Sensors and Actuators B 153 (2011) 232–238

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Nanogold based electrochemical sensor for determination of norepinephrine in biological fluids

Rajendra N. Goyal[a,*], Md. Abdul Aziz[b], Munetaka Oyama[b], Sanghamitra Chatterjee[a], Anoop Raj Singh Rana[a]

[a] Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee 247667, Uttranchal, India
[b] Graduate School of Engineering, Kyoto University, Japan

ARTICLE INFO

Article history:
Received 3 September 2010
Received in revised form 21 October 2010
Accepted 25 October 2010
Available online 30 October 2010

Keywords:
Nanogold particles
Indium tin oxide
Norepinephrine
Square wave voltammetry

ABSTRACT

A stable layer of gold nano particles (AuNPs) was deposited on the surface of indium tin oxide (ITO) and was used as an electrode for the detection of norepinephrine (NE) by square wave voltammetry (SWV) and cyclic voltammetry (CV). This modified electrode exhibits potent and persistent electron-mediating behavior and a well-defined oxidation peak towards NE was observed. The peak potential of NE was observed at less positive potential with increase in peak current as compared to bare ITO and bare gold electrodes. NE exhibited two quasi-reversible couples at AuNPs modified ITO over the potential range from −0.6 to 0.8 V in phosphate buffer solution (PBS) (pH 7.2). At optimal experimental condition, the catalytic oxidative peak current was responsive with the NE concentrations ranging from 100 nM to 25 μM. The detection limit was found to be 87 nM. Also, the effect of pH revealed that the oxidation of NE at the AuNPs modified ITO involved the transfer of equal number of protons and electrons. The interfering effect of common coexisting metabolites in blood and urine has also been reported. The modified electrode exhibited high stability and reproducibility. A comparison of results with high performance liquid chromatography (HPLC) signalizes a good agreement.

1. Introduction

Norepinephrine (NE) (**I**) is a crucial catecholamine neurotransmitter in the mammalian central nervous system. It is an endogenous hormone exuded by the adrenal medulla, and relinquished as a metabotropic neurotransmitter from nerve endings in the sympathetic nervous system and some areas of the cerebral cortex. Its aftermath may be excitatory or inhibitory. Low levels of NE are associated with depression. Its release is elevated by amphetamines and its dispatch from synapses is occluded by cocaine. NE is also a significant transmitter in many parts of the central nervous system, where it is engrossed in emotional arousal, blood pressure regulation, and mood disorders [1]. Ratiocination of systemic changes in levels of NE in animal models is a substantial step in the process of discovery and evaluation of new drugs in many disease areas such as diabetes, heart disease, pain, anxiety, and other neurological disorders [2–5]. For example, systemic changes in this catecholamine typify a counter regulatory response induced by hypoglycemia, which is helpful for understanding diabetes [6]. In addition, quantification of catecholamine concentrations in plasma and urine is considered clinically important for diagnosis and for evaluating hemodynamic function in intensive care patients [7,8] of pheochromocytoma, paraganglioma, and neuroblastoma. Recent reports have indicated that NE enhances adhesion of human immunodeficiency virus-1-infected leukocytes to cardiac microvascular endothelial cells and also accelerates HIV replication via protein kinase [9]. NE is a drug belonging to the stimulants that are on the World Anti-Doping Agency's 2005 prohibited list.

Hence, it is very necessary to develop sensitive, selective, and reliable methods for the direct determination of trace NE due to its physiological function and the diagnosis of some diseases in clinical medicine. Generally, the determination of NE is carried out by various methods, including high performance liquid chromatography [10,11], gas chromatography [12], ion chromatography [13], spectrophotometry [14] and capillary electrophoresis [15]. Nevertheless, most of these methods have several inconveniences, such as the necessity of previously treating the sample or the chromatographic separation by ion pair reagents, which typically hinder gas chromatography–mass spectrometry detection. HPLC-based methods have several disadvantages since they often require a derivatization to convert the neurotransmitter into a fluorescent molecule [16] and a previous extraction for concentrating or previous purification of the sample [17]. Till date, very few publications concerning the electroanalytical determination of NE in nanomolar concentrations is accessible in the literatures [18,19]. Therefore,

* Corresponding author. Tel.: +91 1332 285794/274454; fax: +91 1332 284361.
E-mail addresses: rngcyfcy@iitr.ernet.in, rngcyfcy@rediffmail.com (R.N. Goyal).

0925-4005/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2010.10.041

it would be ideal to ascertain simple, fast and reliable methods for the determination of catecholamines and neurotransmitters at nanostructured modified ITO.

Nanostructured materials have aroused dramatic interests and have become an intensive research area for the past decade due to their finite small size, high specific surface area, high porosity, and unique physical and chemical properties. The enamouring properties and functions related to the size effect incur intriguing applications in many fields [20,21]. In recent years, AuNPs modified electrodes have received much attention owing to their fascinating electrocatalytic properties [22–24]. Among the different AuNPs modified electrodes for the determination of NE, the AuNPs modified ITO electrode has several advantages over carbon based electrodes [25,26]. Multiwall carbon nanotube/nanogold electrode, gold–platinum hybrid film, gold electrodes modified with mercapto compounds have been used for the determination of NE [27–30]. Since ITO can be used in a wide potential window and possesses stable electrochemical property, it can be served as an excellent working electrode substrate for the fabrication of electrochemical sensors [31]. In nanostrutured network with unusual charge/mass transport mechanisms, nanoparticles could improve charge and mass transfer [32,33]. To the best of our knowledge, no study has reported the electrocatalytic determination of NE by using AuNPs modified ITO electrode. Thus, in the present work, we have described initially the preparation and suitability of AuNPs modified ITO electrode as a new electrode in the electrocatalysis and determination of NE in an aqueous buffer solution, then we evaluated the analytical performance of the modified electrode in quantification of NE in the presence of ascorbic acid, uric acid and dopamine.

