually increases with an increase in cyclic time. This indicates the funtionalization of 8-hydroxyquinoline and more reduction of gold over the surface of GCE. It is clear from the cyclic voltammogram that electrochemical reduction of gold takes place at –0.4 V *vs*. Ag/AgCl and further functionalization of 8-HQ at 1.8 V on the reduced gold nanoparticles [26,27].

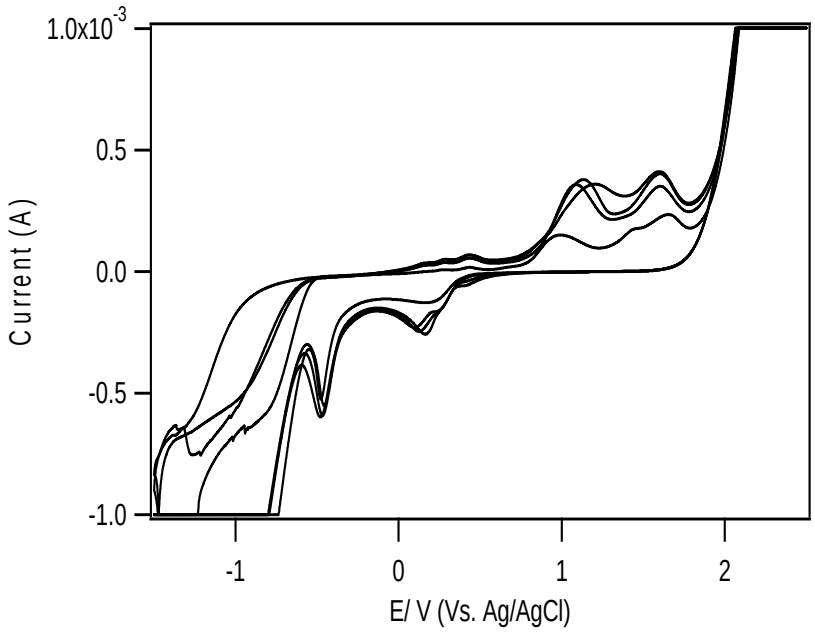


Fig. 1. Cyclic voltammogram of gold nanoparticles functionalized 8-HQ

3.2. SEM and Raman analysis

The SEM and Raman analysis were used to confirm the modification and functionalization over the GCE. From the SEM analysis, surface morphology of the gold-modified GCE and gold functionalized 8-HQ modified GCE was studied. The surface of the

gold-coated GCE seems to have irregular granular shapes varying from 34 to 64 nm (Fig. 2). However, typical small irregular shapes with aggregation was found for gold functionalized 8-HQ (Fig. 3).

