ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2018, *63*(10), 941-952

ELECTROCHEMICALLY GROWN BISMUTH(III) OXIDE NANOPARTICLES ON GOLD AS SENSOR FOR QUANTIFICATION OF METHIMAZOLE

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Received December 11, 2017

This Bismuth (III) oxide modified gold electrode was fabricated succesfully using an electrochemical method and characterized by cyclic voltammetry (CV), potential-controlled electrochemical deposition, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), photoluminescence (PL) and UV visible spectroscopy techniques. The ease of fabrication, excellent electrochemical performance and high electroactive surface area are the promising features of the fabricated sensor. This modified electrode was used for the detection of methimazole in pharmaceutical formation in 1 mM $K_3[Fe(CN)_6]$ containing 0.1 M KCl. The fabricated sensor can be used for sensitive and selective determination of many pharmaceuticals owing to their sensitivity, specificity, speed response and low cost of analysis.

INTRODUCTION

Methimazole (2-mercapto-1-methylimidazole) (Fig. 1) used in the treatment of hyperthyroid by the production of thyroxine, a hormone excreted by the thyroid gland, inhibits the formation of thyroid hormones.[1] It is absorbed by the gastrointestinal tract and acts as an immunosuppressive agent in Graves disease.[2-4] Several analytical procedures have been described for the determination of methimazole including thin layer chromatography,[1] coulometry,[5] conductometry,[6] high-performance liquid chromatography with ultraviolet detection,[7] spectroscopy,[8-10] electrochemistry with a silver-silver sulphide solid-state electrode,[11] liquid chromatography with amperometric detection at a nafion/indium hexacyanoferrate film modified electrode[12] capillary zone electrophoresis with amperometric detection at a carbon electrode[13] potentiometric and voltammetric methods.[14]

However, these methods are complicated and needed expensive instrumentation. Electrochemical methods have proved to be useful for sensitive and selective determination of many pharmaceuticals owing to their sensitivity, specificity, simplicity of samples preparation, simplicity of nanomaterials based modified sensor preparation, speed of response and low cost of analysis.[15]

Nanomaterials have been widely used in constructing electrochemical sensor or biosensors such as carbon nanotubes,[16,17] TiO_2,[18] SiO_2,[19] silver,[20] iridium oxide,[21] graphene,[22,23] bismuth(III) oxide[24-33] and fullerenes,[34] etc. These have been succesfully used for the preparation of sensors or biosensors, with good stability and easy to fabricate.[35-38] Among all the given nanomaterials,

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bismuth (III) oxide is recognized as one of the most promising electrode materials for electrochemical sensing devices due to its characteristic parameters such as energy band gap, large surface area, electrochemical stability and catalytic behavior that are suitable for large applications. Apart from the improvement in the sensitivity, its high electrical conductivity, chemical stability and mechanical strength proved a suitable electrochemical sensor for its application in voltammetric measurement.[39] In addition, bismuth (III) oxide is nontoxic and has excellent chemical inertness and biocompatibility. Nanosized bismuth (III) oxide shows greater advantages and novel characteristics than regular sized particles, such as the much higher specific surface and greater surface free energy, which are favorable for the biomolecules adsorption.[40]

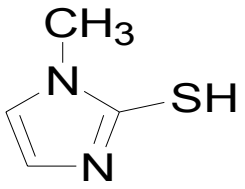


Fig. 1 – Chemical structure of methimazole.

The main objective of this work is the first application of bismuth oxide nanoparticles to develop a new sensor for the trace quantification of methimazole. It was used to investigate the electrochemical response of methimazole on the surface of Bismuth III oxide nanoparticles/Au modified electrode. Structural and morphology characterization, cyclic voltammetry (CV), potential-controlled electrochemical deposition, scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), photoluminescence (PL) and (UV) visible spectroscopy studies performed on the synthesized nano-Bi_2O_3 are presented.

EXPERIMENTAL

Materials

Chemical, Reagents and Analytical Conditions

Methimazole obtained from Sigma (Germany). Thyromazol tablets were purchased from the local pharmacy (Erzurum, Turkey). A stock solution of 100 µg/mL was prepared by dissolving the compound in formation 1 mM $K_3[Fe(CN)_6]$ containing 0.1 M KCl. All other reagents were of analytical grade or equivalent, and obtained from Merck or Fluka.

Standard solutions were prepared by serial dilution of the stock solution with selected supporting electrolyte. The calibration curve for SWV and DPV analysis was constructed by plotting the peak current against the methimazole concentration. The ruggedness and precision were checked at different days, within day and between days. Relative standard deviations were calculated to check the ruggedness and precision of the method.[41] The precision and accuracy of analytical methods are described in a quantitative fashion by the use of relative errors (bias %). One example of relative error is the accuracy, which describes the deviation from the expected results. All solutions were kept in the dark in a refrigerator and were used within several hours to avoid hydrolysis. However, voltammograms of the sample solutions recorded 72 h after preparation did not show appreciable change in assay values.

Voltammetric measurements were obtained with Gamry Potentiostat Interface 1000 controlled with software PHE 200 and PV 220. A three electrode cell system used gold electrode (Polycrystalline Au electrode 99.99% pure) as working electrode and an Ag/AgCl (KCl 3M, BAS) electrode as the reference electrode. All the results in the figures are presented in respect to the Ag/AgCl, 3M KCl reference electrodes.

All pH measurements were made with Model 538 pH meter (WTW, Austria), calibrated with standard buffers (Fixanal, Riedel-deHaen, Germany) at room temperature. All measurements were carried out at ambient temperature of the laboratory (22-25 ^{0}C).

For analytical application, the following parameters being employed: SWV pulse amplitude 25 mV, frequency 15 Hz, potential step 4mV; DPV pulse amplitude 50 mV, pulse width 50 ms, scan rate 20 mV/s.

Cyclic voltammetry: The initial and final potentials were variable, depending on the pH value and the cut-off electrolyte. Scan rate measurements in the range 10-1000 mV/s.

