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Fabrication, Characterization and Application of $NiSO_4$ Modified Carbon Paste Electrode for the Detection of 2-Thiouracil in Biological Fluids

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Abstract- A new carbon paste electrode modified with $NiSO_4$ (NSCPE) was fabricated and employed in electrocatalytic oxidation of 2-thiouracil (2-TU) for the first time using cyclic voltammetry (CV), square wave voltammetry (SWV) and differential pulse voltammetry (DPV) as diagnostic techniques. Characterization of the NSCPE was carried out by CV and scanning electron microscope (SEM) techniques. All experimental parameters have been optimized. The plausible mechanism of oxidation of 2-TU was proposed. The DPV of 2-TU gave a good linear response in the range of $10\text{-}100\times10^{-8}$ M with a limit of detection 2.1×10^{-9} M and limit of quantification 6.9×10^{-9} M. The proposed method was successfully applied for quantitative determination of 2-TU in pharmaceutical formulations and urine as real sample.

Keywords- Sensors, Drug research, Heterogeneous catalysis, Chemically modified electrodes, Redox reactions, 2-thiouracil

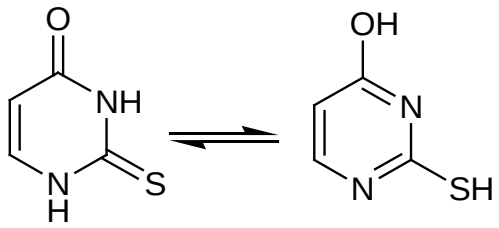
1. INTRODUCTION

It is worthwhile to develop a simple and accurate method for detecting ingredients in drugs since it is important in quality control laboratory. Electrochemical methods are powerful and versatile analytical techniques that offer high sensitivity, accuracy, and precision as well as large linear dynamic range, with relatively low-cost instrumentation. The

electroanalytical studies are more regularly used on industrial [1], environmental applications [2] and on the drug analysis [3] in their dosage forms and especially in biological samples.

The chemically modified electrodes (CMEs) have been widely used for sensitive and selective analytical methods for the trace component analysis of bio-active compounds [4-6]. CMEs have been used due to their ability to catalyze the electrode process through significant decreasing of overpotential with respect to unmodified electrode. It has been known for a long period that certain transition metal complexes with Schiff bases [7] can catalyze the electro-oxidation of some chemical and biological important compounds via reduction of their central metal ions. Chemically modified CPEs have been used for the determination of various species namely dopamine [8], cysteine [9], thioglycolic acid [10], levodopa and carbidopa [11, 12], in recent years.

2-Thiouracil (2-thioxo-2, 3-dihydropyrimidin-4(1H)-one) (2-TU) shown in Scheme 1, is a chemically modified analogue of a DNA base, so it is of interest for its biochemical, pharmacological and biological capabilities [13]. 2-TU has a strong antibacterial effect against Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Streptococcus faecalis, and Mycobacterium phlei due to its antimetabolic effect [14]. In addition 2-TU itself has certain biological activities as antiviral activity, antiprotozoal and antifungal [15] and also some 2-thio derivatives of uracil derivatives have cytotoxic activity [16]. The sulfhydryl compounds are known to undergo electrochemical oxidation at solid electrodes, but their oxidation occurs at considerably high potentials [17]. 2-TU was characterized for the electrocatalytic oxidation of sulfhydryl compounds. 2-TU and its derivatives also act as selective inhibitors of nitric oxide synthase (NOS) [18]. The administration of 2-TU in chicken has been found to cause increase in total protein content and decrease in DNA content [19].



Scheme 1. Chemical structure of 2-thiouracil

The determination of 2-TU from different methods have been reported in complex physiological samples such as liquid chromatography coupled with electrochemical detection [20] and spectral studies [21]. These methods are unsuitable for routine analysis due to some major disadvantages such as high cost, long analysis time, sample pre-treatment, low sensitivity and selectivity. In the present work, $NiSO_4$ modified carbon paste electrode

(NSCPE) possesses high stability and good electrocatalytic activity towards the oxidation of 2-TU. Cyclic voltammetry was used to characterize the electrochemical properties of the NSCPE and to investigate its electrocatalytic effect on 2-TU oxidation. Differential pulse voltammetry (DPV) and square wave voltammetry (SWV) were used to evaluate the analytical performance of the modified electrode in the determination of 2-TU. To the best of our knowledge, electrocatalytic detection of 2-TU using NSCPE has not been reported so far. The aim of the present work is to develop sensitive and selective method for the determination of 2-TU and apply the same in pharmaceuticals and urine as real samples.