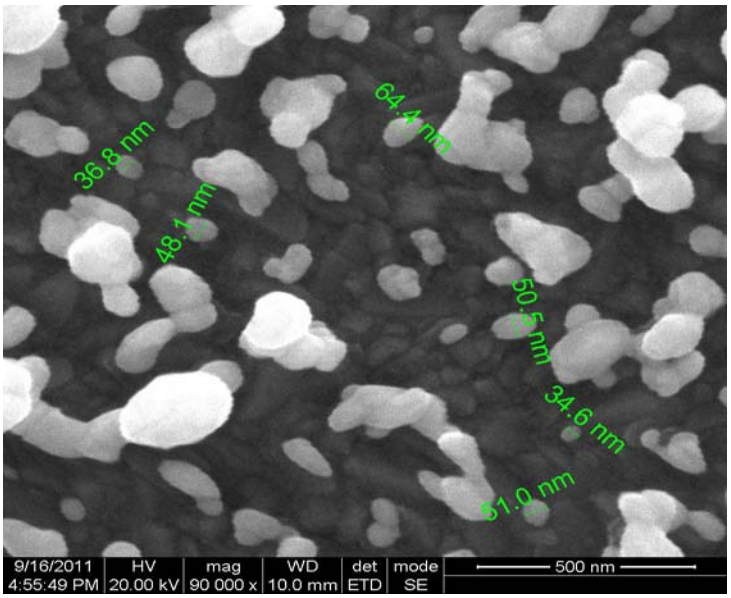


Fig. 2. SEM image of gold nanoparticles

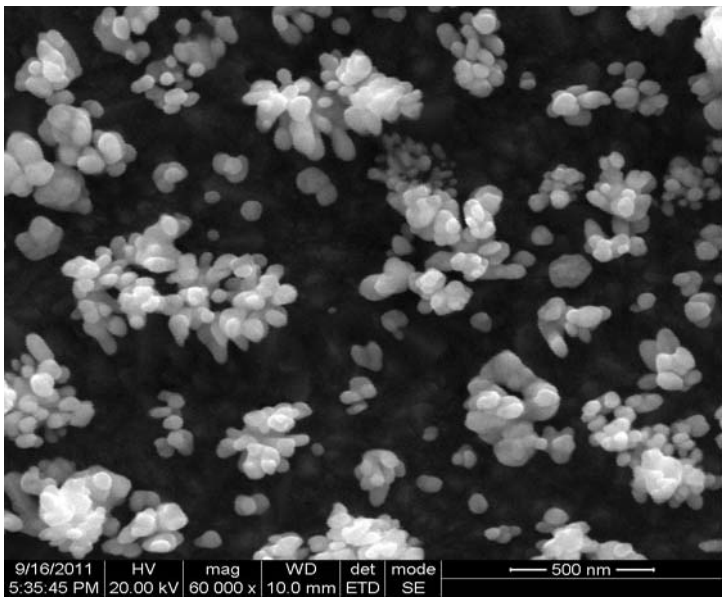


Fig. 3. SEM image of gold nanoparticles functionalized 8-HQ

The Raman spectra of 8-HQ (Fig. 4).shows weak O-H peak at 3246 cm^{-1} but in the case of gold nanoparticles functionalized 8-HQ (Fig. 5), the spectra shows weak OH peak at 3157 cm^{-1}. These shifts in the wave number, confirms the funtionalization of 8-HQ with gold. ITO

substrate peaks were observed in both spectra (Fig. 4, 5) at 1095 cm^{-1} [28].