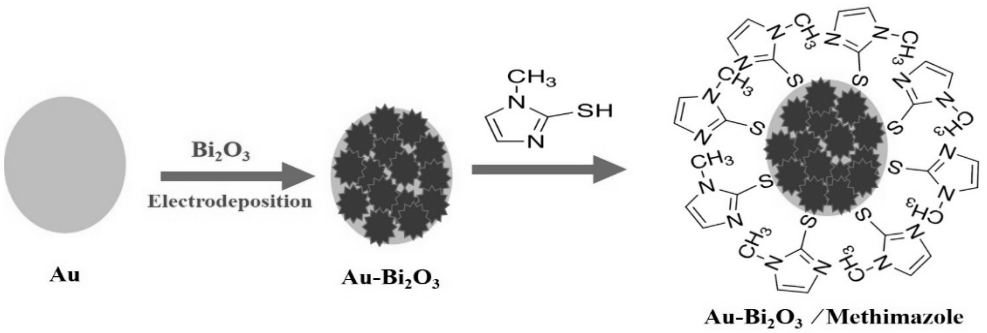
The morphological analysis and elemental composition determination (Bi/O) of the Bi_2O_3 nanostructures were carried out by EDS with a INSPECT S50 system coupled to the scanning electron microscope. Steady-state fluorescence measurements were performed with Shimadzu RF-5301 PC spectrofluorophotometer.

The sensor fabrication

The electrochemical deposition technique employed in the growth of the Bi_2O_3 nanostructures was growth from a solution at 1 mM $Bi(NO_3)_3$ and pH 1.5, dissolved O_2 gas. Electrochemical deposition of Bi film was carried out in room temperature at constant potential of + 300 mV for 30 min. The Bi_2O_3/Au working electrode was dried at room temperature and the film was cured with warm air for about 1 min (Scheme 1).

Procedure for Pharmaceutical Preparations

A total 10 tablets of methimazole (Thyromazol) were accurately weighed and powdered. An amount of this powder corresponding to one tablet methimazole content was weighed and accurately transferred into 100 mL calibrated flask and 50 mL of 1 mM $K_3[Fe(CN)_6]$ containing 0.1 M KCl was added and then the flask was sonicated to 10 min at room tempature. The flask was filled to volume with 1 mM $K_3[Fe(CN)_6]$ containing 0.1 M KCl. The resulting solutions in both the cases were filtered through Whatman filter paper no[42] and suitably diluted to get final concentration within the limits of linearity for the respective proposed method. The drug content of methimazole tablet was calculated from the current potential curve.



Scheme 1 – Schematic illustration of the electrochemically patterned Bi_2O_3 biosensor on Au.

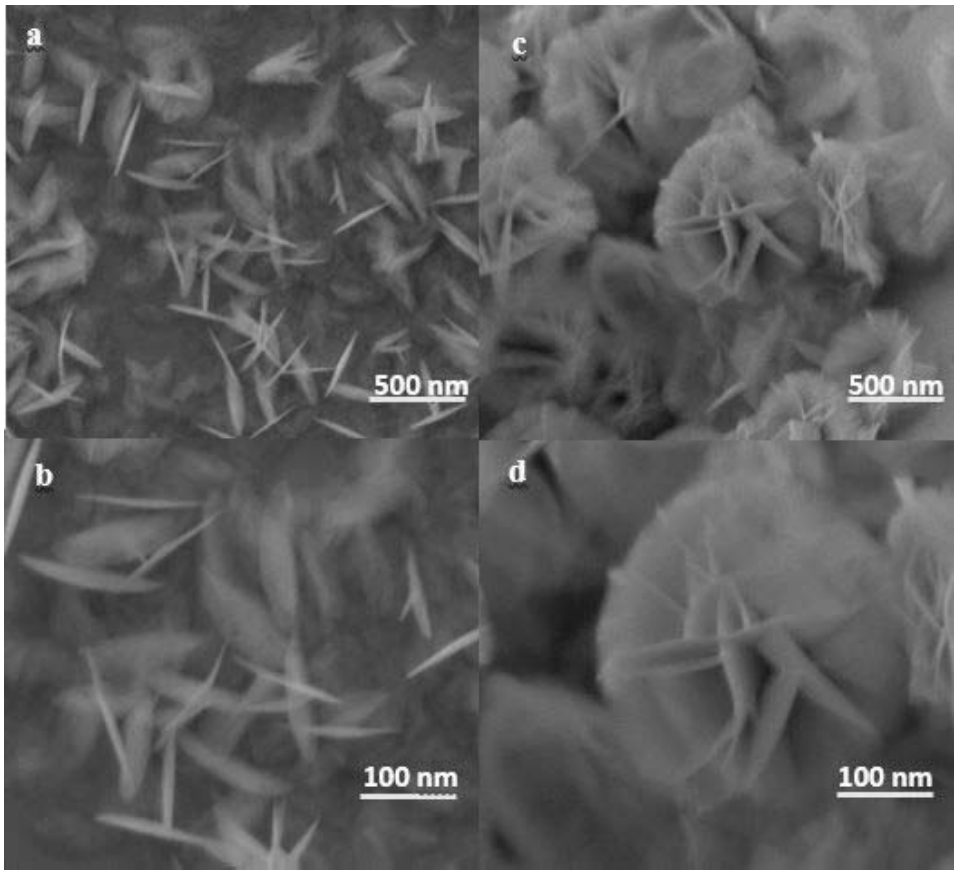


Fig. 2 – SEM images of the electrodeposited Bi_2O_3 nanostructures on Au (111) electrodes (+ 300 mV) for 5 min (a-b) and 15 min (c-d).

RESULTS AND DISCUSSION

Characteristics of Synthesized nano-Bi_2O_3

Fig. 2 (a-b) and (c-d) show the SEM micrographs of the Bi_2O_3 nanostructures, shaped as 3D hierarchical architectures and 2D petal like nanoplates of different shape and electrodeposited on Au (111) electrodes at a potential of Bi_2O_3 (+ 300 mV) for 5 and 15 min. This indicates that the deposition parameters, such as deposition potential and the concentration of Bi^{3+}, remained constant in our experiment.

In Fig. (2a-b), it can be observed that most of the sample's surface is covered by well-defined nanostructures. The magnified SEM image (Fig. 2c) indicates that the nanostructures possess 3D hierarchical architectures of flower like appearance and the diameter of 3D is 100 nm. Fig. 2d shows a single 3D flower, made up of dozens of 2D petal-like nanoplates. These results also revealed that the size of the Bi_2O_3 nanostructures could be tunned simply by controlling the deposition time.