2. Experimental

2.1. Apparatus and procedure

The square wave voltammetric experiments were carried out at room temperature ($27 \pm 2\,^{\circ}C$). Initially the experiments were carried out in the temperature range of 25–35 °C. As peak current was essentially similar in this temperature range, the studies were performed at room temperature. Three electrode single compartment cell equipped with a AuNPs modified ITO working electrode, platinum wire counter electrode and Ag/AgCl (3 M NaCl) reference electrode (Model BAS MF-2052 RB-5B) was used. Phosphate buffers in the pH range 2.4–11.0 ($\mu = 0.5$ M) were prepared according to the method reported by Christian and Purdy [34]. BAS (Bioanalytical Systems, West Lafayette, USA) CV-50 W voltammetric analyzer controlled via a computer by its own software was used for electrochemical measurements. The pH measurements were performed using a Century India Ltd. Digital pH-meter (Model CP-901) after due standardization with 0.05 M potassium hydrogen phthalate (pH 4.0 at 25 °C) and 0.01 M borax (pH 9.2 at 25 °C). HPLC studies were performed on Shimadzu LC-2010A HT system with RP-18e (5 μm) column. The mobile phase used for HPLC experiment was a mixture of acetonitrile:water (80:20) at a flow rate of 1.2 mL min^{-1} and detection was carried out at 210 nm.

The parameters for square wave voltammetry were optimized by recording voltammograms at different conditions. Initially voltammograms of NE were recorded from −0.2 to +1.0 V at different square wave amplitude (E_{sw}) in the range 5–100 mV. The best peak was noticed at 25 mV square wave amplitude. Similarly potential step was varied between 4 and 25 mV and finally square wave frequency was changed between 5 and 200 Hz. The conditions at which well-defined peak was noticed were selected as optimized parameters. As there was no peak after +600 mV, the curves were recorded up to 600 mV. Optimized square wave voltammetry parameters used in the present studies were: initial E: −200 mV, final E: 600 mV, square wave amplitude (E_{sw}): 25 mV, potential step (E): 4 mV, square wave frequency (f): 15 Hz. Cyclic voltammograms were recorded in the sweep rate 10–1000 mV s^{-1} with initial sweep to positive potentials. The solutions were deoxygenated by bubbling high-purity nitrogen for 12–15 min before recording the cyclic voltammograms. Working solutions of NE were prepared by adding required volumes of the respective stock solution (1 mM) to the phosphate buffer solution and then the voltammograms were recorded. Urine and blood samples were received from laboratory personnel. The blood sample with EDTA as anticoagulant was centrifuged and the supernatant was taken for analysis.

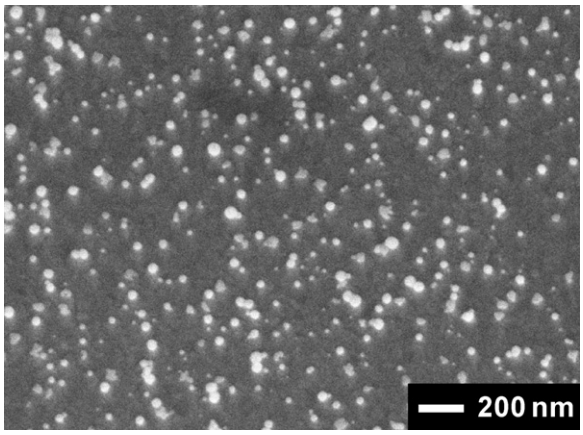


Fig. 1. A FE-SEM image of the surface of a densely coated AuNPs modified ITO electrode.

2.2. Reagents

ITO spurted glass sheets with resistivity 30 Ω cm^{-2} were obtained from Geomatec, Japan. Cetyltrimethylammonium bromide, $HAuCl_4$ and ascorbic acid were purchased from Aldrich (USA). Norepinephrine bitartarate salt monohydrate is incurred from Sigma–Aldrich Chemie, Stenhein, Germany. All other solvents and chemicals used were of analytical grade.

2.3. Fabrication of gold nanoparticles modified ITO electrode

For preparing the AuNPs on ITO electrode, initially the ITO sheet (1 cm^2) was immersed in 2.5 ml solution of 1.34 mM $HAuCl_4$ (aq.) in a test tube. Then 2.5 ml of 10 mM solution of ascorbic acid (aq.) was added to the reaction mass at room temperature. Afterward, the mixed solution was heated at 75 °C for 15 min. After washing with water, the AuNP-attached ITO was dried at 40 °C. The details of this preparation method will be published elsewhere. A FE-SEM image of nano gold modified electrode is shown in Fig. 1 and clearly shows deposition of nano gold particles at the surface of ITO. The size of the nano particles was in the range 20–40 nm and the effective surface coverage based on two-dimensional SEM was found to be 8%.

AuNPs modified ITO sheet was then connected to a thin copper strip and molded between two pieces of scotch tape of size 50 mm × 12 mm. The contact of the electrode to the solution was provided with the help of a 2 mm dia. hole on one side of tape. The electrode is then ready for use. Thus, the exposed area of ∼3 mm^2 contacted the solution as working electrode and diffusion of species from the solution to the electrode surface will be chiefly linear. The prepared electrode was stored in air with contact side upwards.