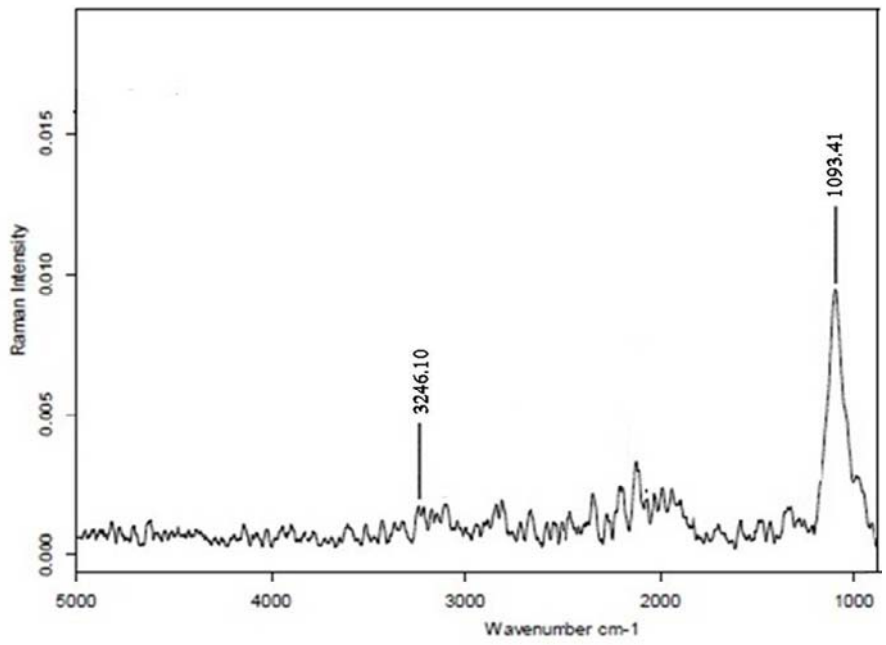


Fig. 4. Raman spectroscopy of 8-HQ

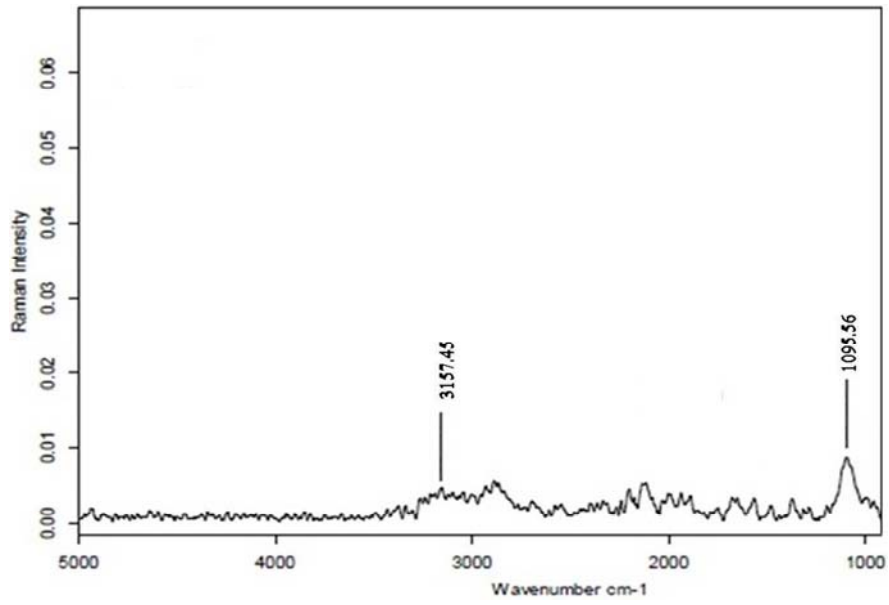


Fig. 5. Raman spectroscopy of gold nanoparticles functionalized 8-HQ

3.3. Electrochemical behavior of L-dopa

The electrochemical behavior of L-dopa was investigated with CV in 0.1 M phosphate buffer solution at pH 7.0. As shown in Fig. 6 a weak response and slow electron transaction

for L-dopa were observed on a bare electrode. In the +0.56 to 0.6 V region, an oxidation peak of L-dopa at +0.56 was noted. The gold functionalized 8-HQ modified electrode shows a good electrocatalytic oxidation toward L-dopa indicating that the gold functionalized 8-HQ modified electrode can effectively decrease the oxidation potential of L-dopa to 0.22 V from 0.56 V.