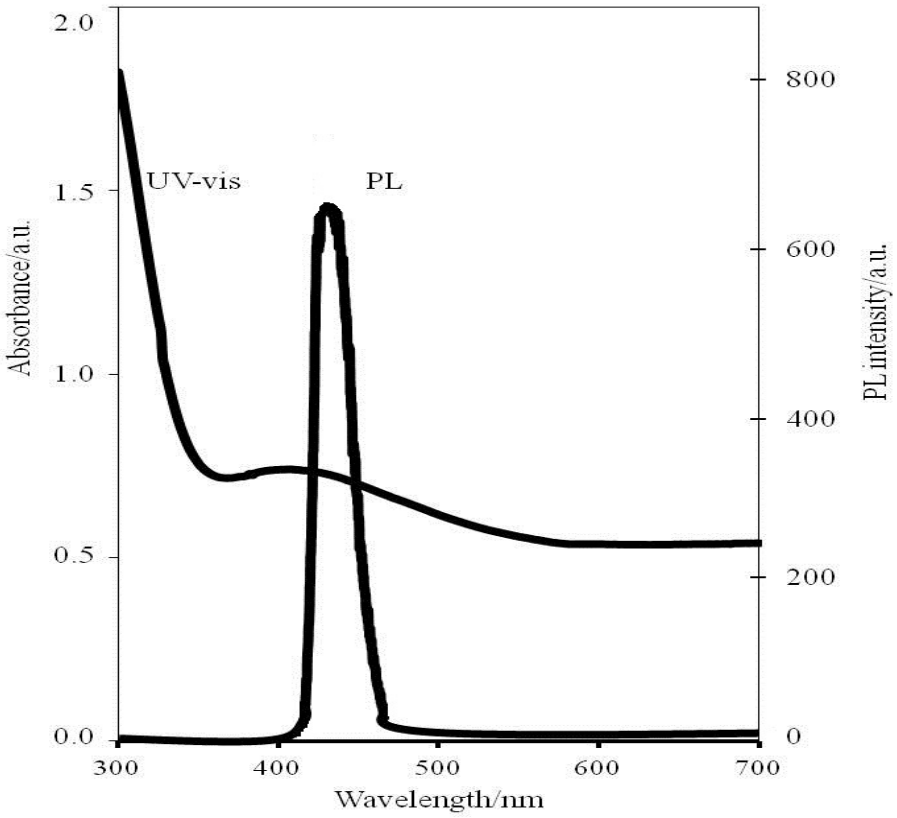


Fig. 3 – PL spectrum and UV-visible absorption spectrum of the electrodeposited Bi_2O_3 nanostructures on ITO/quartz substrates.

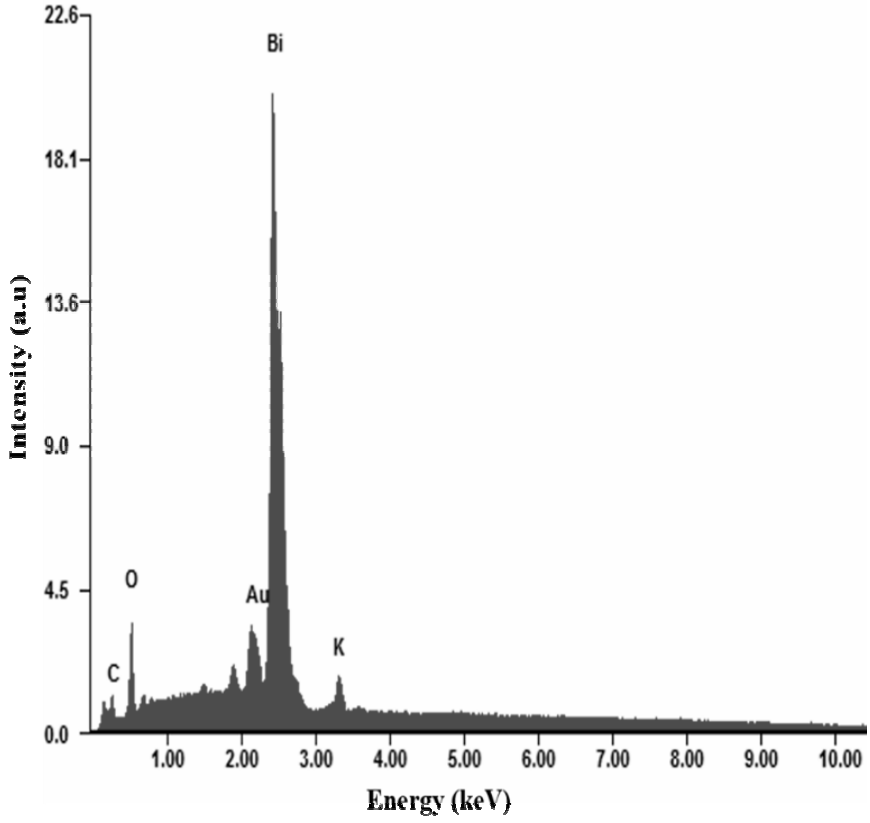


Fig. 4 – EDS spectrum of the electrodeposited Bi_2O_3 nanostructures on a Au electrode at + 300 mV for 15 min.

Fig. 3 is the PL emission spectrum of the Bi_2O_3 monitored at room temperature. The Bi_2O_3 exhibits a strong and sharp PL signal in the range from 300 to 700 nm within a maximum peak to 450 nm (2.75 eV). The strong PL emission band observed from the Bi_2O_3 hierarchical architectures was associated with the oxygen vacancy defects [42]. UV-visible absorption spectra of the Bi_2O_3 as obtained from the nanostructure are shown in Fig. 3. The wide absorption nature of the spectra confirms direct band to band transition caused by the absorbed photon within semiconductor. The extrapolated edge of the absorption spectra intercepts with the wavelength (λ) axis at ~ 420 nm (2.95 eV). EDS (Fig. 4) detection accompained with SEM analysis is also accomplished and the result is shown in Fig. 2 From the corresponding EDS analysis the body of the film is composed of Bi and O, and the ratio of Bi to O approximates to 2:3, which conform to the chemical formula of Bi_2O_3, except for the gold from the working electrode and trace amounts of C and K due to leakage from the reference electrode.

Electrochemical Behavior of Methimazole

The electrochemical behavior of methimazole was investigated at the electrode in 1 mM $K_3[Fe(CN)_6]$ containing 0.1 M KCl as the supporting electrolyte by using cyclic voltammetry (CV). Fig. 5 shows a typical cyclic voltammogram of 30 μg/mL methimazole recorded under these conditions for the scan rate of 0.1 V/s. In the anodic sweep, an oxidation peak is seen at potential of about 0.72 V. Upon reversing the potential scan, no reduction peak corresponding to this oxidation wave is observed, indicating the irreversible nature of the electrode reactions.

In order to gain a deeper insight into the voltammetric waves, the effect of scan rate on the anodic peak currents (Im) and peak potentials (Ep) was studied in the range of 0.01-1 V/s of the potential scan rates in acetonitrile solution containing 30 μg/mL concentration of methimazole (Fig. 6).