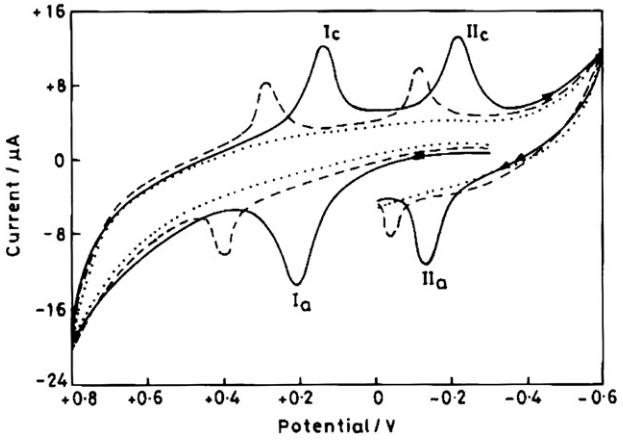


Fig. 2. Cyclic voltammograms obtained for 25 μM NE at pH 7.2 at (a) AuNPs modified ITO (—), (b) bare ITO (- - -) and (c) background PBS at pH 7.2 at AuNPs modified ITO (···) at 20 mV s^{-1}.

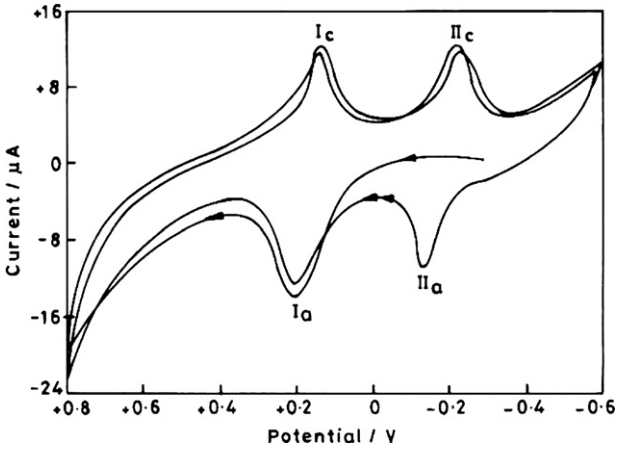


Fig. 3. Cyclic voltammogram of 25 μM NE at pH 7.2 at AuNPs modified ITO showing two complete cycles.

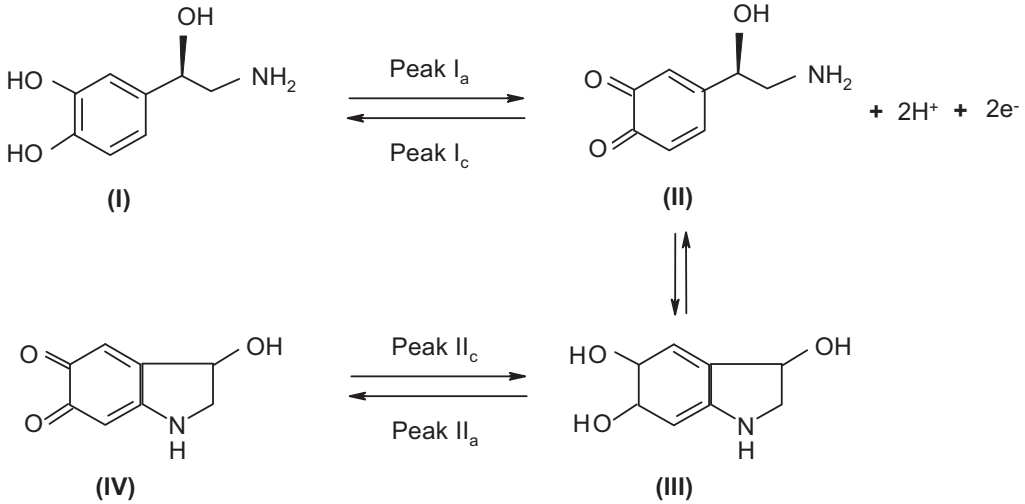
3. Results and discussion

3.1. Cyclic voltammetry

The electrochemical response of NE at bare ITO and AuNPs modified ITO electrode was characterized by CV as shown in Fig. 2. The cyclic voltammograms recorded for 25 μM NE at AuNPs modified ITO in 1 M phosphate buffer solution at pH 7.2 illustrated that NE exhibited two quasi-reversible couples of well-defined redox peaks on AuNPs modified ITO over the potential range from −0.6 to 0.8 V. On the first positive scan, only one peak (I_a) appeared at 218 mV, which corresponds to the electrochemical oxidation of NE (**I**) to the open-chain norepinephrine quinone (**II**) as shown in Scheme 1. On the reverse potential scan, the reduction of this quinone was again observed at 137 mV indicated by peak I_c, and another cathodic peak II_c is also obtained at −206 mV. On the second positive scan, a new anodic peak (peak II_a) at −131 mV emerged and other peaks remain same. Fig. 3 presents a CV with two complete cycles and no new peaks were observed. The quasi-reversible couple, peaks II_a and II_c are obtained by the interconversion of 2,3,5,6-tetrahydro-1 H-indole-3,5,6-triol (**III**) which is obtained by the cyclization reaction of (**II**) and 3-hydroxy-2,3-dihydro-1 H-indole-5,6-dione (**IV**) as reported earlier [35]. To ascertain the nature of the reaction, sweep rate studies were performed in the range 10–1000 mV s^{-1}. Fig. 4 presents effect of sweep arte on peak I_a of NE at different sweep rates. The analyte peak current was found to increase with increasing sweep rates and the plot of $i_p/v^{1/2}$ versus log v clearly indicated that the electrode process is adsorption controlled [36,37].

