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Electrochemical study of methimazole and its direct determination in pharmaceutical preparations and human serum by square wave and differential pulse voltammetry

Bilal Yilmaz[1] , Fatma Bayrakceken Nisanci[2*]

[1]Department of Analytical Chemistry, Faculty of Pharmacy, Ataturk University, 25240, Erzurum, Turkey; [2]Department of Chemistry, Faculty of Science, Ataturk University, 25240, Erzurum, Turkey

ABSTRACT

Two simple and rapid methods were developed for the determination of methimazole in pharmaceutical preparations and human serum by square wave and differential pulse voltammetry. The anodic peak at +0.60 V obtained in a buffer on glassy carbon electrode was used for analysis. The peak current and peak potential depend on pH, scan rate and initial potential. Decrease of the anodic peak with increasing pH, as well as deviations from linear plots of ip = f(C) and ip = f(C) and ip = kv1/2 indicate that this peak at higher concentrations is affected by adsorption-desorption phenomena. The linearity was established over the concentration range of 10-80 ug/mL for both methods in supporting electrolyte and human serum. The repeatability and reproducibility of the methods for all media (such as supporting electrolyte and serum samples) were determined. Precision and accuracy were also checked in all media. Developed methods in this study can be applied to Thyromazol tablet. Also, the proposed techniques were successfully applied to spiked human serum samples. No electroactive interferences from the endogenous substances were found in the serum samples.

Keywords: Methimazole, serum, voltammetry, tablet

***Correspondence to Author:**
Fatma Bayrakceken Nisanci
Department of Chemistry, Faculty of Science, Ataturk University, 25240, Erzurum, Turkey.

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Introduction

Methimazole (2-mercapto-1-methylimidazole) (Figure 1) used in treatment of hyperthyroid by the production of thyroxin, 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]

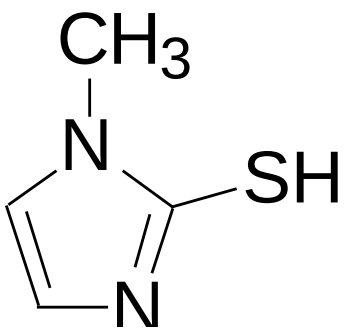

Figure 1. Chemical structure of methimazole

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]

There are some problems encountered in using such methods. Spectrophotometric methods suffer from low sensitivity. Chromatographic methods are relatively slow and expensive and they require derivatization or time consuming extraction procedures. Thus, the use of simpler, faster and less expensive, but still sensitive electrochemical techniques can be considered as a useful alternative.

Only a limited number of articles have been published in the literature on the use of chemically modified glassy carbon electrodes for the determination of methimazole including acetylene black/chitosan film modified glassy carbon electrode,[15] multi-walled carbon nanotube modified glassy carbon electrode (GCE)[16] and a carbon paste electrode modified with a schiff base complex of cobalt.[17]

The development of a new method capable of determining drug amount in pharmaceutical preparations or biological fluids is important. Electroanalytical techniques have been used for the determination of a wide range of drug compounds with the advantages that there are, in most, instances no need for derivatization and that these techniques are less sensitive to matrix effects than other analytical techniques. Additionally, application of electrochemistry includes the determination of electrode mechanism. Redox properties of drugs can give insights into their metabolic fate or their in vivo redox processes or pharmacological activity. Despite the analytical importance of the electrochemical behaviour and oxidation mechanism of methimazole, no report has been published on the voltammetric study of the electrochemical oxidation of methimazole in human serum.

The goal of this work was the development of new voltammetric methods for the direct determination of methimazole in pharmaceutical preparations and spiked human serum samples without any time-consuming extraction or evaporation steps prior to drug assay. This paper describes fully validated, simple, rapid, selective and sensitive procedures for the determination of methimazole employing square wave voltammetry (SWV) and differential pulse voltammetry (DPV) at the glassy carbon electrode. Also, this work was also aimed to study the voltammetric behavior and oxidation mechanism of methimazole using cyclic, SWV and DPV techniques.