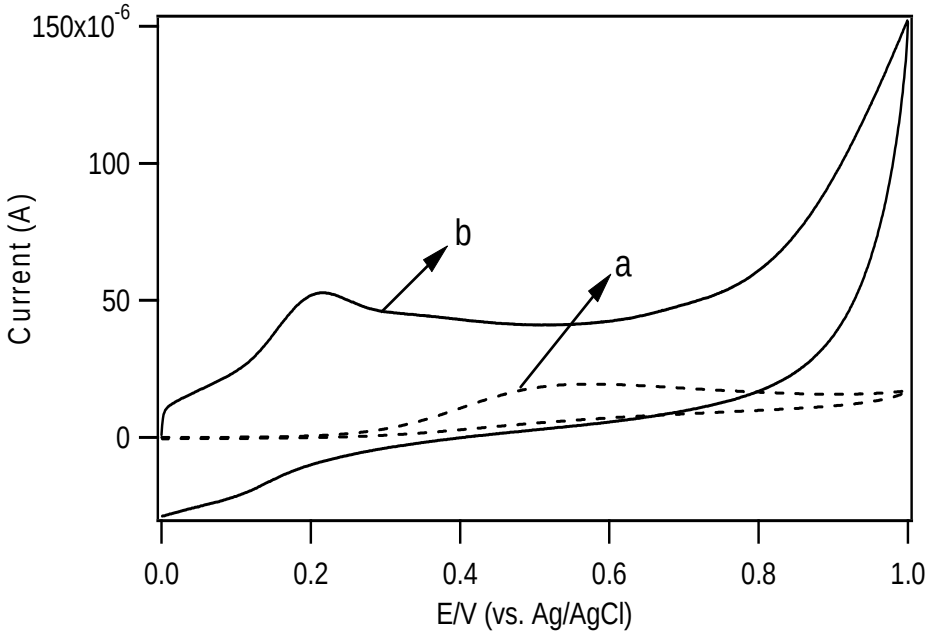


Fig. 6. Cyclic voltammogram of 1 mM L-dopa, (a) bare GCE (b) gold nanoparticles functionalized 8-HQ GCE

Furthermore, the oxidation peak current (I_{pa}) of L-dopa at the gold functionalized 8-HQ modified electrode is two times higher than that of the bare electrode with a better reversibility. These results confirm that the gold nanoparticles functionalized 8-HQ on the surface of the bare electrode can effectively accelerate the electrochemical redox behavior of L-dopa and significantly increase the oxidation current at the modified electrode. The relationship between anodic peak current *vs.* square root of the scan rate was also investigated by the CV method. The anodic currents are linear with the square root of the scan rate from 40 to 120 mV s^{-1} with a correlation coefficient of 0.970 indicating that the process of the electrode is controlled by diffusion and not a surface active reaction [29].

3.4. Effects of solution pH

A CV study was carried out to characterize the effects of solution pH on redox peak potentials of L-dopa at the gold functionalized 8-HQ modified electrode. As shown in Fig. 7 the redox peak potential of L-dopa shifts negatively with the increase in solution pH indicating that protons take part in the electrode reaction process.

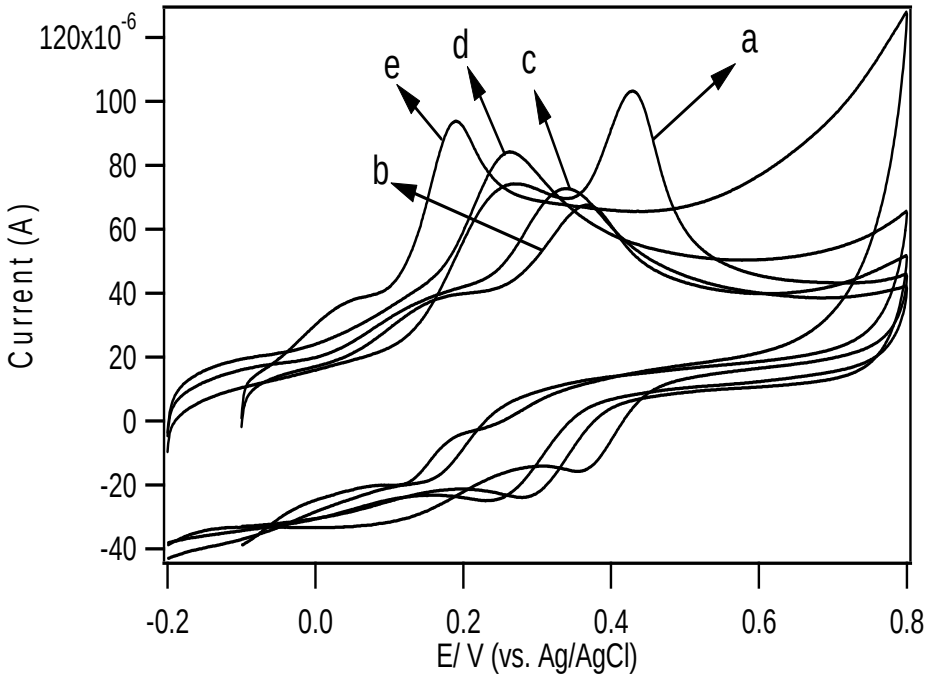
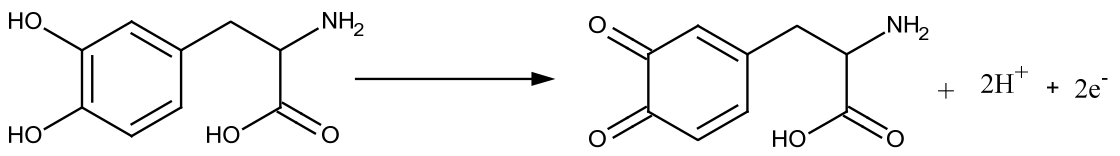