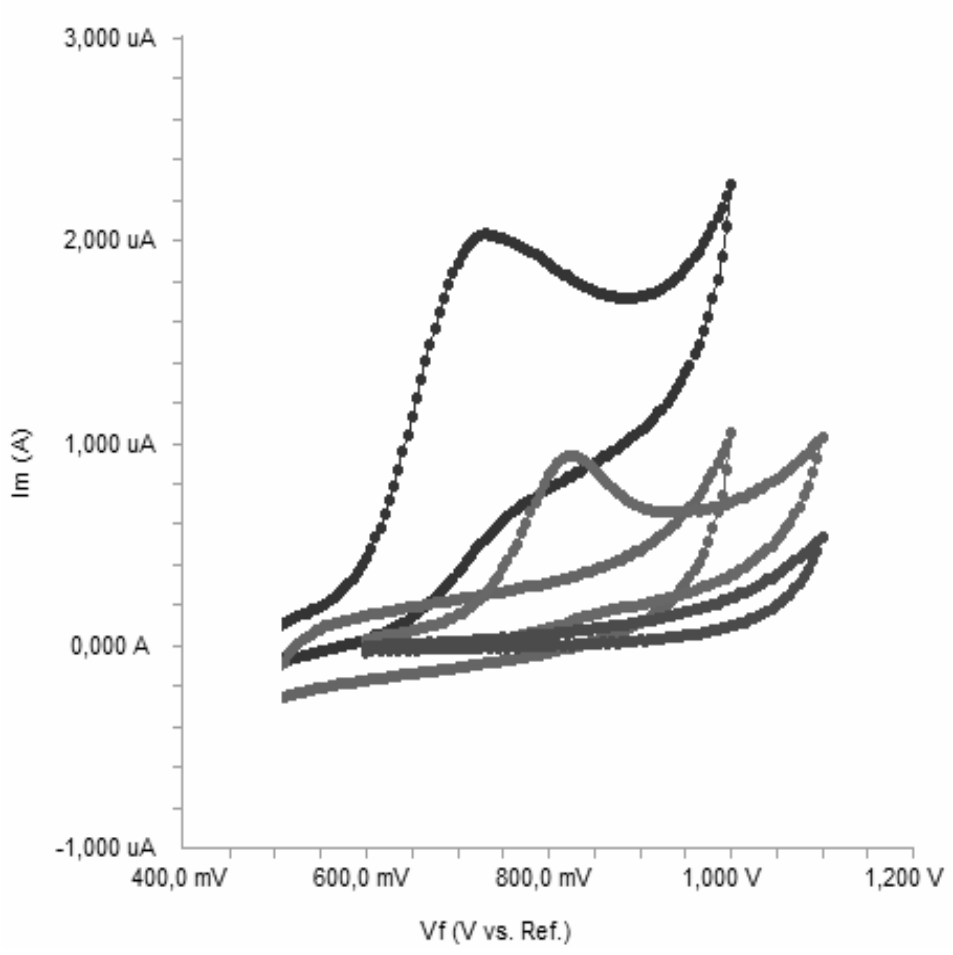


Fig. 5 – Cyclic voltammogram (CV) for the oxidation of 30 μg/mL methimazole in 1 mM $K_3[Fe(CN)_6]$ containing 0.1 KCl at Au (111) electrode, scan rate: 0.1 V/s.

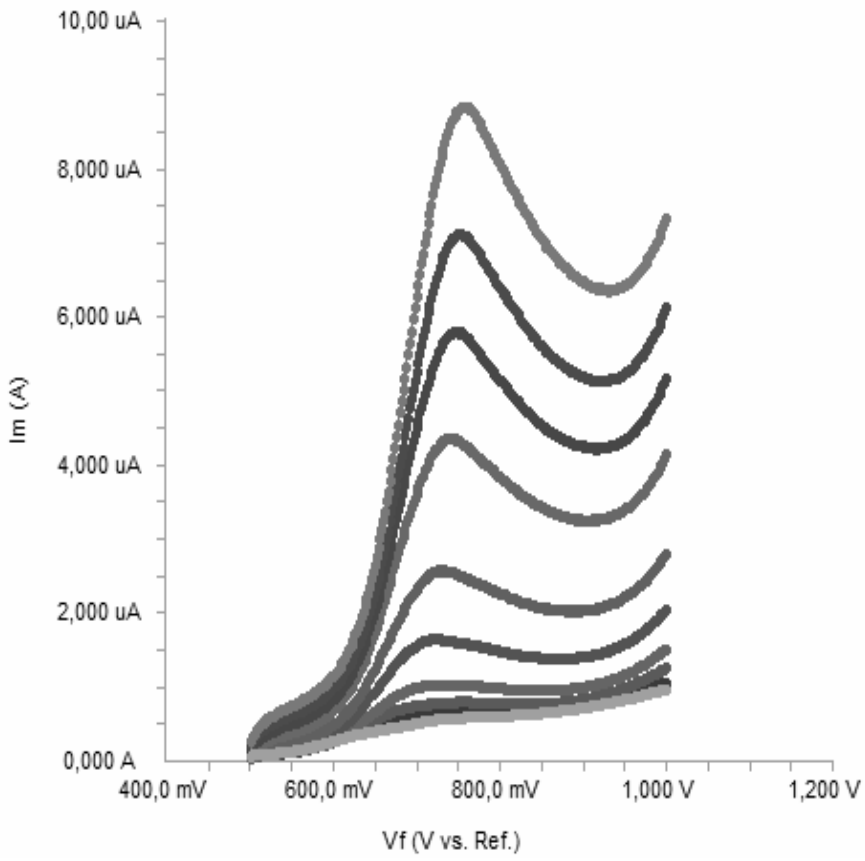


Fig. 6 – Linear sweep voltammograms (LSV) for the oxidation of 30 μg/mL methimazole in 1 mM $K_3[Fe(CN)_6]$ on Bi_2O_3/Au electrode as a function of scan rate; from inner to outer, 5, 10, 25, 50, 100, 200, 400, 600, 800, 1000 mV/s.