3.2. Square wave voltammetry

In order to illustrate the electrocatalytic effect of AuNPs modified ITO electrode towards NE, the square wave voltammograms of NE at three different working electrodes were recorded. Fig. 5 shows the square wave voltammograms obtained at the bare ITO, bare gold (Au) and AuNPs modified ITO electrode for 15 μM NE in 1 M phosphate buffer solution of pH 7.2. At bare ITO electrode surface, a broad oxidation peak with low current was observed at 314 mV. Under identical conditions, the oxidation peak of NE increases slightly at bare gold electrode with peak potential at 251 mV. However, the oxidation peak current of NE at the AuNPs modified ITO electrode increases significantly and the peak potential shifts negatively from 314 to 137 mV, in comparison to that at the bare ITO electrode. The remarkable enhancement in peak current response and the negative shift of the oxidation peak potential support the fact that the gold nanoparticles act as a very efficient promoter to enhance the kinetics of the electrochemical process. Thus, the AuNPs modified ITO shows electrocatalytic activity towards the oxidation of NE. The most likely reasons for such



Scheme 1. Tentative redox mechanism proposed for the oxidation of norepinephrine.

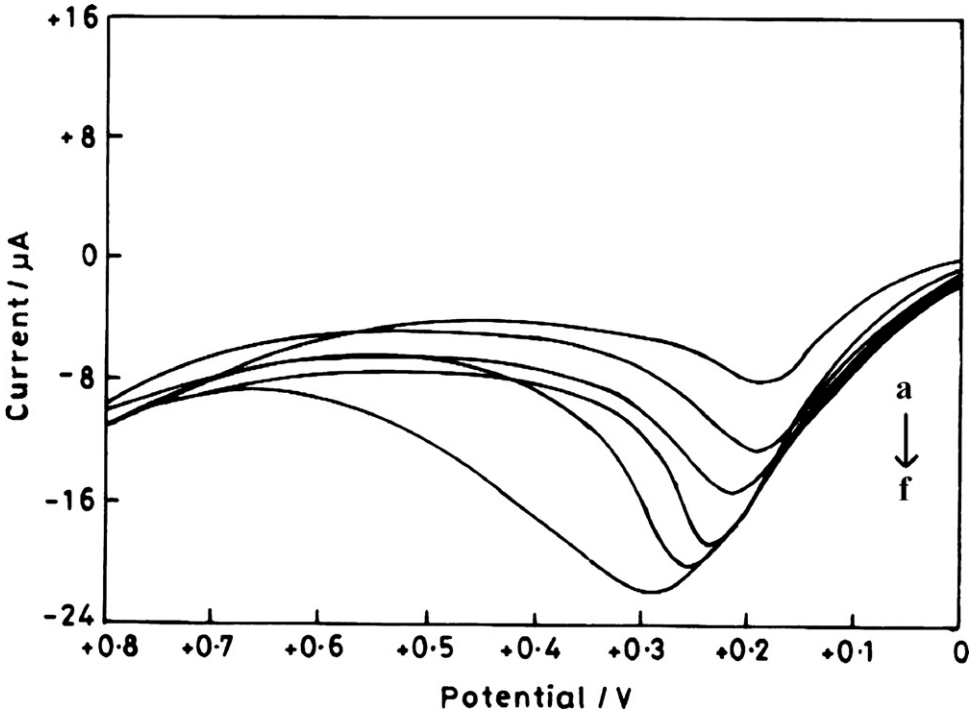


Fig. 4. Effect of sweep rate observed on peak I_a of 25 μM NE at pH 7.2 in cyclic voltammetry at AuNPs modified ITO. Curves were recorded at (a) 10 mV s^{-1}; (b) 20 mV s^{-1}; (c) 50 mV s^{-1}; (d) 100 mV s^{-1}; (e) 250 mV s^{-1} and (f) 500 mV s^{-1}.

electrocatalytic activity of AuNPs is their high specific surface area, superhydrophobicity and surface enhanced Raman scattering [38].

3.3. Effect of pH

SWV measurements were performed for NE oxidation at AuNPs modified ITO electrode over the pH range 2.4–11.0 and the relationship between pH value and the oxidation peak potential (E_p) was investigated. It was found that the peak potential of NE was found to be dependent on pH and shifted to less positive potential with increasing pH from 2.4 to 11.0 at AuNPs modified ITO electrode as shown in Fig. 6(a). The dependence of E_p on pH at AuNPs modified ITO electrode using linear regression analysis can be expressed by the relation:

$$E_p(\text{pH } 2.4\text{–}11.0) = [592\text{–}58.839\,\text{pH}] \text{ versus Ag/AgCl}$$

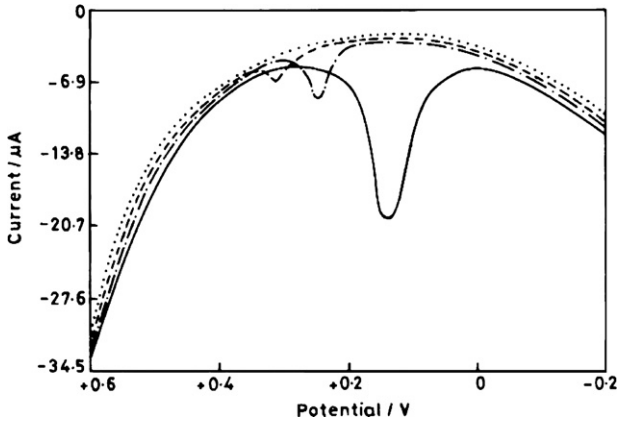


Fig. 5. Comparison of square-wave voltammograms of 15 μM NE (pH 7.2) at (a) AuNPs modified ITO (—), (b) bare gold (– · – · –), (c) bare ITO (- - -) and (d) background PBS at pH 7.2 at AuNPs modified ITO (· · ·).