Fig. 7. Cyclic voltammograms of 1 mM L-dopa and 1mM AA on the gold nanoparticles functionalized 8-HQ GCE in various pH (a) 3.0, (b) 4.0, (c) 5.0, (c) 6.0, (d) 7.0 various PBS

The anodic peak current of L-dopa is proportional to the solution pH in the range of 3.0–7.0. Since pH 7.0 is a biological pH and it shows higher current than the other pH, we choose pH 7.0 for further study by plotting Ep_0 *vs.* pH, the linear regression equation of E_{p0} (V)–(0.058+0.54) pH, with the correlation coefficient of 0.9786 which is shown in (Fig. 8). This demonstrates that the electrode process involves equal proton-electron transfer. The slope of the equation is in close agreement with 59/n mV. So the electrochemical redox reaction of L-dopa in the proposed electrode is a two-electron coupled two-proton transfer mechanism [11]. The electrochemical redox process of L-dopa to give dopaquinone is described in Scheme 1.



Scheme 1. Proposed mechanism for L-dopa on the surface of the electrode

3.5. Interference study

The DPV method is normally used for the determination of compounds because of its high sensitivity [30]. It is well known that AA is an electroactive molecule coexisting in a biological system, which can also be oxidized in the conventional solid electrode [9]. L-dopa in the presence of AA by gold functionalized 8-HQ GCE is able to separate both 1 mM L-dopa and 1 mM AA in the potential range of +0.12 V, –0.08 V *vs.* Ag/AgCl (Fig. 9).

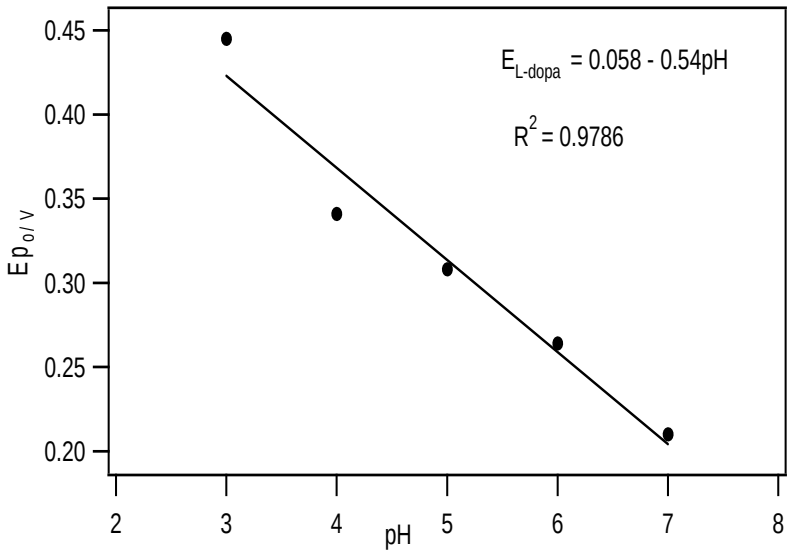


Fig. 8. Plot of Ep_0 (L-dopa) *v*s. pH in gold nanoparticles functionalized 8-HQ GCE