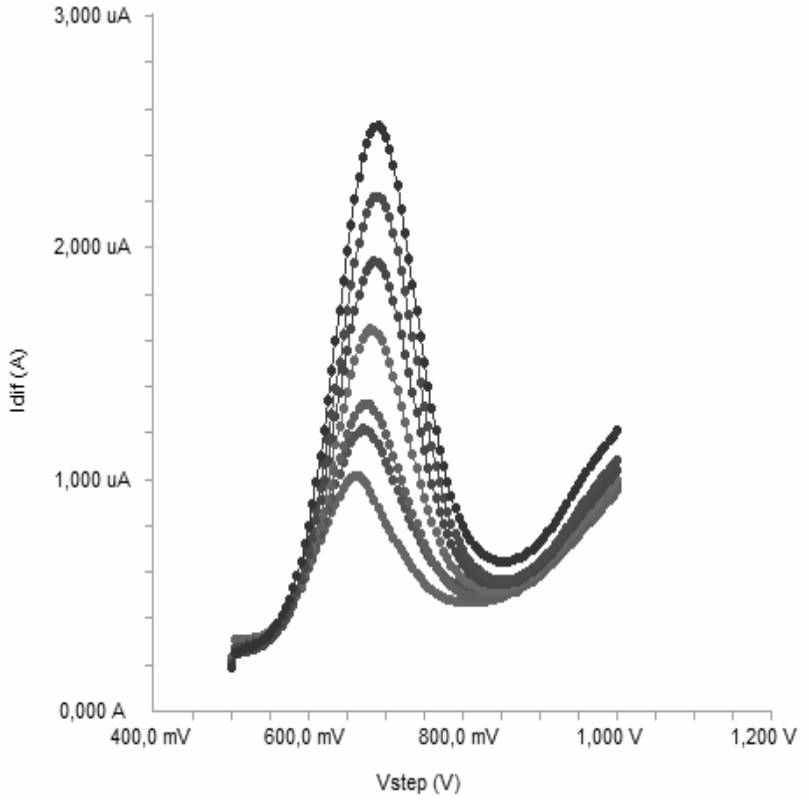


Fig. 7 – Square wave voltammograms (SWV) for different concentrations of methimazole at Bi_2O_3/Au electrode in 1 mM $K_3[Fe(CN)_6]$. Methimazole concentration; 5, 7.5, 10, 15, 20, 25 and 30 μg/mL (down to up).

The representative linear sweep voltammograms obtained at Au-Bi_2O_3 electrode for 30 μg/mL methimazole as a function of the scan rate are presented in Fig. 7. Scan rate dependency experiments show that the peak currents for peak vary linearly with the scan rate (ν) which points out the adsorption-controlled process. However, the plots of logarithm of peak currents *versus* logarithm of scan rates for 30 μg/mL concentration of methimazole display straight lines with 0.5818 slope which are close to theoretical value of 0.5 expected for an ideal diffusion-controlled electrode process.[43] Im-log ν curve is more eligible for this aim, therefore, a diffusional process for peak should be considered. These results suggest that the redox species are diffusing freely from solution and not precipitating onto the electrode surface. The reason for this behavior may be due to the solubility of the intermediate species in potassium ferrocyanide or poor adherence of products on the electrode surface.

Validation of the Method

The validation was carried out by establishing specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), ruggedness, recovery according to ICH Q2B recommendations.[45]

Specificity

Excipients (corn starch, magnesium stearate, lactose, sodium lauryl sulfate, polyethyleneglycol, titanium dioxide, carboxymethylcellulose, hydroxypropylmethylcellulose and talc) were added to the drug for recovery studies, according to the manufacturer's batch formulas for 5 mg methimazole per tablet. The mean percentage recovery of 30 μg/mL methimazole showed no significant excipient interference; thus the procedures were able to assay methimazole in the presence of excipients, and hence it can be considered specific.

Linearity

Standard solutions were prepared as 5-30 μg/mL (5, 7.5, 10, 15, 20, 25 and 30 μg/mL) for SWV and DPV (Figs. 8,9), respectively. Calibration curves were constructed for methimazole standard by plotting the concentration of compound *versus* peak current responses. The calibration curves were evaluated by their correlation coefficients.

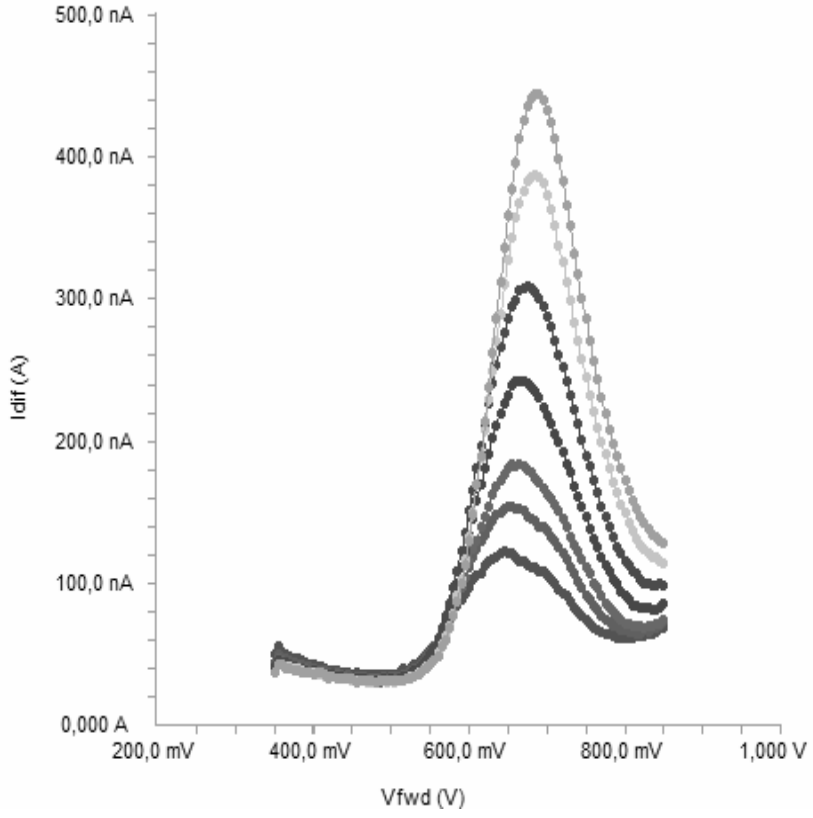


Fig. 8 – Differential pulse voltammograms (DPV) for different concentrations of at Bi_2O_3/Au electrode in 1mM $K_3[Fe(CN)_6]$. Methimazole concentration; 5, 7.5, 10, 15, 20, 25 and 30 μg/mL (down to up).