Experimental

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 methanol. 0.5 M H_2SO_4, 0.2 M phosphate buffer between pH 2 and 12, 0.04 M Britton-Robinson buffer between pH 2 and 12 and 0.2 M acetate buffer between pH 3.5 and 5.7 were used as the supporting electrolytes. 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.[18] 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 was used a glassy carbon electrode (Φ= 3 mm, BAS) 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. Before each experiment, the glassy carbon surface was polished with polishing alumina (prepared from 0.01 µm aluminium oxide) on alumina polish pad then rinsed with purified water.

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 potential were variable, depending on the pH value and the cut-off the electrolyte. Scan rate measurements in the range 10-1000 mV/s.

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 0.04 M Britton-Robinson buffer (pH 2) was added and then the flask was sonicated to 10 min at room tempature. The flask was filled to volume with 0.04 M Britton-Robinson buffer (pH 2). 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.

Analysis of spiked serum samples

Drug free human blood, obtained from healthy volunteers (after obtaining their written consent), was centrifuged (5000 rpm) in 10 min at room temperature and separated serum samples were stored frozen until assay. An aliquot volume of serum sample was fortified with methimazole dissolved in 0.04 M Britton-Robinson buffer (pH 2) to achieve final concentration of 10-80 µg/mL. Acetonitrile

removes serum proteins more effectively, as the addition of 0.5 volume of serum is sufficient to remove the proteins. After vortexing for 30 s, the mixture was then centrifuged for 10 min at 5000 rpm for getting rid of serum protein residues and supernatant was taken carefully. Appropriate volumes of this supernatant were transferred into the volumetric flask and diluted up to the volume with Britton-Robinson buffer at pH 2.0. The analyses were carried out using a standard addition method. The concentration of methimazole was varied in the range of 10-80 µg/mL in human serum sample.

Results and discussion

Electrochemical behavior of methimazole

Methimazole yields in solution between pH 2 and 12 a main anodic peak which is accompanied by a smaller peak at more negative potentials. The main peak was the best developed and suitable for analytical purposes in solution of sulphuric acid and in buffer solutions at pH < 4. Absence of a cathodic peak on reverse sweep at pH 2.0 indicates irreversibility of the electrode process. Decrease of the main anodic peak with repeated cycling indicates filming of the electrode by an adsorbed product. The potential of the main anodic peak in shifted with increasing pH to less positive potentials (Figure 2) with d*E*p = 1030.4-30.45 mV/pH (r^2: 0.99 between pH 2 and 12).

