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Electrochemical Determination of 2-thiouracil in Pharmaceuticals and Real Samples Using Gold Electrode

Vijay P. Pattar, Atmanand M. Bagoji, Naveen M. Gokavi and Sharanappa T. Nandibewoor*

P. G. Department of studies in Chemistry, Karnatak University, Dharwad-580003, India

* Corresponding Author, Tel.: +91-83622152861; Fax: +91-8362747884

E-Mail: stnandibewoor@yahoo.com

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Abstract- In this study, the electrochemical behaviour of 2-thiouracil (2TU) has been investigated using cyclic, differential pulse and square wave voltammetric techniques. The electrochemical studies were carried out in pH 3 with 0.2 M phosphate buffer as supporting electrolyte. Cyclic voltammetric studies were performed in a wide range of sweep rates and various concentrations of 2TU. The effect of surfactants was studied. The anodic peak was characterized and process was adsorption-controlled. According to the linear relation between the peak current and the 2TU concentration, differential-pulse voltammetric method for the quantitative determination in pharmaceuticals was developed. The linear response was obtained in the range of 1–11 µM with a detection limit of 2.05×10^{-8} M with good selectivity and sensitivity. The proposed method was successfully applied to 2TU determination in pharmaceutical samples and for the detection of 2TU in urine as a real sample.

Keywords- 2-thiouracil, Voltammetry, Gold electrode, Analytical applications, Surfactant

1. INTRODUCTION

2-thiouracil (2TU) (2-mercapto-4-hydroxypyrimidine), (Scheme 1) is a thio-derivative of uracil, one of the nucleic acid bases (NABs). Its biological importance has been clearly established as an antithyroid drug [1,2], an anticarcinogenic agent [3] and an antiviral agent

[4]. Thiouracil derivatives attract attention not only because of their unclear role in nucleic acid structures, but also because of exhibited pharmacological activities, such as, a dietary product due to the effects on thyroid activity suppression [5,6], or an increase of the hypothyroidism effect on blood [7]. Thiouracils are also of interest because of kidney stone formation inhibition [8] and their antidote properties for mercury poisoning [9].

In the thiouracils, 2TU offers special importance. 2TU has been identified in t-RNA and it plays an important role in antiviral and anticancer activity [10]. The chemotherapeutic activity of 2TU is due to its ready incorporation into the nucleic acid [11], impeding the melanoma tumours growth [12,13]. 2TU also induces modifications in the thyroid gland [14,15]. The synthesis, structural studies, and an anti-HIV activity of some new complexes of metal ions of 2TU were recently reported [16].

Various analytical methods for therapeutic monitoring have been reported in the literature for the determination of 2TU in complex physiological samples [17-19]. The main problems encountered in using such methods are either the need for derivatization or the need for time-consuming extraction procedures.

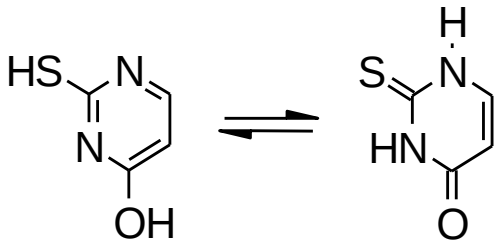
The gold electrode has been widely used in electrochemical studies and electro analysis for various substrates for wide potential window and fast electron transfer rate [20-21].
Electrochemical methods, especially square wave voltammetry (SWV) and differential pulse voltammetry (DPV), make it possible to decrease analysis time as compared to the time required by classical methods. The advantages of DPV over other electroanalytical techniques are greater speed of analysis, fewer problems with blocking of the electrode surface and lower consumption of electroactive species.

To the best of our knowledge, to date there are no studies in the literature on the voltammetric method for the determination of 2TU by gold electrode. Here, we have investigated an electrochemical oxidation process of 2TU on gold electrode by cyclic, square wave voltammetry and to develop a differential pulse voltammetric method for the direct determination of 2TU in real samples like pharmaceuticals and urine.

2. EXPERIMENTAL

2.1. Reagents and Chemicals

A stock solution of 2TU (Sigma Aldrich), 1 mM was prepared in millipore water. The phosphate buffers from pH 3 – 11 were prepared in millipore water as described by Christian and Purdy [22]. In this study, Sodium lauryl sulphate (S.D. Fine), cetyltrimethylammonium bromide (Hi-media) and Triton X-100 were used as anionic, cathodic and nonionic surfactants respectively. 2TU containing tablet Propylthiouracil® (Macleods pharmaceuticals Ltd., batch no. HPB203B) were purchased from a local pharmacy. All other reagents used were of analytical or reagent grade and their solutions were prepared with millipore water.