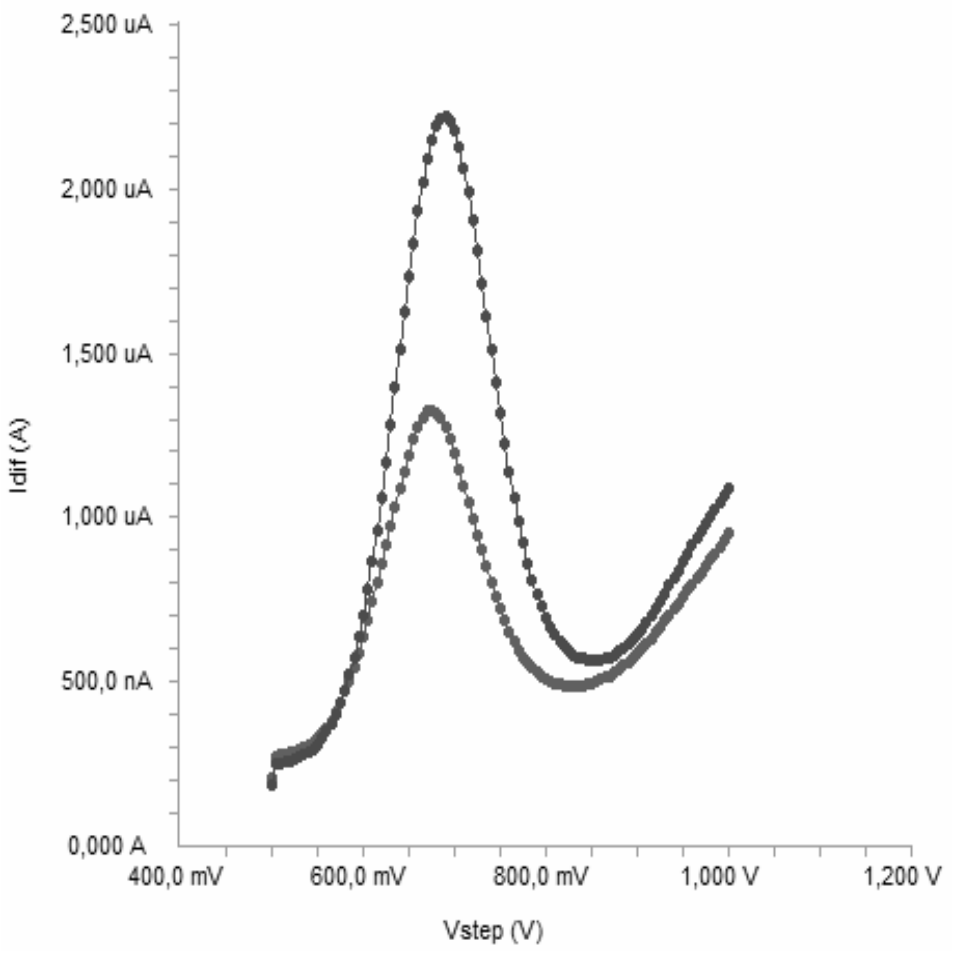


Fig. 9 – Thyromazol tablet SWV 2 for different two concentrations 10 and 25 µg/mL.

Table 1

Regression data of the calibration lines for quantitative determination of methimazole

Parameters	DPV	
Measured potential (V)	+0.69	+0.68
Linearity (µg/mL)	5-30	5-30
Slope	0.059	0.013
Intercept	0.739	0.054
R	0.998	0.998
S_a	0.232	0.373
S_b	0.025	0.014
LOD (µg/mL)	0.06	0.12
LOQ (µg/mL)	1.18	0.36
Precision (RSD%)	2.10	2.08
Accuracy (% relative error)	-2.18	2.33
Repeatability of peak current (RSD%)[a]	1.52	1.73
Repeatability of peak potential (RSD%)	1.07	1.43
Reproducibility of peak current (RSD%)	2.36	2.86
Reproducibility of peak potential (RSD%)	1.79	2.62

RSD: Relative standard deviation, [a]Average of six replicate determinations, S_a: Standard deviation of intercept of regression line, S_b: Standard deviation of slope of regression line, R: Coefficient of correlation, LOD: Limit of detection, LOQ: Limit of quantification.

The correlation coefficients (r) of all the calibration curves were consistently greater than 0.99. The linear regression equations were calculated by the least squares method using Microsoft Excel® program and summarized in Table 1.

Accuracy and Precision

Accuracy of the assay methods was determined for both intra-day and inter-day variations using the six times analysis of the QC samples. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time that was evaluated by assaying the QC samples during the same day. Intermediate precision was assessed by comparing the assays on different days (3 days). The intra-day accuracy ranged from -2.18 % to 2.33% and precision from 1.52% to 1.73%. The results obtained from intermediate precision (inter-day) also indicated a good method precision.

Limits of detection (LOD) and Quantitation (LOQ)

For the LOD and LOQ of methimazole by the proposed methods were determined using calibration standards. LOD and LOQ values were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y-intercept of regression equation (n=3)[43].The LOD and LOQ values of the methods were summarized in Table 1.

Ruggedness

In this study, the SWV and DPV determinations of methimazole were carried out by a different analyst in the same instrument with the same standard. The results showed no statistical differences between different operators suggesting that the developed method was rugged.

Stability

To evaluate the stability of methimazole, standard solutions were prepared separately at concentrations covering the low, medium and higher ranges of calibration curve for different temperatures and times. These solutions were stored at room temperature, refrigeratory (4 ^{o}C) and frozen (-20 ^{o}C) temperature for 24 h and 72h. Stability measurements were carried out with SWV and DPV method. The results were evaluated comparing these measurements with those of standards and expressed as percentage deviation and methimazole was found as stable at room temperature, 4 and -20 ^{o}C for at least 72h.

Recovery

To determine the accuracy of the SWV and DPV methods and to study the interference of formulation additives, the recovery was checked as three different concentration levels. Analytical recovery experiments were performed by adding a known amount of pure drugs to pre-analyzed samples of commercial tablet form (Figs. 9,10). The recovery values were calculated by comparing the concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table 2.

Comparison of the Methods

Voltammetry has been recently proposed as a promising new analytical method for electrochemical detection of drugs. Owing to the high sensitivity, low cost, simplicity of instrumentation and short analysis time voltammetric techniques are important methods for pharmaceutical analysis.[46,47]

SWV and DPV voltammetry methods were applied for the determination of the commercial tablets (Table 2). The results show high reliability and reproducibility of the two methods. The best results were statistically compared using the t-test. At 95% confidence level, the calculated t-values do not exceed the theoretical values (Table 4). Therefore, there is no significant difference between SWV and DPV voltammetry methods.

Voltammetric determination of methimazole at glassy carbon electrodes in thyromazol tablets was referred to the regression equation. The relative standard deviations (RSD) were 1.02% using the proposed methods for the voltammetric analysis of thyromazol tablets. The validity of the proposed procedures applied to thyromazol tablets was also assured by the recovery of standard additions. A mean recovery of 99.9% with RSD of 1.34 was obtained. The results of the drug analysis obtained from the proposed methods are in close agreement with the claimed value. At the same time, the results obtained are also comparable with the results obtained from liquid chromatography and capillary zone electrophoresis.[12,13] Also, the results

obtained using the proposed method in this study are well compared with several electrochemical methods for the determination of methimazole as shown in Table 4. However, the results obtained from the proposed method indicate that this method is more precise and accurate for the determination of methimazole in drug samples.