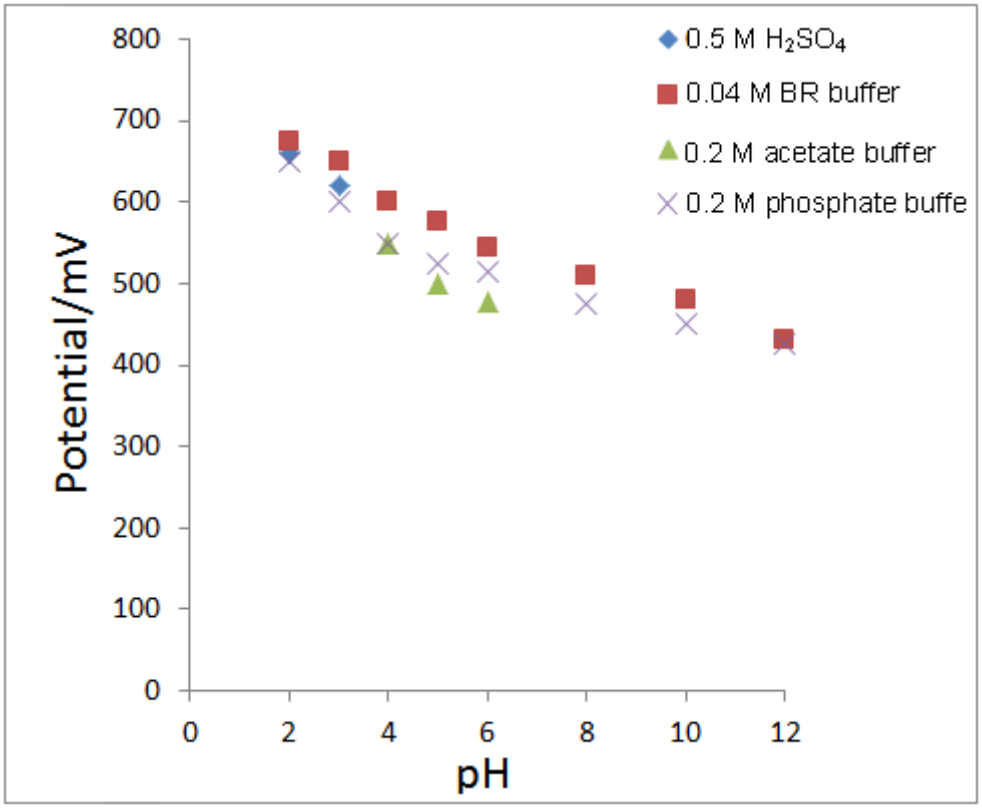

Figure 2. Effects of pH on methimazole anodic peak potential, methimazole concentration 50 µg/mL; 0.5 M H_2SO_4 (◆); 0.04M Britton-Robinson (■); 0.2 M acetate (▲); 0.2 M phosphate (×) buffers.

That indicates participation of a proton transfer in the electrode process. Dependence of peak currents on pH presents a more complex situation: whereas in Britton-Robinson buffers the peak current increases gradually from 2 to 12, in simple acetate and phosphate buffers the current reaches a limiting value at about pH 4 and decreases with further decrease in pH

(Figure 3). The solution pH influenced the peak current considerably. The peak current decreased linearly with the increase in pH of solution. So, the buffer solution with pH 2.0 was selected for further experiments.

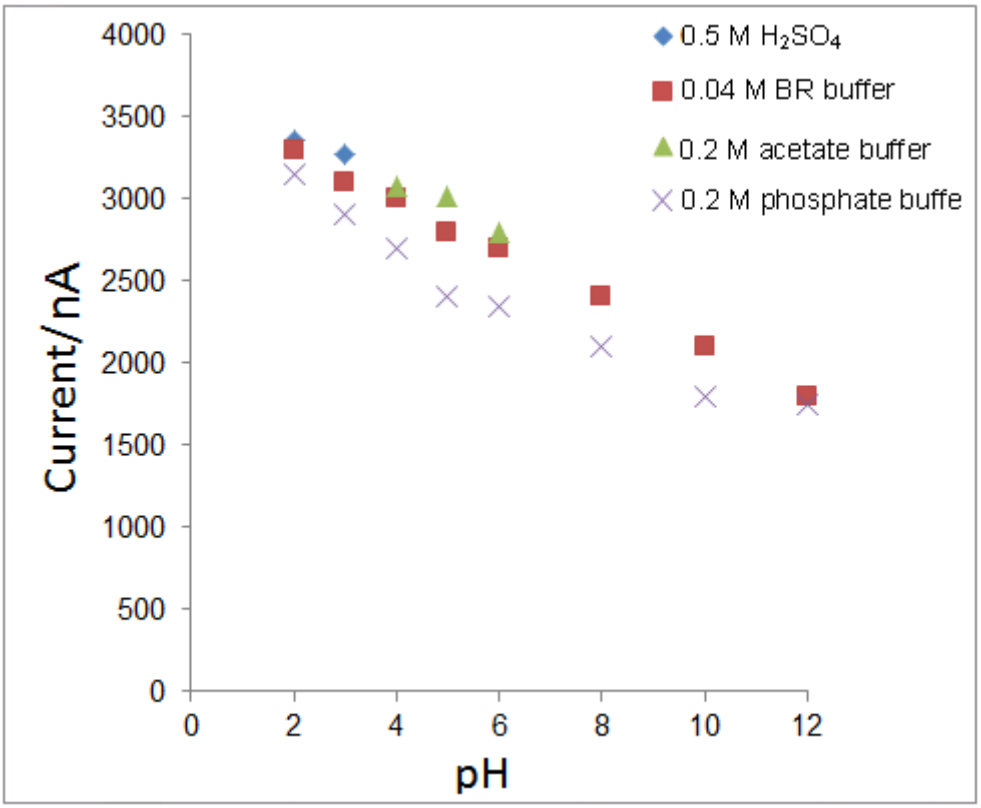

Figure 3. Effects of pH on methimazole anodic peak current, methimazole concentration 50 µg/mL; 0.5 M H_2SO_4 (◆); 0.04 M Britton-Robinson (■); 0.2 M acetate (▲); 0.2 M phosphate (×) buffers.