Scheme 1. Chemical structure of 2TU

2.2. Instrumentation

Electrochemical measurements were carried out on a CHI 630D electrochemical analyzer (CH Instruments Inc., USA). The voltammetric measurements were carried out in a 10 mL single-compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode, and a 2 mm diameter gold electrode as a working electrode (part no.CHI101). All experiments were carried out at an ambient temperature of 25±0.2^{o}C. All the potentials are given against the Ag/AgCl (3M KCl). PH measurements were performed with Elico LI120 pH meter (Elico Ltd., India).

At different scan rates, the area of the electrode was calculated using 1.0 mM $K_3Fe(CN)_6$ as a probe. For a reversible process, the Randles-Sevcik formula has been used [23].

$$I_{pa} = (2.69\times10^{5})n^{3/2}AD_o^{1/2}C_o\,\upsilon^{1/2} \qquad (1)$$

Where I_{pa} refers to the anodic peak current, n is the number of electrons transferred, A is the surface area of the electrode, D_O is diffusion coefficient, m is the scan rate and C_O is the concentration of $K_3Fe(CN)_6$. For 1.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, n=1, D_O=7.6 x 10^{-6} cm^2 s^{-1} [23], then from the slope from the plot of I_{pa} *vs.* $\upsilon^{1/2}$, the surface area of the electrode can be calculated. In our experiment the slope was 1.939×10^{-4} $\mu A(Vs^{-1})^{-1/2}$ and the area of electrode was calculated to be 0.0948 cm^2.

2.3. Pretreatment of working electrode

To provide a reproducible active surface and to improve the sensitivity and resolution of the voltammetric peaks, the gold electrode was polished to a mirror finish with 0.3 micron alumina on a smooth polishing cloth and then rinsed with methanol and millipore water prior to each electrochemical measurement. All the measurements were carried out at room temperature (25 ± 0.2°C).

3. RESULTS AND DISCUSSION

3.1. Cyclic Voltammetry Behavior of 2TU

In order to understand the electrochemical behavior of 2TU at gold electrode cyclic voltammetry experiment was carried out between pH 3.0 and 11 of phosphate buffer. The cyclic voltammograms obtained for 1.0 mM 2TU solution at a scan rate of 50 mVs^{-1} exhibits a well-defined irreversible anodic peak at about 1.41 V at gold electrode. The cathodic peak that appeared corresponds to the reduction of gold oxides [24]. The results are shown in Fig. 1. The blank solution without 2TU was shown by curve (b) and anodic peaks corresponding to 2TU oxidation appeared at 1.41V as shown in curve (a). Here in peak 'b' lower intensity broader peak is observed and at curve 'a' intensity of the peak increased due to electro catalytic behavior of the gold. Gold electrodes are very weak chemisorbers due to filled d-orbitals, yet display a higher electroactivity towards the oxidation of drugs. The electrocatalytic behavior of gold is highly complex. The catalytic component of gold electrode is believed to be hydrous gold oxide, AuOH, which is formed by the chemisorption of hydroxide anions to the gold surface. This process occurs at potentials of 0.4 to 0.8 V *vs.* Ag/AgCl (3 M KCl) depending on the surface structure of the gold electrode. Therefore, the gold oxide formation and its reduction is pH dependent (Fig. 2) [25].

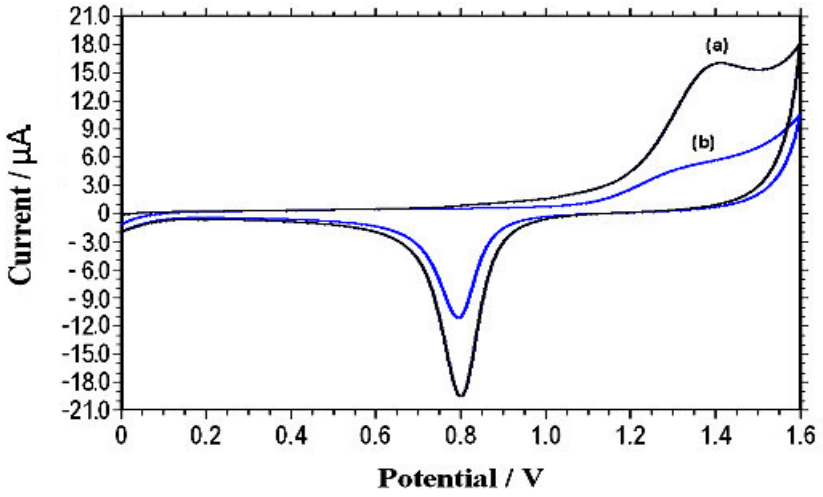


Fig. 1. Cyclic voltammogram obtained for 1 mM 2TU on gold electrode in pH 3, 0.2 M buffer: (a) 2TU and (b) blank run without 2TU at υ=50 mVs^{-1}

The voltammograms corresponding to the first cycle were generally recorded because of decrease in the oxidation peak current occurs with the number of successive sweeps. This phenomenon may be attributed to the adsorption of the oxidation product on the electrode surface [26].