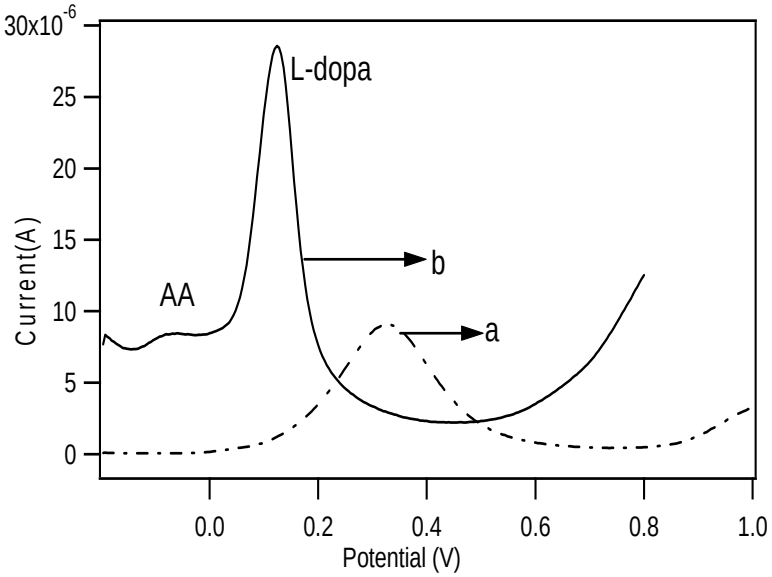


Fig. 9. DPV of 1 mM L-dopa and 1 mM AA, (a) bare, (b) gold nanoparticles functionalized 8-HQ

The DPV curves at different concentration of L-dopa at the modified electrode clearly show that the anodic peak current increases linearly with an increase in L-dopa concentration from 3.0 to 10 μM (Fig. 10).

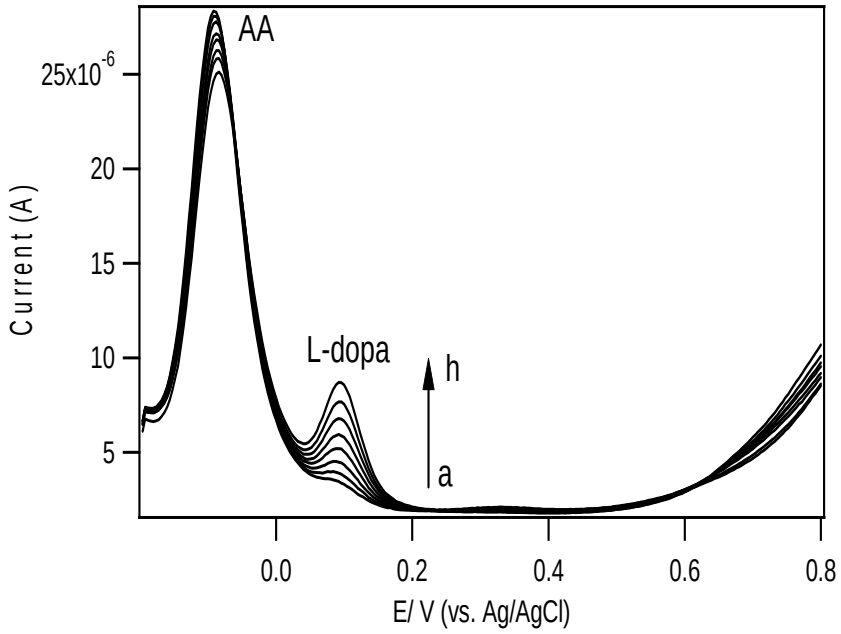


Fig. 10. Dpv of 1 mM AA at gold nanoparticles functionalized 8-HQ GCE in the presence of different concentration of L-dopa: (a) 3.0, (b) 4.0, (c) 5.0 (d) 6.0 (e) 7.0 (f) 8.0 (g) 9.0 (h) 10.0 μM