As a general rule, a base is more easily oxidized than its conjugate acid. Thus anodic waves controlled by the rate of formation of the basic form from the conjugate acid, reach the diffusion controlled limiting value at sufficiently high pH value, when all electroactive species in present in the basic form. In such cases the anodic current decreases with decreasing pH. As in the present case the peak current oppositely increases with decreasing pH, the observed dependence can not be attributed to the oxidation of the basic form of methimazole. The observed dependence of $i_p = f(\text{pH})$ for methimazole can then be attributed to a difference in adsorption of its acidic and basic form. The role of adsorption is further supported by the sharp form of the main anodic peak and by the dependence of the peak current on scan rate (v). For diffusion current the plot of log i_p as a function of log v should have a slope of 0.5 and for a purely adsorption current a slope of 1.0.

The observed value the slope of such plot of 0.67 indicates a strong role of adsorption in a solution containing 50 µg/mL methimazole. In order to understand the mechanisms responsible for the oxidation of methimazole at glassy carbon electrode cyclic voltammograms of methimazole were recorded at various scan rates. The peak potential shifted to more positive values on increasing the scan rate which confirms the irreversibility of the oxidation process. The anodic peak current was proportional to the scan rate over the range of 10-1000 mV/s.

The results indicated that the electrochemical oxidation of methimazole at glassy carbon electrode is a surface-controlled process. In order to get information about the number of electrons involved in the oxidation of methimazole, the value of αn for the electrocatalytic oxidation of methimazole was determined. The catalytic oxidation peak potential of methimazole is proportional

to $\log_v$ with a slope of 0.049. The oxidation of methimazole is a one electron transfer process assuming the electron transfer coefficient α is approximately 0.5 in a totally irreversible electrode process.

In addition, the effect of the pH value of the Britton-Robinson buffer solution on peak potential of methimazole at glassy carbon electrode was also investigated. The anodic peak potential of methimazole shifted in the negative direction with increasing pH (Figure 2). The shift in Ep with pH refers to a proton transfer in the electrochemical oxidation of methimazole. It has also been reported that the identical numbers of electrons and protons are involved in the oxidation process of methimazole.[16] Combining the fact that identical numbers of electrons and protons should be involved in the electrode process, it might be concluded that the electrochemical reaction of methimazole at glassy carbon electrode could be realized the oxidation of disulphydryl group in the molecule.[16,17]

The best results with respect to signal enhancement and peak shape accompanied by sharper response was obtained with Britton-Robinson buffer at pH 2.0. This supporting electrolyte was chosen for the subsequent experiments. The electrochemical behavior of methimazole was investigated at glassy carbon electrode in 0.04 M Britton-Robinson buffer (pH 2) as the supporting electrolyte by using cyclic voltammetry (CV). Figure 4 shows a typical cyclic voltammogram of 50 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 about potential of +0.60 V.