3.2. Effect of pH

Cyclic voltammograms of 2TU recorded from pH 3.0–11.0 at a scan rate of 0.05 Vs^{-1} was presented in Fig. 2. With the increase of pH of the solution, the peak potentials shifted to less positive values. It can be concluded that 2TU in buffered solutions with pH greater than 11 exist mainly as thiolate anions and therefore, their equilibrium concentrations do not vary by increasing the pH of solution. Hence pH variation is restricted to 11.

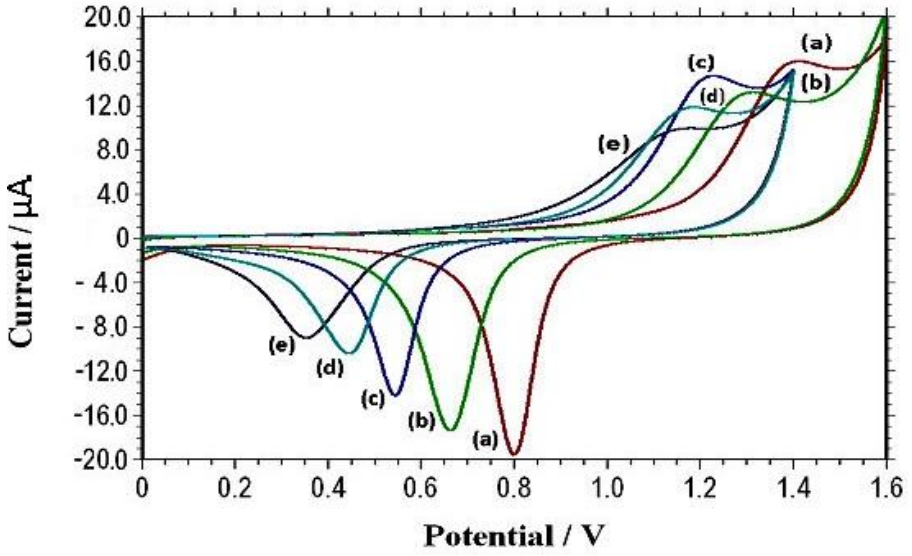


Fig. 2. Influence of pH on the shape of the peaks in phosphate buffer solution at (a) pH 3.0, (b) pH 5.0, (c) pH 7.0, (d) pH 9.0 and (e) pH 11.0 with potential scan rate 50 mVs^{-1}. Other conditions are as in Fig. 1

The plot of E_{pa} versus pH (Fig. 3A) shows that the peak potential is pH dependent. The variation of peak current with pH is as shown in Fig. 3B. With the increase in solution pH, the peak potential linearly shifts to less positive values and the linear relation between E_{pa} and pH can be expressed as:

$$E_{pa} = 1.532 - 0.043pH \qquad r^2 = 0.989$$

The intersection point of the E_{pa}–pH curve was at about 9.5. This type of treatment of the data was reported in literature [27]. This value would correspond to the pKa value for the amine group. Although pKa value is not exactly matching with the literature value (10.7), the value obtained was in the neighborhood. The slope of this equation is found to be 43 mV/pH. This closeness of the slope to the expected theoretical value of 59 mV/pH suggests that the number of electrons transferred is equal to that of the hydrogen ions taking part in the electrode reaction.

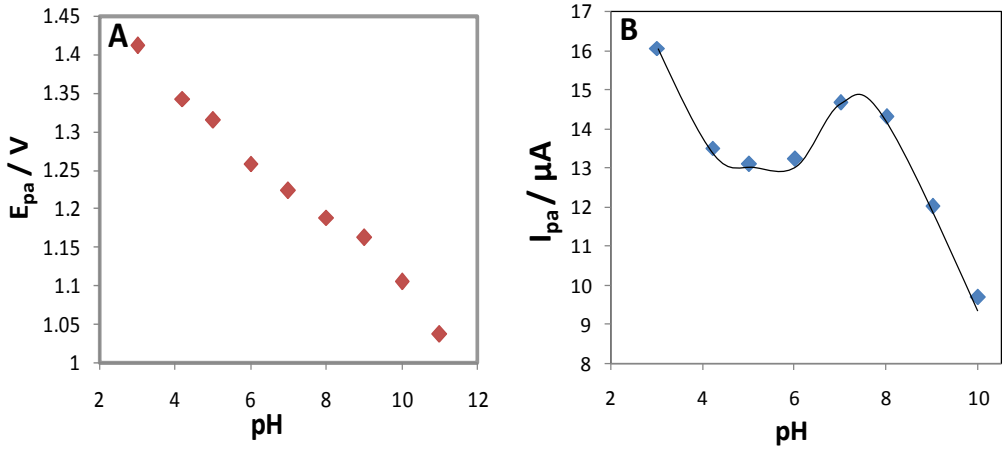


Fig. 3. (A) Influence of pH on the peak potential of 2TU; **(B)** Variation of oxidation peak currents with pH