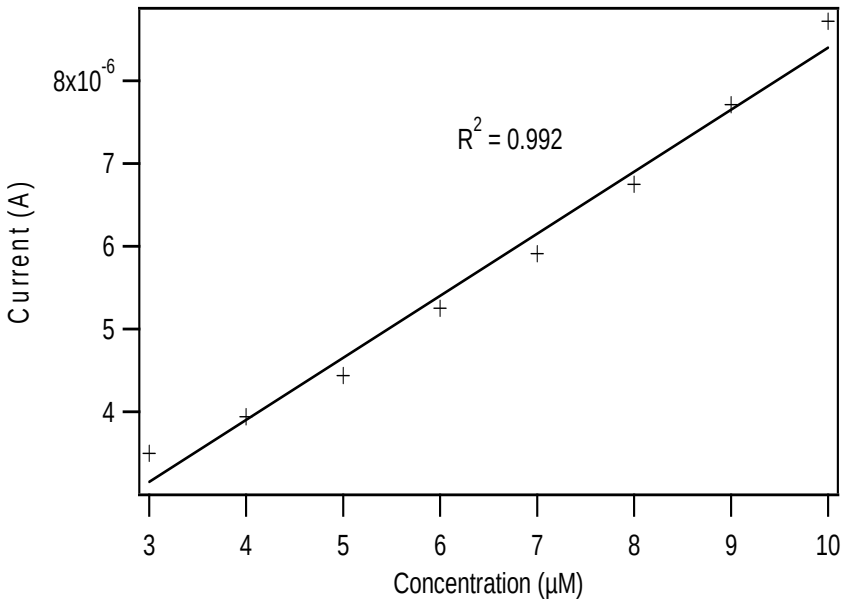


Fig. 11. Plot of i_{pa} *v*s. Concentration of L-dopa

The correlation coefficient of the various concentrations of L-dopa *v*s. current is found to

be 0.992 which is shown in (Fig. 11). This result illustrates that the coexistence of AA has no influence on L-dopa determination. The limit of detection is 0.31 μM (S/N=3) in the presence of 1 mM ascorbic acid. Compared with other electrochemical methods (Table 1), the present method has the advantages of simple electrode preparation, good sensitivity and reproducibility in the determination of L-dopa.

3.6. Analytical application

To investigate the applicability of the proposed method for the determination of L-dopa, six commercial tablets were emptied. A portion (0.197 g) was weighed accurately and dissolved in 1 mL of 5% acetic acid and made up to 100 mL using 0.1 M PBS at pH 7.0. The standard addition method was used for the determination of L-dopa; the results are shown in Table 2, the average recovery is 95%, indicating applicability and reliability of the proposed method.

Table 1. Comparison of the efficiency of some modified electrodes used in the electrocatalysis of L-dopa

Analyte	Types of electrode	Method	Linear range (µM)	Limit of detection(µM)	Reference
UA	Gold screen printed electrode	Amperometry	99 – 1200	68	[10]
	SWCNT	DPV	0.5 -20	0.3	[11]
	Trinuclear ruthenium ammine complex (Ru-red) supported on Y-type zeolite	CV	120 – 10000	85	[12]
	MWCNT / nafion	ASV	0.35 – 15	0.05	[13]
	3, 3', 3" , 3"' – tetraminopthalocyanatonic kel (II) film	Amperometry	0.1 – 0.7	0.1	[14]
	MWCNT and poly-pyrrole doped with tiron	DPV	1 - 100	0.1	[15]
	PEDOT /SWCNT	DPV	0.1 - 20	0.1	[16]
	Gold nano particle self assembled CNT modified pyrolytic graphite	DPV	0.1 – 150	0.5	[17]
	Nickel hexacyanoferrate	Amperometry	0.8 – 2000	0.53	[18]
	Gold functionalized 8-HQ	DPV	3 – 10	0.31	This work

Table 2. Real sample analysis of L-dopa in commercial tablets

Powder tablets samples	Concentration of L-dopa (μM)		Recovery (%)
	Added	Found	
Sample I	4 μM	3.72 μM	93%
Sample II	4 μM	3.84 μM	96%

4. CONCLUSION

A good conducting, gold functionalized 8-HQ material has been successfully synthesized by the electrochemical method. This modified electrode can be considered as a good, sensitive and selective sensor in voltammetric determination of L-dopa. The proposed material shows good electrocatalytic activity toward L-dopa and AA. It shows significant enlargement in peak current and a great decrease in the peak potential. The reproducibility as well as the selectivity is good.