SWV and DPV are effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background current and low detection limits.[4] Two calibration graphs from the bulk solution of methimazole according to the procedures described above were constructed by using SWV and DPV.

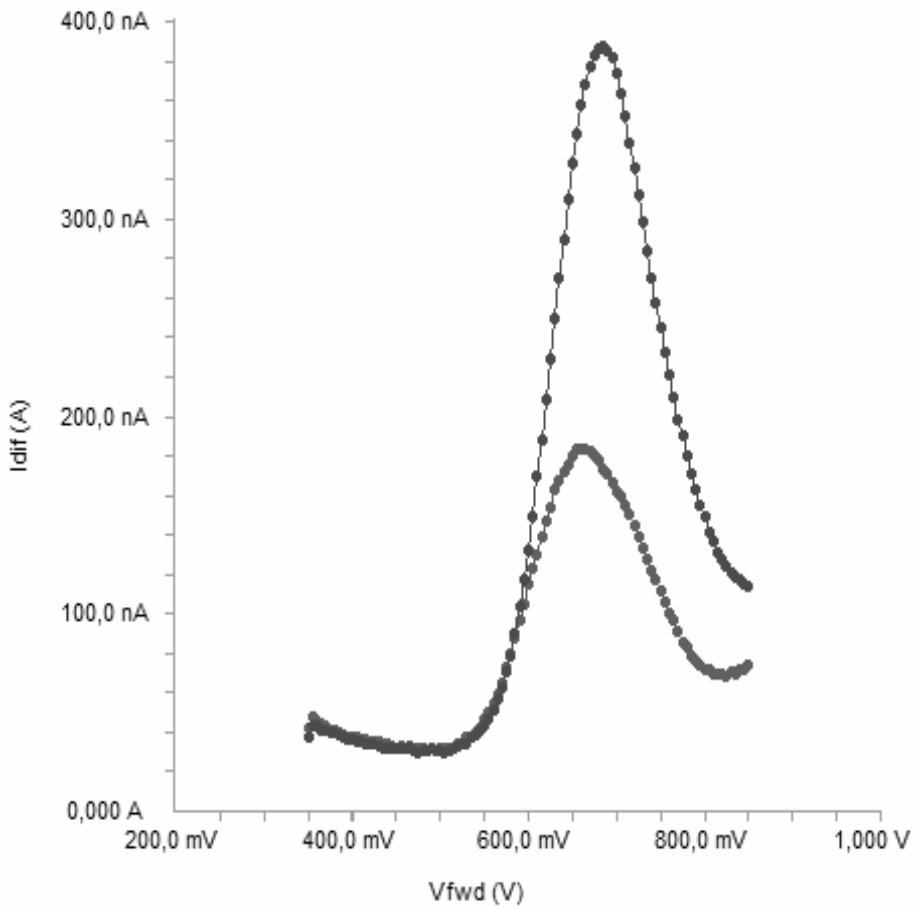


Fig. 10 – Thyromazol tablet DPV 2 for different two concentrations (10 and 25 µg/mL).

Table 2

Recovery of methimazole in pharmaceutical preparation by proposed methods

Pharmaceutical preparation	Added (µg/mL)	SWV			DPV		
		Found ± SD	Recovery (%)	RSD[a] (%)	Found ± SD	Recovery (%)	RSD[a] (%)
Thyromazol (10 µg/mL)	5	4.9 ± 0.11	98.0	2.24	5.1 ±0.22	102.0	4.31
	15	14.9 ± 0.23	99.3	1.54	14.7 ± 0.34	98.0	2.31
	25	24.89±0.418	99.6	1.68	25.16±0.597	100.6	2.37

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, [a]Average of six replicate determinations
SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation
SE: Standard error, [a]Theoretical values, Theoretical values at p=0.05, Ho hypothesis: no statistically significant difference exists between four methods, $F_t > F_c$: Ho hypothesis is accepted (P > 0.05).

Table 3

Comparison of the proposed and reported methods for determination of methimazole

Electrode construction	Linear range (μM)	LOD[a] nM	Reproducibility (%RSD)	Reference
Acetylene black/chitosan film modified glassy carbon electrode	0.1–20	20 nM	3.10	15
Multi-walled carbon nanotube modified glassy carbon electrode	0.1–500	30 nM	2.64	16
Carbon paste electrode modified with a Schiff base complex of cobalt	1.0–100	500 nM	<4.5	17
Gold electrode	5-30 μg/mL (0.044-0.263 μM)	0.06 μg/mL (52.5 nM)	2.76	Proposed work

Table 4

Comparison of analytical parameters of proposed work with previously reported in the literature

Parameters	**SWV**	**DPV**	**Reported method [16]**	**Reported method[17]**
Mean (recovery %)	99.8	100.1	98.6	96.9
SD	0.634	1.344	-	-
% RSD	0.635	1.343	2.64	4.50
Variance	0.402	1.806	-	-
t-test (2.228)[a]	0.921		-	-
F- test (5.1)[a]	4.05		-	-

LOD[a]: Limit of detection

CONCLUSIONS

In this study, Bi_2O_3/Au nanostructure sensor was fabricated and a validated square wave and differential pulse voltammetric procedure was developed and successfully applied to the estimation of methimazole in tablet samples. SWV and DPV are effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background current and low detection limits. And the methods are requiring less than 3 min to run samples. Therefore, the methods can be used effectively without separation for routine analysis of methimazole in pure form and its formulations. Also to proposed sensor has easy fabrication and low cost.

Acknowledgements: Atatürk University is gratefully acknowledged for the financial support of this work.