From the plot of I_{pa} versus pH, the best result with respect to sensitivity accompanied with sharper response was obtained with pH=3.0, so pH=3.0 was selected for further experiments.

3.3. Effect of Scan Rate

The influence of the potential scan rate on the electrochemical oxidation was studied at pH 3.0 by cyclic voltammetry (Fig. 4). Scan rate studies were carried out to assess whether the processes on gold electrode were under diffusion or adsorption-controlled.

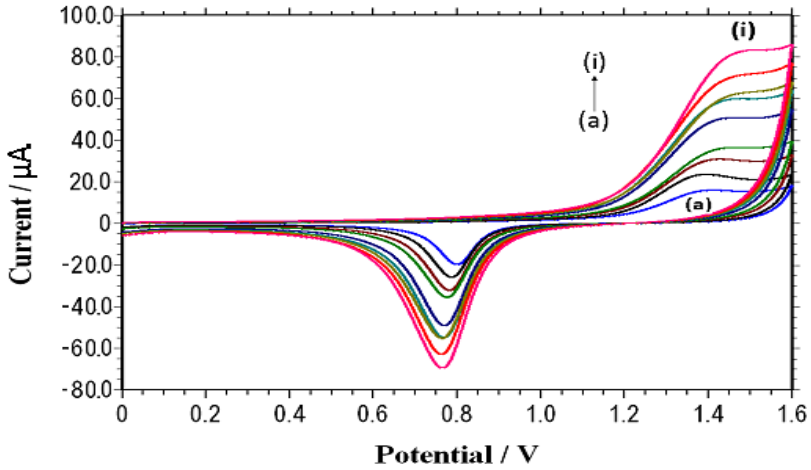


Fig. 4. Cyclic voltammograms obtained for 1.0 mM 2TU in buffer solution of pH 3 at scan rates of (a) 0.05, (b) 0.1, (c) 0.15, (d) 0.2, (e) 0.25, (f) 0.3, (g) 0.35, (h) 0.4 and (i) 0.45 Vs^{-1}

When the scan rate was varied from 50 to 450 mVs^{-1} in 1.0 mM solution of 2TU, a linear dependence of the peak intensity I_{pa} (µA) upon the scan rate (υ) was found [28],

demonstrating an adsorption behavior (Fig. 5A). The equation for this line is given below in phosphate buffer at pH 3.0: I_{pa} (μA) = 13.95 υ (V s^{-1}) + 0.421, r^2 = 0.992.