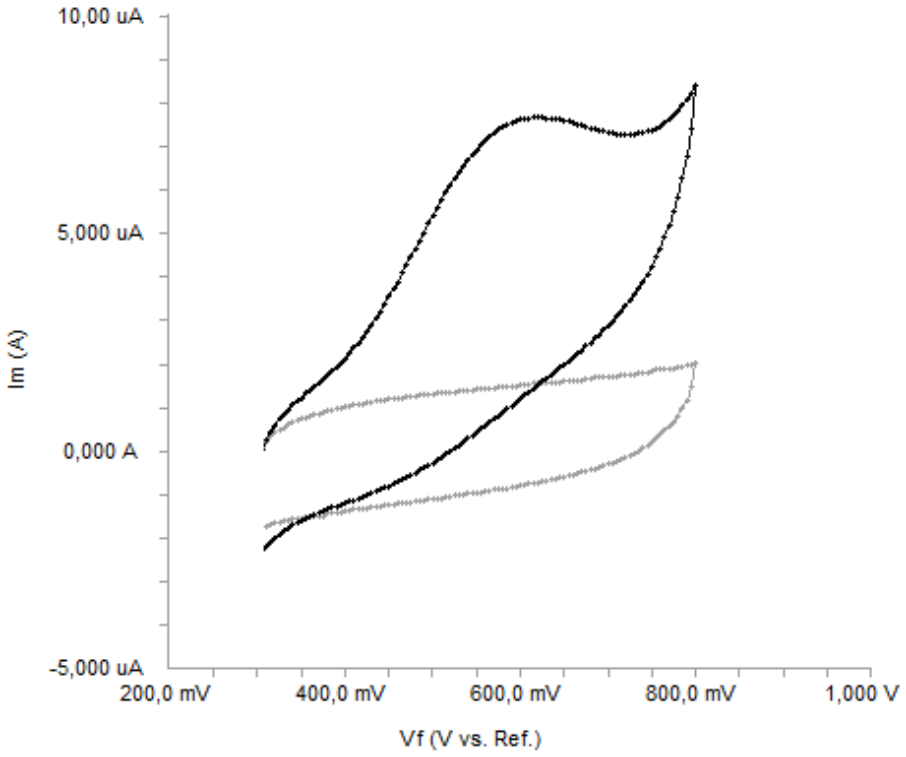

Figure 4. Cyclic voltammogram for the oxidation of 50 µg/mL methimazole in 0.04 M Britton-Robinson buffer (pH 2) at glassy carbon electrode, scan rate: 0.1 V/s.

In order to develop a voltammetric method for determination of the methimazole, we selected the SWV and DPV techniques, since the peaks were sharper and better defined at lower concentration of methimazole than those obtained by cyclic and linear sweep voltammetry with a lower background current, resulting in improved resolution. SWV and DPV are effective and rapid electroanalytical techniques with well-established advantages, including good discrimination against background currents and low detection and determination limits.[18-21).

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.[22]

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 50 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 10-80 µg/mL (10, 20, 30, 40, 50, 60, 70 and 80 µg/mL) for SWV and DPV (Figures 5,6), respectively.