Having correlation coefficient ~0.992. The observed slope of ~59 mV/pH clearly indicates that equal number of electrons and protons are involved in the electrode reaction. The detailed studies for analysis were carried out at pH 7.2, which is close to physiological pH.

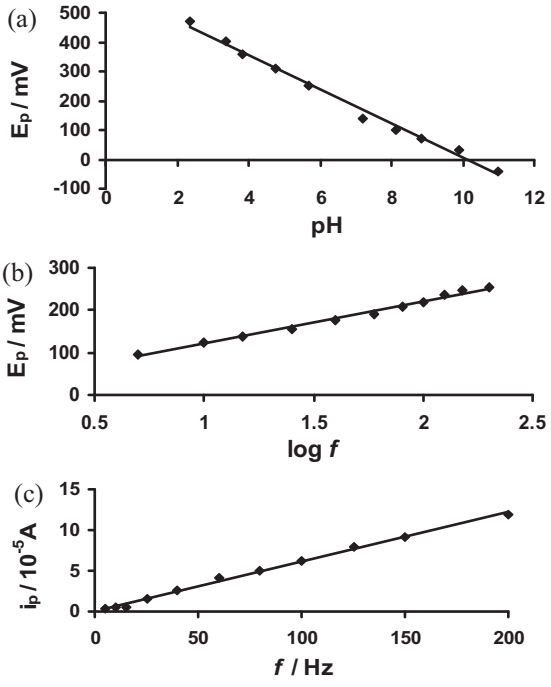


Fig. 6. (a) Observed dependence of peak potential (E_p) on pH for 15 μM NE at AuNPs modified ITO. (b) E_p versus log f plot observed for 15 μM NE at AuNPs modified ITO at pH 7.2. (c) Linear dependence of peak current (i_p) on square wave frequency for 15 μM NE at pH 7.2 at AuNPs modified ITO.

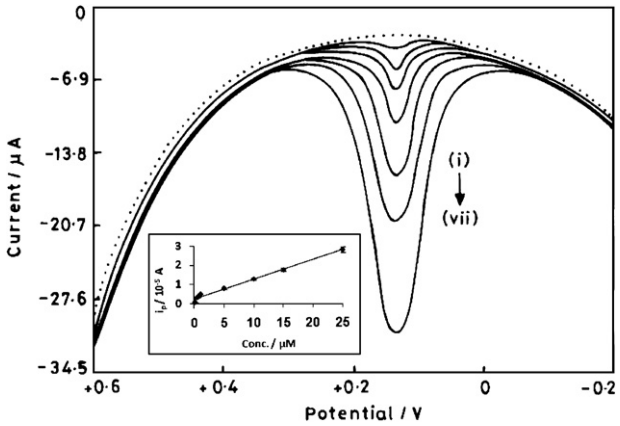


Fig. 7. Square wave voltammograms recorded for (a) phosphate buffer solution (background) at AuNPs modified ITO (···) and (b) increasing concentration of NE at the modified electrode (—) [curves were recorded at (i) 0.1 μM; (ii) 0.5 μM; (iii) 1 μM; (iv) 5 μM; (v) 10 μM; (vi) 15 μM and (vii) 25 μM concentration in phosphate buffer solution of pH 7.2]. The linear calibration plot at AuNPs modified ITO at pH 7.2 is presented in inset.

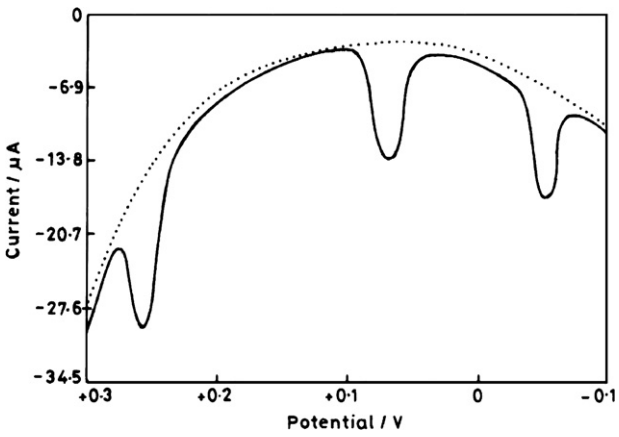


Fig. 8. Square wave voltammogram observed for a mixture of ascorbic acid, dopamine and uric acid at pH 7.2.

3.4. Effect of square wave frequency

The effect of square wave frequency on the oxidation of NE was studied in the frequency range 5–200 Hz. The peak potential of NE was found to shift towards more positive potentials with increase in square wave frequency (f). The plot of E_p versus log$f$ was linear as represented in Fig. 6(b). The dependence of E_p on log$f$ can be represented by the relation having correlation coefficient 0.993:

$$E_p\,(\text{mV}) = 100.87 \log f + 19.82$$

The effect of square wave frequency on the peak current of analyte was also studied in the square wave frequency range 5–200 Hz. The oxidation peak current of NE was found to increase with increase in square wave frequency as shown in Fig. 6(c). The dependence of peak current on square wave frequency was linear and can be expressed by the relation:

$$i_p\,(10^{-5}\text{A}) = 0.0609 f + 0.023$$

Having correlation coefficient 0.996. These observations are in agreement with a reversible, adsorption controlled electrode reaction [39–41] which supports the inferences obtained from cyclic voltammetric studies.