2. EXPERIMENTAL

2.1. Chemicals

2-TU was purchased from Sigma Aldrich. A stock solution (1.0 mM) of 2-TU was prepared in Millipore water. The phosphate buffers from pH 3.8–9.2 were prepared in Millipore water as described by Christian and Purdy [22]. All other reagents used were of analytical or reagent grade and their solutions were prepared in Millipore water.

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 $NiSO_4$ modified carbon paste electrode (NSCPE) as the working electrode. All the potentials are given against the Ag/AgCl (3 M KCl). All experiments were carried out at an ambient temperature of 25±0.1°C. The pH measurements were performed with Elico LI120 pH meter (Elico Ltd., India).

2.3. Preparation of NSCPE

The conventional CPE was prepared by hand mixing of graphite powder with paraffin at a ratio 70/30(w/w) in a mortar. The homogeneous paste was packed into a cavity of Teflon tube, the electrical contact was made with a copper wire connected to the paste in the tube. The NSCPE was prepared by inserting the CPE into the 10 ml single compartment containing 0.5 M $NiSO_4$. The NSCPE was then activated by successive cyclic voltammetric sweeps between −1.2 and 2.0 V until stable cyclic voltammograms were obtained. Then electrode was transferred into another 10 ml of phosphate buffer containing proper amount of 2-TU. After accumulating for 10 s at open circuit, potential scan was initiated and cyclic voltammograms were recorded between -0.4 and 0.8 V, with a scan rate of 50 mVs^{-1}.

2.4. Electrochemical measurements

The NSCPE in the three-electrode system was immersed in 0.2 M phosphate buffer (pH 6.0) containing a known amount of 2-TU. Then CV, SWV and DPV were performed. The common parameter for CV was a scan rate of 100 mVs^{-1}. The parameters for DPV and SWV were initial potential E: 0.05 V; final potential E: 0.45V; sample interval: 0.001 V; frequency: 15 Hz; quiet time: 2 s; sensitivity: 1.0×10^{-6} A/V. Voltammograms were recorded with the phosphate buffer in the absence of 2-TU also.

2.5. Sample preparation and analytical procedure

Ten pieces of 2-TU containing tablet i.e. propyl-thiouracil (local pharmacy) were accurately weighed and finely powdered in a mortar. An adequate amount of the powder to a stock solution of concentration of about 1.0 mM was weighed and transferred to a 100 ml calibrated flask and dissolved with distilled water. Appropriate solutions were prepared by taking suitable aliquots from this stock solution and diluted them with phosphate buffer solutions. The standard addition method was used for analyzing the pharmaceutical samples.
To check the interference from excipients used in the dosage form, DPV experiments were carried out for 1.0 μM 2-TU in the presence of 1.0 mM of each of the interferents in phosphate buffer solution as a supporting electrolyte.

Urine samples were collected during 24 h from healthy volunteers. 2 ml of aliquot of urine sample was transferred to 100 ml calibrated flask and diluted to the mark with phosphate buffer solutions. These urine samples were analyzed immediately or they were stored at −18 °C until analysis. Further, DPV technique has been carried for the analysis of 2-TU in urine samples by using standard addition method. The content of the drug in pharmaceutical, urine and effect of excipients was determined referring to the calibration graph.

3. RESULTS AND DISCUSSION

3.1. Surface morphology of CPE and NSCPE

Characterization of the prepared carbon paste electrode (CPE) and $NiSO_4$ modified electrode (NSCPE) was carried out using scanning electron microscopy (SEM). The SEM (Figure 1A) images of the bare carbon paste electrode showed a microstructure with a discontinuous grain growth with a large unclear crystal structure. The surface structure of the bare carbon paste electrode also shows that the graphite particles are covered by a very thin film of silicone oil. Figure 1B shows that the surface of the carbon paste electrode modified with $NiSO_4$ is relatively homogeneous and better dispersed than the bare carbon paste.