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REFERENCES

[1] J. Hardy, and K. Gwinn-Hardy, Science 282 (1998) 1075.

[2] E. R. Ritvo, A. Yuwiler, E. Geller, A. Kales, S. Rashkis, A. Schicor, S. Plotkin, R. Axelrod, and C. Howard, J. Autism Childhood Schizophrenia 1 (1971) 190.

[3] A. H. V. Schapira, J. Neurol. Neurosurg. Psychiatry 76 (2005) 1472.

[4] Barbeau, Adv. Neurol. 5 (1974) 347.

[5] W. K. Kim, M. M. Karim, and S. H. Lee, Anal. Chim. Acta 619 (2008) 2.

[6] E. J. Greenhow, and L. E. Spencer, Analyst 98 (1973) 485.

[7] A. Hasan, K. D. Khalaf, and M. D. L. Guardia, Talanta 42 (1995) 627.

[8] P. Siddhuraju, and K. Becker, Food Chem. 72 (2001) 389.

[9] R. N. Adams, Anal. Chem. 48 (1976) 1126.

[10] M. F. Bergamini, A. L. Santos, N. R. Stradiotto, and M. V. B. Zanon. J. Pharm. Biomed. Anal 39 (2005) 54.

[11] X. X. Yan, D.W. Pang, Z. X. Lu, J. Q. Lu, and H. Tong, Electroanal. Chem. 569 (2004) 47.

[12] M. F. S. Teixeira, M. F. Bergamini, C. M. P. Marques, and N. Bocchi, Talanta 63 (2004) 1083.

[13] Xiang, Y. Zou, J. Xie, and X. Fei, Anal. Lett 39 (2006) 2569.

[14] A. Sivanesan, and S. A. John, Biosens. Bioelectron. 23 (2007) 708.

[15] S. Shahrokhian, and E. Asadian, Electroanal. Chem. 636 (2009) 40.

[16] J. Mathiyarasu and L. Nyholm, Elactroanalysis 22 (2010) 449.

[17] G. Hu, L. Chen, Y. Guoa, X. Wang, and S. Shao, Electrochimica Acta 55 (2010) 4711.

[18] P. Prabhu, S. Babu and R. S. Narayanan, Sens. Actuators B 156 (2011) 606.

[19] S. Lü, Microchem. J. 77 (2004) 37.

[20] J. Di, C. Shen, S. Peng, Y. Tu, and S. Li, Anal. Chim. Acta 553 (2005) 196.

[21] M. E. B. Calvo, O. D. Renedo, and M. J. A. Martínez, Talanta 74 (2007) 59.

[22] L. Zhang, and X. Jiang, Electroanal. Chem. 583 (2005) 292.

[23] G. Hu, Y. Guo, and S. Shao, Electroanalysis 21 (2009) 1200.

[24] R. N. Goyal, V. K. Gupta, M. Oyama, and N. Bachheti, Talanta 72 (2007) 976.

[25] S. Thiagarajan, and S. M. Chen, Talanta 74 (2007) 212.

[26] Badocco, A. Dean, V. Di Marco, and P. Pastore, Electrochimica Acta 52 (2007) 7920.

[27] B. Brunetti, and E. Desimoni, Electroanalysis 23 (2011) 1116.

[28] Y. Furukawa, K. Seto, K. Nakajima, Y. Itoh, J. Eguchi, T. Sugiyama, and H. Fujimura, Vib. spectrosc. 5 (2012) 60 .

[29] M. F. S. Teixeira, M. F. Bergamini, C. M. P. Marques, and N. Bocchi, Talanta 63 (2004) 1083.

[30] H. R. Zare, N. Nasirizadeh, and M. M. Ardakani, Electroanal. Chem. 577 (2005) 25.