3.5. Calibration plot

The quantitative determination is based on the dependence of the peak current (i_p) on concentration of NE. The current values are obtained by subtracting the background current at peak potential and are reported as an average of three replicate determinations. Fig. 7 depicts the set of square wave voltammograms with increasing concentration of NE in 1 M phosphate buffer solution (pH 7.2). With increasing concentration of NE, the peak current was found to increase in the range 100 nM to 25 μM at AuNPs modified ITO electrode at pH 7.2. The plot of i_p versus concentration was linear and presented as inset in Fig. 7. The dependence of peak current (after background current correction in peak current) can be expressed by the equation:

$$i_p\,(10^{-5}\text{A}) = 0.1011\, C\,(\text{mM}) + 0.2559$$

where C is the concentration (μM) having correlation coefficient of 0.989 with sensitivity of 0.1011×10^{-5} A μM^{-1} at AuNPs modified ITO electrode. This behavior indicated that NE can be safely estimated in the concentration range 100 nM to 25 μM at AuNPs modified ITO electrode. The detection limit ($3\sigma/b$), where σ is the blank standard deviation and m is the sensitivity, was found to be 87 nM.

3.6. Stability and reproducibility of the modified electrode

The reproducibility and stability of the AuNPs modified ITO for the determination of NE was investigated. The stability of the modified electrode was evaluated by measuring the current response at a fixed concentration of 5 μM of NE over a period of 10 days. The electrode was used daily and stored in the air. The experimental results indicated that the current response deviated intraday by 1.8% and interday by 3.7%, suggesting that the modified electrode possesses good stability. To characterize the reproducibility of the modified electrode, repetitive determinations of NE were carried out at 5 μM concentration at pH 7.2. The results of six replicate measurements showed a relative standard deviation of 1.4% indicating that the results are reproducible. Thus, the AuNPs modified ITO electrode exhibits good stability and reproducibility for the determination of NE.

3.7. Specificity

The specificity of the optimized procedure for the assay of NE was investigated by observing any interference encountered from endogenous substances present in complex matrices such as biological fluids (e.g. urine and plasma). The effect of the interferents (viz. uric acid, ascorbic acid and dopamine) was examined by carrying out the determination of 0.5 μM NE in the presence of different concentrations of the interferents. The tolerance limit was defined as the concentrations of foreign substances, which gave an error less than ±5.0% in the detection of the drug. It was observed that up to 100-fold excess of each of the interferents there was no remarkable change in the peak current response of NE. A square wave voltammogram of mixture of ascorbic acid, dopamine and uric acid is presented in Fig. 8 and three peaks at $E_p \sim -50$, 70 and 260 mV are observed corresponding to the oxidation of ascorbic acid, dopamine and uric acid. A typical voltammogram of NE in presence of interferents is shown in Fig. 9. It was found that a clear peak at E_p 137 mV was observed at pH 7.2 corresponding to oxidation of NE. in addition to peaks at $E_p \sim -50$, 70 and 260 mV. The oxidation peaks of these interferents did not interfere with peak of NE up to 100-fold concentration of NE. As square wave voltammogram of the modified electrode in PBS containing uric acid, dopamine and ascorbic acid is able to adequately identify the three species, thus, the developed sensor can also be used for the simultaneous determination of NE, uric acid, dopamine and ascorbic acid. This indicates that

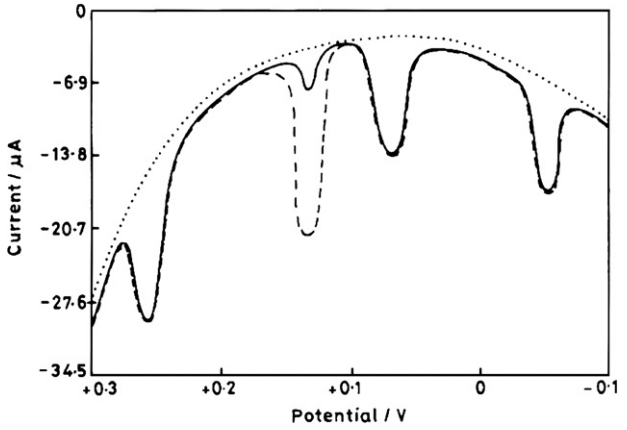


Fig. 9. Voltammograms observed for (a) background pH 7.2 (···), (b) 0.5 μM NE in presence of interferents ascorbic acid, dopamine and uric acid (—) and (c) normal human urine sample spiked with 15 μM NE (- - -).

Table 1
Recovery data of NE added to human blood plasma.

Added (μM)	Found (μM)	Recovery (%)[a]
Sample 1		
5.0	4.92	98.40
15.0	15.37	102.47
25.0	24.84	99.36
Sample 2		
5.0	5.19	103.80
15.0	14.74	98.27
25.0	25.27	101.08
Sample 3		
5.0	5.14	102.80
15.0	15.19	101.27
25.0	24.75	99.00

[a] The R.S.D. value for determination was less than 2.1% for $n = 3$.

the method can be safely applied to the determination of NE in biological fluids in presence of these interferents.

3.8. Recovery test

To study the accuracy of the proposed method, attempts were made to obtain samples of blood and urine from the patients undergoing treatment with NE. However, due to major use of this compound as a life saving drug, no samples could be obtained inspite of our best efforts. Therefore, recovery experiments were carried out by standard addition method in biological samples obtained from healthy volunteers. Fig. 6 depicts a voltammogram obtained for normal human urine sample spiked with 15 μM of NE. The results observed are listed in Tables 1 and 2 for blood and urine samples, respectively. The recoveries for the peak varied in the range from 98.27% to 103.80% in the case of human blood plasma and from 99.40% to 104.47% in the case of urine samples. Thus, it is quite evident that the recovery data lie in the acceptable range and the sensor can be used for such determinations.