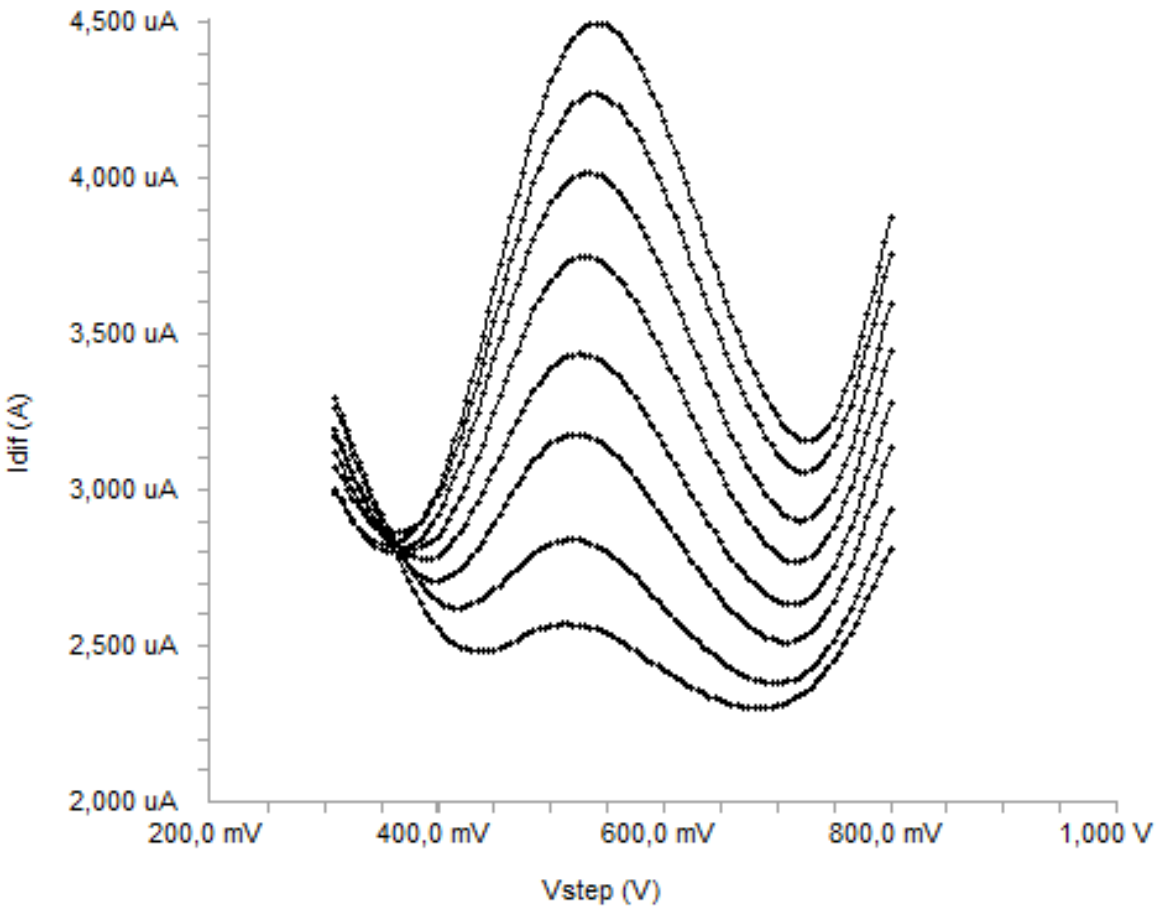

Figure 5. Square wave voltammograms for different concentrations of methimazole in 0.04 M Britton-Robinson buffer (pH 2) (10, 20, 30, 40, 50, 60, 70 and 80 µg/mL)

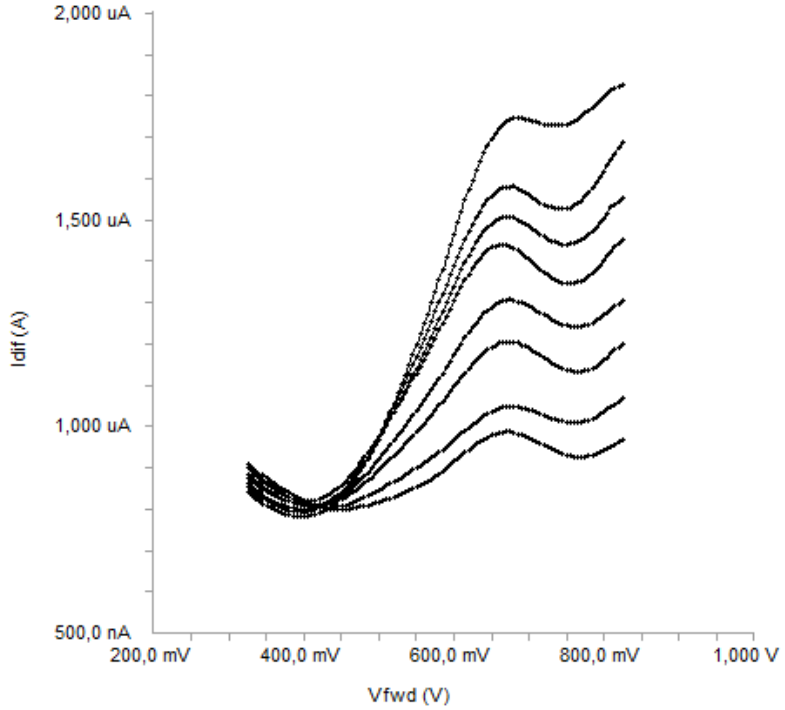

Figure 6. Differential pulse voltammograms for different concentrations of methimazole in 0.04 M Britton-Robinson buffer (pH 2) (10, 20, 30, 40, 50, 60, 70 and 80 μg/mL)

Calibration curves were constructed for methimazole standard by plotting the concentration of compound versus peak current responses. The calibration curves were evaluated by its correlation coefficients. 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.