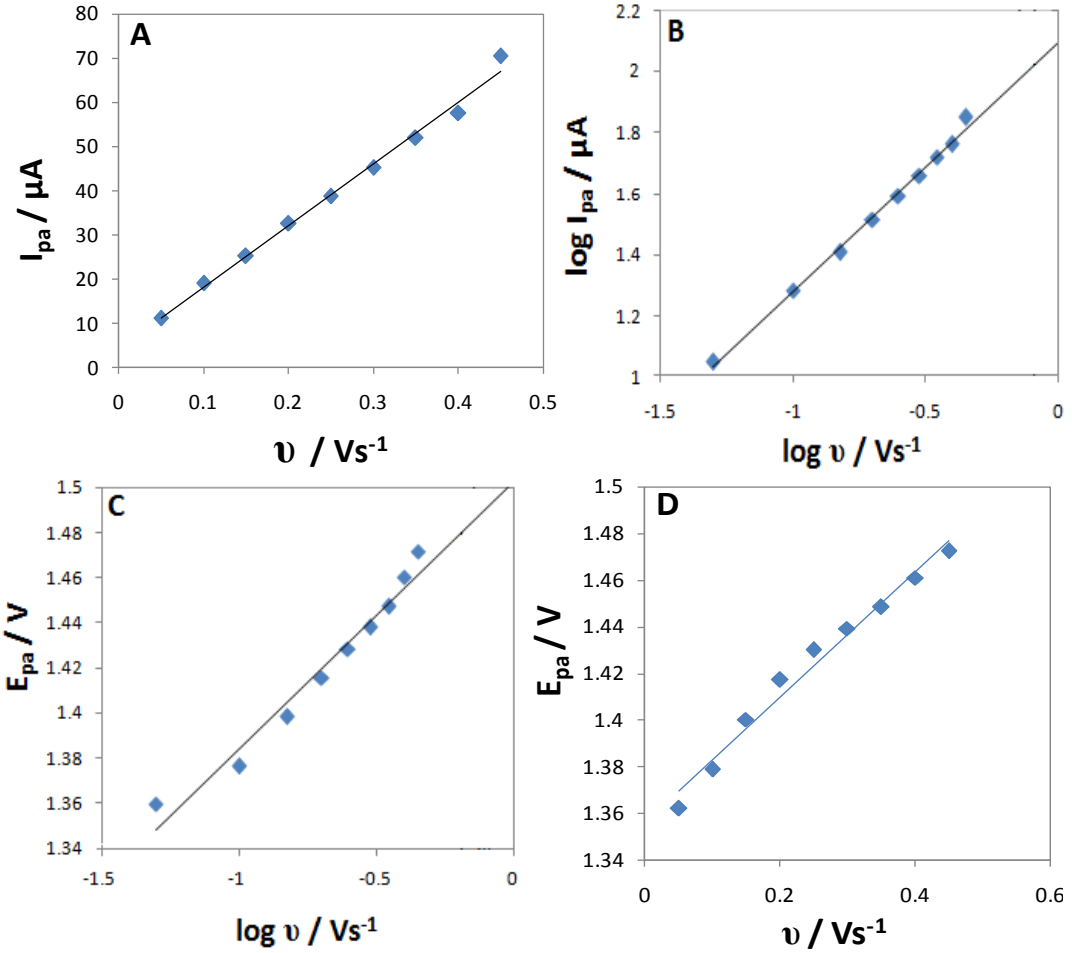


Fig. 5. (A) Dependence of the oxidation peak current on the scan rate**; (B)** Dependence of the logarithm of peak current on logarithm of scan rate**; (C)** Relationship between peak potential and logarithm of scan rate; **(D)** Relationship between peak potential and scan rate

A plot of logarithm of anodic peak current *vs*. logarithm of scan rate gave a straight line with a slope of 0.816 (Fig. 5B) close to the theoretical value of 1.0, which is expected for an ideal reaction for the adsorption-controlled electrode process [28]. The equation obtained was: log I_{pa} (μA) = 0.816 log υ (V s^{-1}) +1.091, r^2=0.995. The E_{pa} of the oxidation peak was also dependent on scan rate. The plot of E_{pa} versus log υ was linear having a correlation coefficient of 0.965 (Fig. 5C) and the relation between E_{pa} and υ (Fig. 5D) can be expressed by the equation E_{pa} (V) = 0.268 υ (Vs^{-1}) + 1.356.

For an adsorption-controlled and irreversible electrode process, according to Laviron, E_{pa} is defined by the following [29]:

$$E_{pa} = E^0 + \left(\frac{2.303RT}{anF}\right) log\left(\frac{RTk^0}{anF}\right) + \left(\frac{2.303RT}{anF}\right) log\upsilon \quad (2)$$

Where α is the transfer coefficient, k^0 is the standard heterogeneous rate constant of the reaction, n is the number of electron transferred, υ is the scan rate and E^0 is the formal redox potential. Other symbols have their usual meanings. Thus the values of αn can be easily calculated from the slope of E_{pa} versus log υ. In this system the slope is 0.117, taking T=298 K, R=8.314 J $K^{-1}mol^{-1}$ and F=96,480 C, αn was calculated to be 0.5054. Generally, α is assumed [30] to be 0.5 in totally irreversible electrode process. According to Bard and Faulkner [31], α can be given as:

$$\alpha = \frac{47.7}{E_{pa} - E_{pa/2}} mV \quad (3)$$

Where $E_{pa/2}$ is the potential where the current is at half the peak value. So, from this we got the value of α to be 0.3006. Further, the number of electron (n) transferred in the electro oxidation of 2TU, was calculated to be 1.69 ~ 2. The value of k^0 can be determined from the intercept of the above plot if the value of E^0 is known. The value of E^0 in Eq. (2) can be obtained from the intercept of E_{pa} versus υ curve by extrapolating to the vertical axis at υ=0 [32]. In our system the intercept for E_{pa} *vs*. log υ plot was 1.503 and E^0 was obtained to be 1.356 V, the k^0 was calculated to be 355.1 s^{-1}.