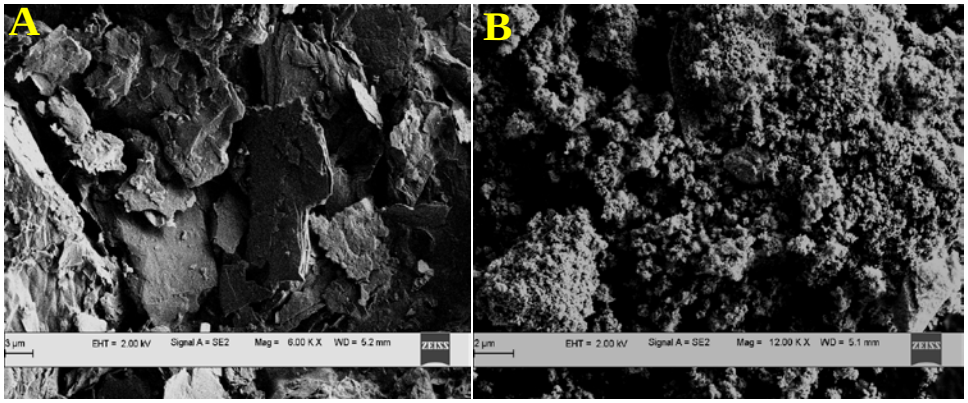


Fig. 1. SEM images of (A) CPE and (B) NSCPE

3.2. Electrochemical characterization and surface Area of the NSCPE

The ferro-ferricyanide system is one of the most common model systems for testing electrodes. Figure 2 shows the cyclic voltammogram of 1.0 mM $K_3Fe(CN)_6$ at bare and NSCPE in the potential range -200 mV to 600 mV at 50 mV/s scan rate. In both the cases, 1.0 mM $K_3Fe(CN)_6$ showed identical reversible cycles. The NSCPE possesses a very good electrochemical response compared to bare CPE.

The surface area of the CPE and NSCPE were obtained by the cyclic voltammetric technique by recording the current voltage curve at different scan rates. For a reversible process, the Randles-Sevcik formula can be used [23].

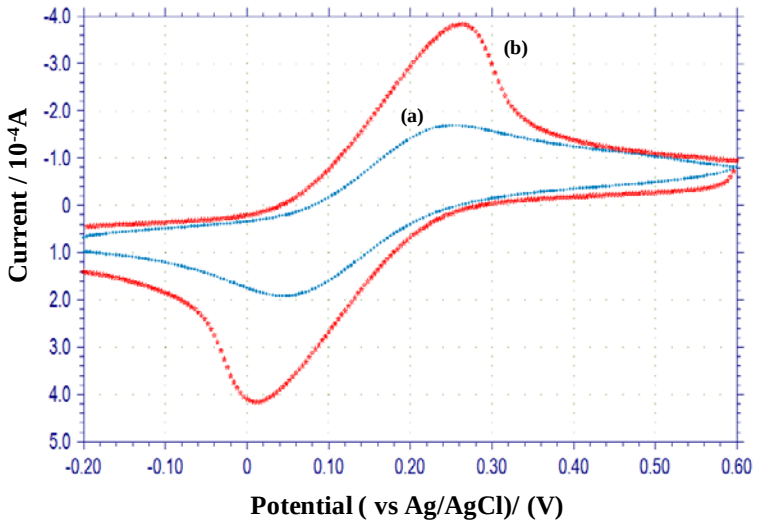


Fig. 2. Cyclic voltammograms for 1 mM $K_3[Fe(CN)_6]$ at (a) bare CPE and (b) NSCPE at 50 mV/s

$$I_{pa} = (2.69x10^5)n^{3/2}AD_o^{1/2}C_o\upsilon^{1/2} \quad (1)$$

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_0 is diffusion coefficient, υ is the scan rate and C_0 is the concentration of $K_3Fe(CN)_6$.

For 1.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, n=1, $D_0=7.6\times10^{-6}$ cm^2s^{-1}, then from the slope of the plot of I_{pa} versus $\upsilon^{1/2}$ relation. The slope obtained for CPE was 1.898×10^{-4} cm^4Ms^{-1} and the surface area of CPE was found to be 0.0256 cm^2. Similarly, for the NSCPE, the slope was 3.048×10^{-4} $cm^4Ms^{-1}$and the surface area of NSCPE was calculated to be 0.0412 cm^2. This reveals that NSCPE provides considerably larger surface area for the electrochemical oxidation of 2-TU than the unmodified CPE. This can also be explained by the SEM images as shown in Figure 1B.

3.3. Cyclic voltammetric behavior of 2-thiouracil at NSCPE

Figure 3 demonstrates the CV measurement for 1 mM 2-TU in phosphate buffer solution of pH 6.0 at both bare CPE and NSCPE to unveil the electro-catalytic effect of NSCPE. The modified electrode has no electrochemical activity in blank supporting electrolyte (Figure 3 curve a). From the Figure 3(curve b), it can be noticed that the redox peaks of 2-TU at bare CPE are broad and poor in sensitivity with anodic and cathodic peak potentials at 297 mV and -173 mV respectively.