Table 1. Regression data of the calibration lines for quantitative determination of methimazole

Parameters	SWV		DPV	
	Supporting electrolyte	Serum	Supporting electrolyte	Serum
Measured potential (V)	+0.54	+0.53	+0.67	+0.66
Linearity (μg/mL)	10-80	10-80	10-80	10-80
Slope	0.0279	0.0321	0.0107	0.0341
Intercept	2.3082	3.2543	0.8691	4.2436
R	0.999	0.990	0.999	0.992
S_a	1.182	2.402	3.484	2.447
S_b	0.705	1.644	0.735	1.654
LOD (μg/mL)	0.50	1.20	1.00	1.30
LOQ (μg/mL)	1.50	3.60	3.00	3.90
Precision (RSD%)	2.10	3.19	2.08	3.17
Accuracy (% relative error)	-1.26	2.64	2.46	2.12
Repeatability of peak current (RSD%)[a]	1.38	2.67	1.38	2.78
Repeatability of peak potential (RSD%)	1.94	2.46	1.04	2.46
Reproducibility of peak current (RSD%)	2.26	3.31	2.76	3.41
Reproducibility of peak potential (RSD%)	1.69	2.24	2.54	3.01

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

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 -1.26 % to 2.64% and precision from 2.13% to 3.19%. The results obtained from intermediate precision (inter-day) also indicated a good method precision.

Limits of detection (LOD) and quantification (LOQ)

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 ($n=3$).[22] The LOD and LOQ values of the methods were summarized in Table 1.

Ruggedness

In this study, the SWV and DPV determination of methimazole were carried out by a different analyst in 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 temperature and times. These solutions were stored at room temperature, refrigeratory (4 °C) and frozen (-20 °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 °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 known amount of pure drugs to pre-analyzed samples of commercial tablet form. The recovery values were calculated by comparing concentration obtained from the spiked samples with actual added concentrations. These values are also listed in Table 2.

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 (20 µg/mL)	15	15.20±0.372	101.3	2.45	15.33±0.419	102.2	2.73
	25	24.89±0.418	99.6	1.68	25.16±0.597	100.6	2.37
	50	48.86±0.479	97.7	0.98	49.07±0.682	98.1	1.39

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, [a]Average of six replicate determinations

Analysis of spiked serum samples

The optimized procedure was successfully applied for the determination of methimazole in protein-free spiked human serum samples. Acetonitrile was tried as a serum precipitating agents. No extraction steps other than the centrifugal protein separation were required prior to the assay of drug. Figure 5 illustrates the response of successive standard additions of methimazole. Calibration equation parameters and necessary validation data were shown in Table 1. Obtained recovery results of spiked human serum samples were given in Table 3.

Table 3. Recovery of methimazole in spiked human serum

Method		Intra-day			Inter-day		
	Added (µg/mL)	Found ± SD	Recovery (%)	RSD[a] (%)	Found ± SD	Recovery (%)	RSD[a] (%)
SWV	15	14.5 ± 0.29	96.7	2.00	14.6 ± 0.28	97.3	1.92
	25	25.2 ± 0.63	100.8	2.50	25.3 ± 1.02	101.2	4.03
	60	58.4 ± 1.92	97.3	3.29	59.1 ± 2.14	98.5	3.62
DPV	15	14.7 ± 0.34	98.0	2.31	14.8 ± 0.27	98.7	1.82
	25	25.4 ± 0.92	101.6	3.62	25.1 ± 0.84	100.4	3.34
	60	61.1 ± 1.82	101.8	2.98	59.2 ± 2.11	98.7	3.56

SD: Standard deviation of six replicate determinations, RSD: Relative standard deviation, [a]Average of six replicate determinations

The recovery results of methimazole in serum samples were calculated from the related linear regression equations, which are given in Table 1. The LOD and LOQ values were also calculated and shown in Table 1. Repeatability and reproducibility of peak potential were also shown in Table 1. Typical SWV and DPV curves of methimazole examined in serum samples are shown in Figures 7 and 8.

As can be seen in Figures 7 and 8; no oxidation compounds and no extra peaks present in biological material peak occurred in the potential range where the analytical peak appeared.

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.[23,24]

SWV and DPV voltammetry methods were applied for the determination of the commercial tablets (Table 2). The results show that high reliability and reproducibility of 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 deviation (RSD) was 1.02% using the proposed method 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 99.2%

with RSD of 3.19 was obtained. The results of the drug analysis obtained from the proposed method 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 5.