3.4. Effect of surfactant

Surfactants can exert a strong effect on the electrode process. Adsorption of such substances at the electrode may inhibit the electrolytic process, bring about the irregularity in the voltammograms, and cause shift in the wave to more negative potentials [33,34]. Surface-active substances have the common tendency of accumulation at interfaces. The lack of affinity between the hydrophobic portion of the surfactant and water leads to a repulsion of these substances from the water phase as a consequence of oxidation of the microscopic 2TU-water interface. It was found that addition of the anionic surfactant, sodium dodecyl sulfate shifted the anodic potential of 2TU to less positive values and with increase in the current (Table 1). Whereas the cationic surfactant, cetyltrimethyl ammonium bromide shifted the anodic peak potential of 2TU to more positive values and there was a decrease in the current. The non-ionic surfactant, Triton X-100 had no effect on the voltammograms.

3.5. Mechanism

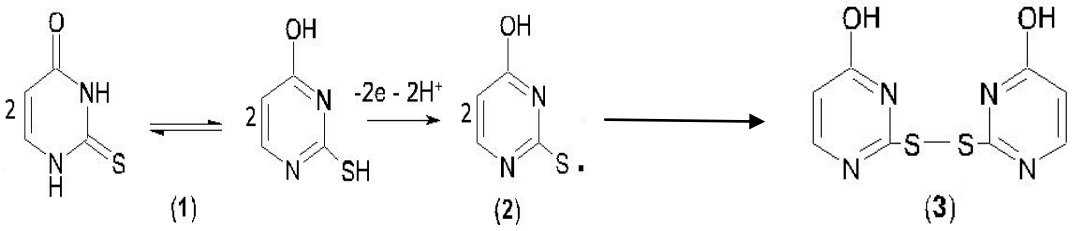
As evidenced from the above studies, it is inferred that electrochemical oxidaton of 2TU occurs in a single well-defined oxidation peak current (I_a). Mercapto compounds have been reported to oxidise by readily losing an electron and proton to give the corresponding

disulfides [35]. Thus, it seems reasonable to conclude that 2TU loses a proton and an electron in the peak I_a process to give a free radical (2), which rapidly dimerizes to give the corresponding disulphide (3) (Scheme 2). An UV-Vis absorption study shows the decrease in the absorption peak after electrolysis due to the formation of product. Some chemical reactions of 2TU were examined and gave the following results: (a) Iodine oxidizes 2TU very rapidly with a formation of the dimer, the composition of which has been established by Miller et al. [36] and the dimer is 2-thiouracil disulfide. (b) According to Miller et al. [36], this dimer behaves as a strong acid and forms a disodium salt that can be easily precipitated from the solution by acetone. (c) Unlike 4-thiouracil, the 2TU does not undergo any chemical or electrochemical reduction reactions. The results obtained provide some additional insights into the formation of disulfide.

Table 1. Effect of surfactants on the voltammetric behavior of 1.0 mM 2TU at gold electrode with scan rate of 50 mVs^{-1}

Concentration (mM)	**I_p (µA)**	**E_p (V)**	**Concentration (mM)**	**I_p (µA)**	**E_p (V)**
SDS[a]			CTAB[b]		
0.00	16.01	1.3722	0.00	16.01	1.3722
0.10	16.12	1.3686	0.10	15.88	1.3730
0.30	16.30	1.3641	0.30	15.56	1.3748
0.50	16.52	1.3607	0.50	15.24	1.3759
0.70	16.75	1.3576	0.70	14.94	1.3772
1.00	17.12	1.3461	1.00	14.64	1.3794

[a]Sodium dodecyl sulfate [b]Cetyl trimethyl ammonium bromide



Scheme 2. Tentative mechanism proposed for the electrooxidation of 2TU

3.6. Calibration Curve and detection limit

In order to develop a voltammetric method for determining the drug, we selected the square wave voltammetric (SWV) and differential pulse voltammetric (DPV) modes, because the peaks are sharper and better defined at lower concentration of 2TU, than those obtained by cyclic voltammetry, with low background current, resulting in improved resolution. According to the obtained results, it was possible to apply these techniques to the quantitative analysis of 2TU. The phosphate buffer solution of pH 3.0 was selected as the supporting electrolyte for the quantification of 2TU as it gave maximum peak current at pH 3.0. The peak at about 1.191 V in DPV and 1.223 V in SWV was considered for the analysis. Differential pulse voltammo grams and square wave voltammograms obtained with increasing amount of 2TU showed that the peak current increased linearly with increasing concentration, as shown in Fig. 6(A) and (B).

Using the optimum conditions described above, linear calibration curves were obtained for 2TU in the range of 1 to 15 μM. The linear equation was

$$I_{pa}\ (\mu A) = 0.070C\ (\mu M) + 0.833 \qquad (r^2 = 0.996)$$

and

$$I_{pa}\ (\mu A) = 0.281C\ (\mu M) + 4.969 \qquad (r^2 = 0.993)$$

For DPV and SWV respectively. The DPV presents a good linear response as compared to SWV in view of a less intercept of linear plot of I_{pa} versus concentrations. Deviation from linearity was observed for more concentrated solutions, due to the adsorption of oxidation product on the electrode surface. It was also observed that the peak potential (E_{pa}) and half peak potential ($E_{pa}/2$) were shifted towards more positive value suggesting that product undergoes adsorption at the surface of gold electrode.