Table 2
Recovery data of NE added to human urine samples.

Spiked (μM)	Detected (μM)	Recovery (%)[a]
Sample 1		
5.0	5.15	103.00
15.0	15.67	104.47
25.0	24.85	99.40
Sample 2		
5.0	5.09	101.80
15.0	14.93	99.53
25.0	25.16	100.64

[a] The R.S.D. value for determination was less than 3.5% for $n = 3$.

Table 3
A comparison of observed concentration of NE in human blood plasma and urine sample 1 at AuNPs modified ITO electrode and by using HPLC.

Spiked (μM)	Observed (μM) by SWV	Observed (μM) by HPLC
Human blood plasma		
Sample 1		
5.0	4.92	4.97
15.0	15.37	15.30
25.0	24.84	24.82
Human urine		
Sample 1		
5.0	5.15	5.10
15.0	15.67	15.64
25.0	24.85	24.88

3.9. Comparison by HPLC

To prove the reliability of data obtained, the results obtained by the voltammetric method were compared with HPLC analysis. Initially, various concentrations of NE were analyzed using HPLC and the peak area was calculated. A well defined peak is obtained at $R_t \sim 4.665$ min in the standard sample of NE. Blood plasma and urine sample of normal person used as control was then injected in HPLC and the peak of NE was found to be absent. The plasma and urine sample of control was then spiked with known concentration of NE. A calibration curve was obtained by plotting the peak area of the analyte peaks against the analyte concentration. The resulting calibration plot was linear. A comparison of the values obtained by HPLC and the proposed voltammetric method (as listed in Table 3) clearly indicated that the results obtained by two methods are in good agreement.

4. Conclusions

The present work reveals the fact that nano-sized Au deposited on ITO shows a high electrocatalytic activity over earlier reported nanogold modified ITO electrode with about 5% coverage [42–44]. The voltammetric behavior of NE was studied at bare ITO, bare Au and Au-nanoparticles deposited electrode in PBS (pH = 7.2). It was found that performance degradation of the ITO electrode towards the oxidation of NE can be suppressed by modifying the electrode surface with densely coated gold nanoparticles, which were formed on ITO electrode. Compared with bare ITO electrode, AuNPs modified ITO electrode demonstrates electrocatalytic activity because the peak potential of NE was observed at a less positive value when densely coated AuNPs modified ITO electrode was employed. The densely coated AuNPs modified ITO electrode demonstrates unique catalytic behavior and stability towards the oxidation of NE. The response of AuNPs modified ITO electrode on the oxidation of related catecholamine and neurotransmitter was also examined. Epinephrine and dopamine showed oxidation peaks at ~125 and ~70 mV confirming thereby that this voltammetric sensor is specific for the oxidation of NE at ~137 mV.

The NE species as detected by SWV shows the dependence of anodic peak current on concentration and is linear from 100 nM to 25 μM with a detection limit of 87 nM. The advantage of the present electrode lies in the fact that the electron transfer for the oxidation of NE is a surface-adsorption controlled process and it shows excellent sensitivity and good selectivity. The method described above requires less time and is easy to perform. A comparison of the results with HPLC indicates that the method is sensitive and the results are comparable. This electrode can detect NE at the nanomolar level in presence of ascorbic acid, uric acid and dopamine. The

normal concentration of NE in blood and urine is 1.2 and 120 nM, respectively. The concentration of NE rises abnormally in traumatic brain injury to a concentration of 500 nM which can be easily detected at AuNPs modified ITO by using SWV. Thus, in the present study, the potential application of densely coated nano-sized gold for the fabrication of a voltammetric NE sensor is reported.

Acknowledgements

Authors (SC and ARSR) are thankful to the Council of Scientific and Industrial Research, New Delhi and Department of Science and Technology, New Delhi for the award of Senior and Junior Research Fellowship, respectively. Financial assistance for this work was provided by Department of Biotechnology, New Delhi.