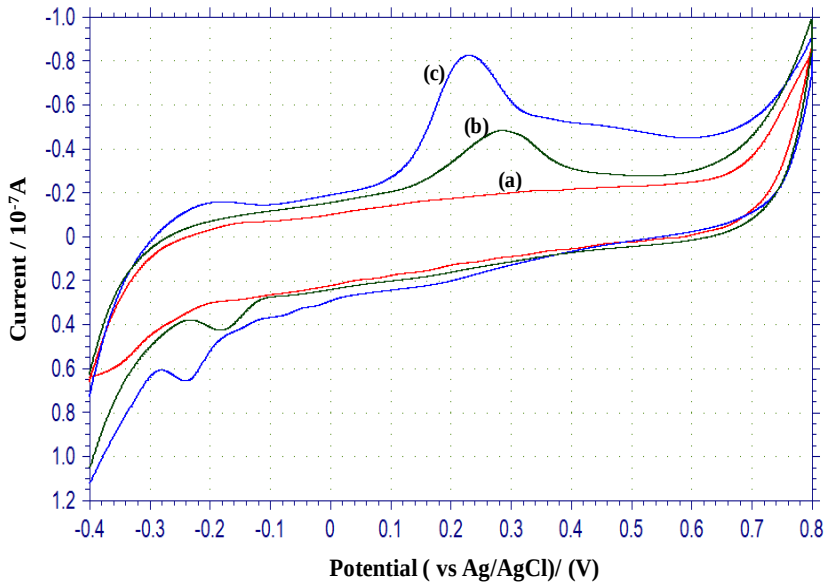


Fig. 3. Cyclic voltammogram for (a) 0.2 M phosphate buffer solution of pH 6.0 (without 2-TU) at NSCPE (b) 1 mM 2-TU at unmodified CPE and (c) 1 mM 2-TU at NSCPE

However, under the similar conditions, the NSCPE exhibited a well-defined voltammogram for 2-TU having oxidation and reduction peak potentials at 200 mV and -240 mV respectively with better peak intensity as compared to the peaks at CPE as shown in Figure 3(curve c). The peak to peak separations (ΔE_p) in CPE and NSCPE were found to be 440 mV and 470 mV respectively, suggesting that the electrochemical reactions were totally irreversible.

3.4. Influence of pH

The electro-oxidation of analyte under investigation might be affected by pH of the supporting electrolyte.