REFERENCES

1. M. Aletrari, P. Kanari, D. Partassides and E. Loizou, *J. Pharm. Biomed. Anal.,* **1998**, *16*, 785-792.
2. J. E. F. Reynolds, in "The Extra Pharmacopoeia", 29th edition,The Pharmaceutical Press, London, 1985, p. 682-688.
3. H. Y. Aboul-Enein and A. A. Al-Badr, "Analytical Profiles of Drug Substance", K. Forey (Ed.), Chap. 18, Academic Press, NewYork, 1979, p. 351-371.
4. A. P. Weetman, A. M. McGregor and R. Hall, *Clin. Endocrinol.*, **1984**, *21*, 163-172.
5. K. Nikolic, K. Velasevic, *Pharmazie,* **1987**, *42*, 698-700.
6. A. Berka, K. Velasevic and K. Nikolic, *Pharmazie*, **1989**, *44*, 499-500.
7. G. Moretti, P. Betto, P. Cammarata, F. Fracassi, M. Giambenedetti and A. Borghese, *J. Chromatogr. B*, **1993**, *616*, 291-295.
8. M. G. Elbardicy, Y. S. Elsaharty and M. S. Tawakkol, *Spectrosc. Lett.,* **1991**, *24*, 1079-1096.
9. C. Sanchezpedreno, M. I. Albero, M. S. Garcia and V. Rodenas, *Anal. Chim. Acta,* **1995**, *308*, 457-461.
10. M. S. Garcia, M. I. Albero and C. Sanchezpedreno, *L. Tobal, Analyst*, **1995**, *120*, 129-133.
11. S. Pinzanti, G. Papeschi and E. LaPorta, *J. Pharm. Biomed. Anal.,* **1983**, *1*, 47-53.
12. S. Zhang, W. Sun, W. Zhang, W. Qi, L. Jin, K. Yamamoto and S. Tao, *J. Jin, Anal. Chim. Acta,* **1999**, *386*, 21-30.
13. A. Wang, L. Zhang, S. Zhang and Y. Fang, *J. Pharm. Biomed. Anal.,* **2000**, *23*, 429-436.
14. M. Aslanoglu and N. Peker, *J. Pharm. Biomed. Anal.,* **2003**, *33*, 1140-1143.
15. W. Yazhen, *Bioelectrochem.* **2011**, *81*, 86-90.
16. Z. H. Zhang, Y. F. Hu, H. B. Zhang, L. J. Luo and S. Y. Yao, *J. Electroanal. Chem.*, **2010**, *644*, 7-16.
17. R. Jain and J. A. Rather, *Colloids and Surfaces B: Biointerfaces*, **2011**, *83*, 340-348.
18. H. Lin, X. Ji, Q. Chen, Y. Zhou, C. E. Banks and K. Wu, *Electrochem. Commun.,* **2009**, *11*, 1990-1998.

19. W. Sun, D. Wang, J. Zhong and K. Jiao, *J. Solid State Electrochem*., **2008**, *12*, 655-661.
20. P. Qiu, X. Cao and L. Jin, *Electrochim. Acta,* **2008**, *53*, 5368-5772
21. A. Salimi, R. Hallaj, B. Kavosi and B. Hagighi, *Anal. Chim. Acta*, **2010**, *661*, 28-34.
22. R. Jain Dhanjai, *Colloids Surf. B: Biointerfaces*, **2013**, *105*, 278-283.
23. R. Jain, R. Sharma, R. Shrivastava and S. P. Satsangee, *J. Electrochem. Soc.,* **2013**, *160*, H1-H6.
24. V. Urbanova, M. Bartos, K. Vytras and A. Kuhn, *Electroanalysis,* **2010,** *22*, 1524-1530.
25. I. Svancara, C. Prior, S. B. Hocevar and J. Wang, *Electroanalysis,* **2010**, *22*, 1405-1420.
26. M. Khairy, R. O. Kadara, D. K. Kampouris and C. E. Banks, *Analytical Methods*, **2010**, *2*, 645-649.
27. A. Bobrowski, A. Krolicka, K. Pacan and J. Zar, *Electroanalysis*, **2009**, *21*, 2415-2419.
28. M. Zidan, T. W. Tee, A. H. Abdullah, Z. Zainal, G. J. Kheng, *Int. J. Electrochem. Sci.,* **2011**, *6*, 279-288.
29. R. Jain and R. Sharma, *J. Appl. Electrochem*., **2012**, *42*, 341-348.
30. S. N. Ding, D. Shan, H. G. Xue and S. Cosnier, *Bioelectrochem.*, **2010**, *79*, 218-222.
31. G. H. Hwang, W. K. Han, J. S. Park and S. G. Kang, *Sens. Actuators B*, **2008,** *135*, 309-316.
32. B. Nigovic, B. Simunic and S. Hocevar, *Electrochim. Acta*, **2009,** *54*, 5678-5683.
33. L. C. S. Figueiredo-Filho, V. B. D. Santos, B. C. Janegitz, T. B. Guerreiro, O. Fatibello-Filho, R. C. Faria and L. H. Marcolino-Junior, *Electroanalysis*, **2010**, *22*, 1260-1266.
34. R. Jain, J. A. Rather and A. Dwivedi Vikas, *Electroanalysis,* **2010**, *22*, 1-7.
35. G. N. Dar, A. Umar, S. A. Zaidi, A. A. Ibrahim, M. Abaker, S. Baskoutas and M. S. Al-Assiri, *Sens. Actuators B: Chem.,* **2012**, *173*, 72-78.
36. Y. Xie, L. Zhou and H. Huang, *Biosens. Bioelectron.*, **2007**, *22*, 2812-2818.
37. Y. Tao, Z. Lin, X. Chen, X. Huang, M. Oyama, X. Chen and X. Wang, *Sens. Actuators B: Chem*., **2008**, *129*, 758-763.
38. S. S. Kalanur, S. Jaldappagari and S. Balakrishnan, *Electrochim. Acta*, **2011**, *56*, 5295-5301.
39. R. Jain, D. C. Tiwari and S. Shrivastava, *Mater. Sci. Eng. B*, **2014**, *185*, 53-59.
40. D. Shan, J. Zhang, H. G. Xue, Y. C. Zhang, S. Cosnier and S. N. Ding, *Bios. Bioelectr.,* **2009**, *24*, 3671-3676.
41. M. E. Swartz and I. S. Krull, "Analytical Method Development and Validation", Marcel Dekker, New York, 1997.
42. R. Jain, Dhanjai and S. Sharma, *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* **2013**, *436*, 178-184.
43. P. T. Kissenger, in "Laboratory Techniques in Electroanalytical Chemistry", W. R. Heineman (Ed.), 2rd edition, Marcel Dekker, New York, 1996.
44. J. Wang, (Ed.), "Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine", VCH Publishers, New York, 1996.
45. GPMP/ICH/281/95, "The European Agency for the Evaluation of Medicinal Products", ICH Topic Q2B Note for Guideline on Validation of Analytical Procedures: Methodology, 1996.
46. G. B. El-Hefnawey, I. S. El-Hallag, E. M. Ghoneim and M. M. Ghoneim, *J. Pharm. Biomed. Anal.,* **2004**, *34*, 75-84.
47. P. Corti, G. Corbini, P. Gratteri, S. Furlanetto and S. Pinzauti, *Int. J. Pharm*., **1994**, *111*, 83-87.