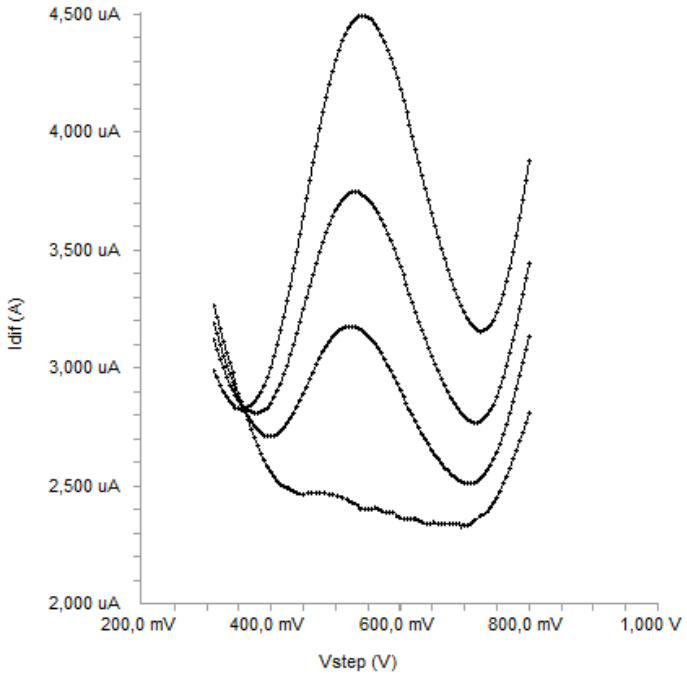

Figure 7. SWV voltammograms obtained for the determination in spiked serum (blank, 30, 50 and 80 μg/mL).

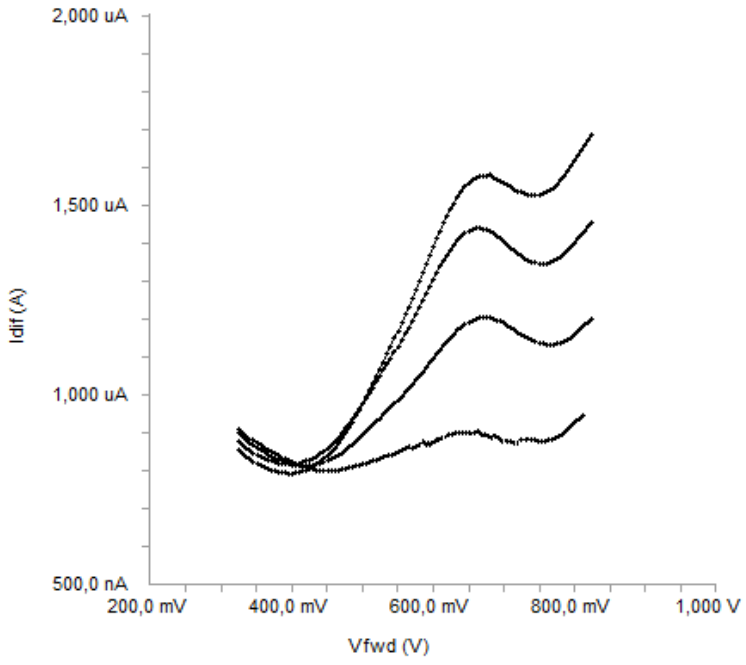

Figure 8. DPV voltammograms obtained for the determination in spiked serum (blank, 30, 50 and 80 μg/mL).

Table 4. Comparison of the proposed and reported methods for determination of methimazole

Parameters	SWV	DPV	Reported method (16)	Reported method[17]
Mean (recovery %)	99.9	100.1	98.6	96.9
SD	0.624	1.730	-	-
% RSD	0.624	1.728	2.64	4.50
Variance	0.389	2.993	-	-
SE	0.254	0.706	-	-
t-test (2.228)[a]	0.921		-	-
F- test (5.1)[a]	4.05		-	-

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

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]**
GCE	10-80 µg/mL (0.0875-0.7007 µM)	0.50 µg/mL (437.9 nM)	2.76	**Proposed work**

[a] LOD: Limit of detection

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.[21] Two calibration graphs from the bulk solution of methimazole according to the procedures described above were constructed by using SWV and DPV. And these methods are requiring less than 3 min to run samples.

Conclusions

A validated square wave and differential pulse voltammetric procedure was developed and successfully applied to the estimation of methimazole in tablet and human serum samples. For the analysis in the presence of biological material standard addition method is preferred. 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, its formulations and human serum samples.

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