Related statistical data of the calibration curves were obtained from five different calibration curves. The limit of detection (LOD) and quantification (LOQ) were 2.05×10^{-8} M and 6.82×10^{-8} M, respectively. The LOD and LOQ were calculated using the following equations:

$$LOD = 3s/m, \qquad LOQ = 10s/m \qquad (4)$$

Where *s* is the standard deviation of the peak currents of the blank (five runs) and *m* is the slope of the calibration curve. Comparison of earlier methods shows the present method was better for the determination of 2TU (Table 2).

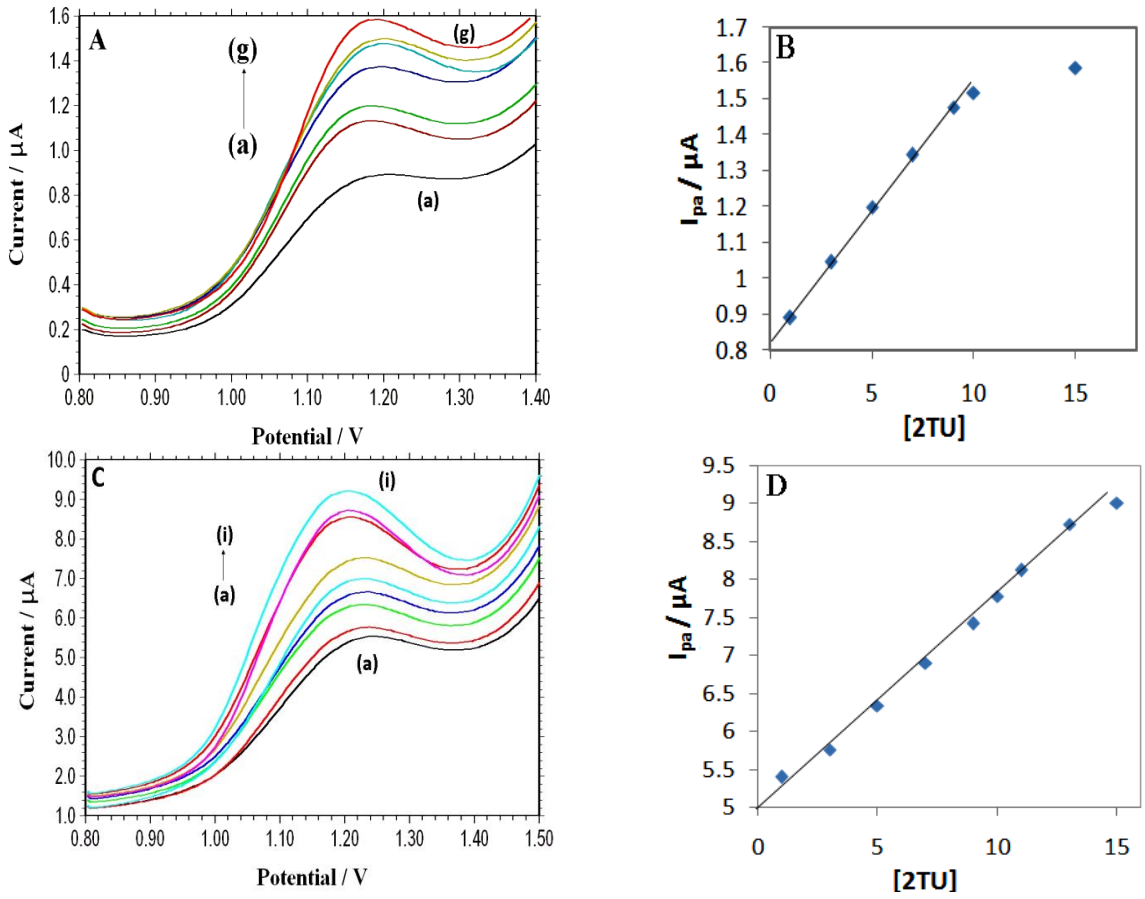


Fig. 6. (A) Differential-pulse voltammograms of gold electrode in 2TU solution at different concentrations at (a) 1, (b) 3, (c) 5, (d) 7, (e) 9, (f) 11 and (g) 15 µM; **(B)** plot of the peak current against concentration of 2TU for DPV; **(C)** Square wave voltammograms of gold electrode in 2TU solution at different concentrations at (a) 1, (b) 3, (c) 5, (d) 7, (e) 9, (f) 10, (g) 11, (h) 13 and (i) 15 µM; **(D)** plot of the peak current against concentration of 2TU for SWV