References

[1] D. Voet, J.G. Voet, Biochemistry, 2nd ed., Wiley, New York, 1995.
[2] J. Slavikova, J. Kuncova, O. Topolcan, Clin. Cardiol. 30 (2007) 326.
[3] E.M. Cotella, I.M. Lascano, G.M. Levin, M.M. Suarez, Int. J. Neurosci. 119 (2009) 664.
[4] B. Mravec, A. Tillinger, I. Bodnar, G.M. Nagy, M. Palkovits, R. Kvetnansky, Ann. N. Y. Acad. Sci. 1148 (2008) 196.
[5] A. Flaa, I.K. Eide, S.E. Kjeldsen, M. Rostrup, Hypertension 52 (2008) 336.
[6] R.S. Sherwin, Diabetes 57 (2008) 2259.
[7] S.D. Parker, M.J. Breslow, S.M. Frank, B.A. Rosenfeld, E.J. Norris, R. Christopherson, P. Rock, S.O. Gottlieb, H. Raff, B.A. Perler, Crit. Care Med. 23 (1995) 1954.
[8] M.M. Kushnir, F.M. Urry, E.L. Frank, W.L. Roberts, B. Shushan, Clin. Chem. 48 (2002) 323.
[9] S.W. Cole, Y.D. Korin, J.L. Fahey, J.A. Zack, J. Immunol. 161 (1998) 610.
[10] V. Carrera, E. Sabater, E. Vilanova, M.A. Sogorb, J. Chromatogr. B 847 (2007) 88.
[11] H. Wang, H. Jin, H.S. Zhang, Fresenius, J. Anal. Chem. 365 (1999) 682.
[12] N. Shafi, J. Chem. Soc. Pak. 17 (1995) 103.
[13] C.L. Guan, J. Quyang, Q.L. Li, B.H. Liu, W.R.G. Baeyens, Talanta 50 (2000) 1197.
[14] M. Zhu, X.M. Huang, J. Li, H.X. Shen, Anal. Chim. Acta 357 (1997) 261.
[15] S. Wei, G. Song, J.M. Lin, J. Chromatogr. A 1098 (2005) 166.
[16] E.C. Chan, P.Y. Wee, P.Y. Ho, P.C. Ho, J. Chromatogr. B 749 (2000) 179.
[17] C. Sabbioni, M.A. Saracino, R. Mandrioli, S. Pinzauti, S. Furlanetto, G. Gerra, M.A. Raggi, J. Chromatogr. A 1032 (2004) 65.
[18] S.H. Huang, H.H. Liao, D.H. Chen, Biosens. Bioelectron. 25 (2010) 2351.
[19] G. Wang, X. Liu, B. Yu, G. Luo, J. Electroanal. Chem. 567 (2004) 227.
[20] W. Shen, F.E. Huggins, N. Shah, G. Jacobs, Y. Wang, X. Shi, G.P. Huffman, Appl. Catal. A: Gen. 351 (2008) 102.
[21] M. Yamada, M. Kawana, M. Miyake, Appl. Catal. A: Gen. 302 (2006) 201.
[22] M.C. Daniel, D. Astruc, Chem. Rev. 104 (2004) 293.
[23] E. Majid, S. Hrapovic, Y. Liu, K.B. Male, J.H.T. Luong, Anal. Chem. 78 (2006) 762.
[24] P. Kannan, S.A. John, Anal. Biochem. 386 (2009) 65.
[25] W. Cheng, S. Dong, E. Wang, Langmuir 18 (2002) 9947.
[26] J. Lin, L. Zhang, S. Zhang, Anal. Biochem. 370 (2007) 180.
[27] C.C. Ti, Y. Umashankar, S.M. Chan, Electroanalysis 21 (2009) 1855.
[28] T. Luczak, Electroanalysis 21 (2009) 1539.
[29] Y. Sun, L. Jia, F. Shang, Fenxi Kexue Xuebao 22 (2006) 425.
[30] S. Thiagarajan, S.M. Chen, J. Solid State Electrochem. 13 (2009) 445.
[31] M. Lahav, A.N. Shipway, I. Willner, J. Chem. Soc., Perkin Trans. 2 (1999) 1925.
[32] F.N. Crespilho, V. Zucolotto, C.M.A. Brett, O.N. Oliveira Jr., F.C. Nart, J. Phys. Chem. B 110 (2006) 17478.
[33] F. Bedioui, N. Villeneuve, Electroanalysis 15 (2003) 5.
[34] G.D. Christian, W.C. Purdy, J. Electroanal. Chem. 3 (1962) 363.
[35] C. Bian, Q. Zeng, H. Xiong, X. Zhang, S. Wang, Bioelectrochemistry 79 (2010) 1.
[36] R.S. Nicholson, I. Shain, Anal. Chem. 36 (1964) 706.
[37] E.R. Brown, R.F. Large, A. Weissberger, B.W. Rossiter (Eds.), Physical Methods of Chemistry, Wiley Interscience, Rochester, New York, 1964.
[38] H. Zhang, J.J. Xu, H.Y. Chen, J. Chem. Phys. 112 (2008) 13886.
[39] M.R.C. Massaroppi, S.A.S. Machado, L.A. Avaca, J. Braz. Chem. Soc. 14 (2003) 113.
[40] S. Komorsky-Lovric, S. Gagic, R. Penovski, Anal. Chim. Acta 389 (1999) 219.
[41] F. Quentel, C. Elleouet, Electroanalysis 13 (2001) 1030.
[42] R.N. Goyal, M. Oyama, A.A. Umar, A. Tyagi, N. Bachheti, J. Pharm. Bioanal. Chem. 44 (2007) 1147.
[43] R.N. Goyal, M. Oyama, A. Tyagi, S.P. Singh, Talanta 72 (2007) 140.
[44] R.N. Goyal, M. Oyama, S.P. Singh, J. Electroanal. Chem. 611 (2007) 140.

Biographies

R.N. Goyal obtained Ph.D. in 1975 from Roorkee University and D.Sc. from Agra University, India in 1991. He worked as postdoctoral fellow at University of Oklahoma during 1979–1982 and again during 1989–1991. He was British Council fellow at University of Loughborough, UK during 1979 and was visiting professor at Kyoto University, Japan during 2007. Presently he is working as a professor in Department of Chemistry, IIT Roorkee, Roorkee, India. His current research area includes molecular electrochemistry, electrochemistry at nano-material modified electrodes and sensors and biosensors for doping agents.

Md. Abdul Aziz received his Ph.D. from Pusan National University in 2009. He is now a post doctoral fellow of Japan Society for the promotion of Science in Kyoto University. His research interest is electroanalytical chemistry with nanomaterials.

Munetaka Oyama received his D.Sc. degree from Kyoto University in 1991. From 1999, he is a associate professor of Kyoto University. His research interest is electroanalytical chemistry with nanomaterials.

Sanghamitra Chatterjee did her M.Sc. in 2005 from Lucknow University, India. She is working for Ph.D. as a Senior Research Fellow of Council of Scientific and Industrial Research, New Delhi.

Anoop Raj Singh Rana did his M.Sc. in 2007 from Agra University. He is working as Junior Research Fellow of Department of Science and Technology, New Delhi.