Table 2. Comparison of some methods for the determination of 2TU with the proposed method

Linearity range (mM)	Detection limit (µM)	Ref.
0.5 - 4.0	0.429	17
0.7 - 800	40.00	18
0.01 - 0.05	6.000	37
0.001 - 0.011	0.021	**Present work**

3.7. Stability and Reproducibility

In order to study the stability and reproducibility of the electrode, a 10 μM 2TU solution were measured with the same electrode (renewed every time) for every several hours within day, the RSD of the peak current was 0.60% (number of measurements=5). As to the between day reproducibility, it was similar to that of within a day if the temperature was kept almost unchanged which could be attributed to the excellent stability and reproducibility of gold electrode.

3.8. Effect of Excipients

For the analytical applications of the proposed method, the effects of potential excipients that are likely to be in biological samples were evaluated under the optimum experimental conditions. Differential pulse voltammetric experiments were carried out for 1.0 μM 2TU in the presence of 1.0 mM of each of the excipients. The experimental results (Table 3) showed that thousand-fold excess of citric acid, maltose, glucose, sucrose and oxalic acid did not interfere. However, thousand-fold excess of starch and gum acacia were interfering.

Table 3. Influence of potential excipients on the voltametric response of 1.0 μM 2TU

Excipients (1mM)	Potential observed (E_p)	Signal Change (%)
Citric acid	1.1598	3.141
Maltose	1.1801	1.111
D-Glucose	1.1919	-0.069
Gum acacia	1.249	-5.779
Oxalic acid	1.2066	-1.539
starch	1.2602	-6.899
Sucrose	1.1592	3.201

3.9. Determination of 2TU in pharmaceutical dosages

To evaluate the applicability of the proposed method, the tablet containing 2TU from Propylthiouracil®**,** India, was studied. The tablets were grounded to powder, dissolved in millipore water and then further diluted so that 2TU concentration falls in the range of calibration plot. Differential pulse voltammograms were then recorded under exactly identical conditions that were employed while recording differential pulse voltammograms for plotting calibration plot. The results are in good agreement with the content marked in the label. The recovery studies were carried out after the addition of known amounts of the drug

to various pre-analyzed formulations of 2TU. The F and Student t tests were also calculated. All these results are listed in Table 4.

Table 4. Analysis of 2TU in tablet by DPV and recovery studies

	Propylthiouracil Tablet
Labeled claim (mg)	50.0
Amount found (mg)[(a)]	49.2
RSD (%)	0.32
Added (mg)	2.00
Found (mg)[(a)]	1.95
Recovered (%)	97.5
RSD (%)	0.99
Calculated F	1.14
Calculated t	2.10

(a)Mean average of five determinations.

3.10. Determination of 2TU in Urine Samples

The developed differential voltammetric method for the determination of 2TU in spiked urine was investigated. The recoveries from urine were measured by spiking drug free urine with known amounts of 2TU. The urine samples were diluted 100 times with the phosphate buffer solution before analysis without further pretreatments. A quantitative analysis can be carried out by adding the standard solution of 2TU into the detect system of urine sample. The detection results of four urine samples obtained are listed (Table 5). The recovery determined was in the range from 97.00% to 102.54% and the standard deviation and relative standard deviations are given in Table 5. The proposed methods are suitable for quality control laboratories as well as pharmacokinetic studies where economy and time are essential.

Table 5. Determination of 2TU in urine samples

Urine	Spiked (μM)	Detected[(a)] (μM)	Recovery (%)	SD ± RSD (%)
Sample 1	2	1.9406	97.03	0.0062±0.319
Sample 2	4	3.9607	99.02	0.0130±0.329
Sample 3	6	5.9111	98.52	0.0082±0.138
Sample 4	8	8.2030	102.54	0.0406±0.497

(a)Mean average of five determinations

4. CONCLUSION

An electrochemical study has been presented on the oxidation of an antithyroid drug, 2TU at a gold electrode. The influences of several physicochemical parameters (pH, potential scan rate, concentration) were investigated. A probable reaction mechanism was proposed. A two electrons, two proton mechanism, irreversible and adsorption controlled were observed for the oxidation reaction of 2TU. By selecting the anodic peak of 2TU, the DPV procedure was developed for its assay in tablets. The recoveries obtained for pharmaceutical formulations and spiked urine samples show the applicability of this technique to control analysis of 2TU drug. Further this method may be considered as a suitable alternative to the existing chromatographic methods. Finally the proposed method was not time-consuming and less expensive than other methods in the literature.

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