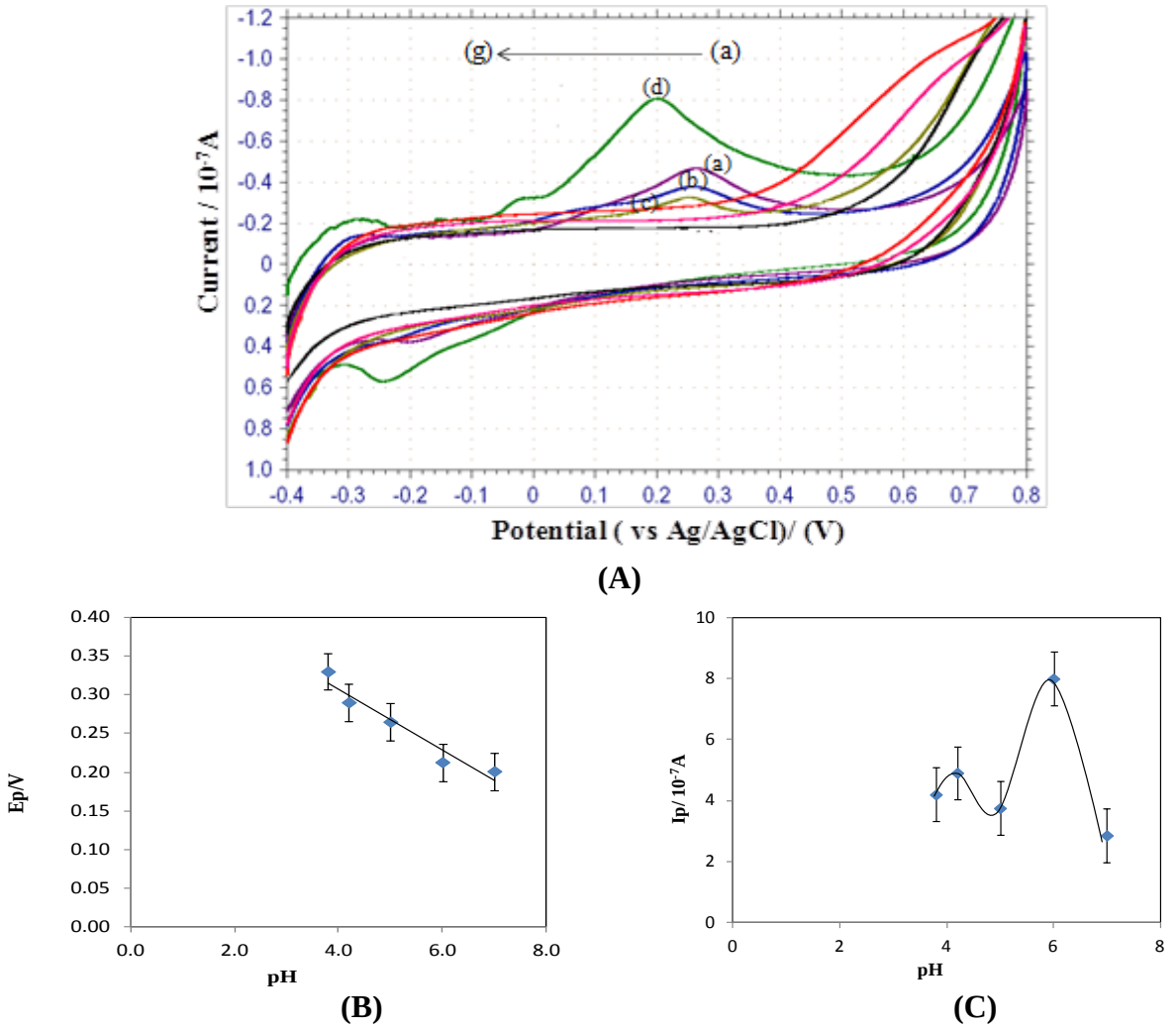


Fig. 4. (A) Influence of pH on the shape of the oxidation peak for 1 mM 2-TU in 0.2 M phosphate buffer solution at (a) pH 3.8, (b) pH 4.2, (c) pH 5.0, (d) pH 6.0, (e) pH 7.0,(f) pH 8.0 and (g) pH 9.2 at NSCPE, 50mV/s scan rate; **(B)** Variation of peak potential Ep (V) with pH for 1.0 mM 2-TU at NSCPE; **(C)** Variation of peak current of 1.0 mM 2-TU (at NSCPE) with pH

By using different supporting electrolytes such as Britton-Robinson buffer and phosphate buffer within the range of pH 3.8–9.2, in the phosphate buffer media NSCPE exhibited better voltammetric loops with enhanced peak current as compared to the Britton-Robinson supporting electrolyte. Also phosphate buffer is stable for several weeks compared to other buffers. Hence phosphate buffers were taken as a supporting electrolyte. The voltammetric response on pH is shown in Figure 4(A).

The plot of Ep versus pH shows that the peak potential is pH dependent, a linear relationship is obtained as shown in Figure 4(B). The peak potential shifted to less positive values with increase in the pH of the buffer solution. The plot of Ip versus pH shows that peak current is affected by the pH of the solution. However, the peak intensity increases to a high value at pH=6.0 and the peak intensity decreased linearly with the increase in pH of solutions as shown in Figure 4(C) suggesting pH 6.0 is optimal pH value. However, the peaks were not observed pH 8.0 onwards. Hence, the voltammetric response of 2-TU was restricted in the pH range from 3.8 to 7.0.

3.5. Influence of scan rate

The cyclic voltammograms of 1 mM 2-TU on the NSCPE at different scan rates are shown in Figure 5(A).The observation was made to investigate the kinetics of the electrode reaction. With the increase of the scan rate, the oxidation peak current also increased gradually, indicating the direct electron transfer between 2-TU and the modified electrode surface. As increasing the scan rate, the peak potential shifted to more positive values. Scan rate studies were carried out to assess whether the process on NSCPE was diffusion or adsorption controlled.

The relationship between logarithms of anodic peak current versus logarithm of scan rate (Figure 5(B)) was a straight line with a slope of 0.308, which was close to the theoretical value of 0.5 for purely diffusion–controlled process [24] and corresponding linear equation is as follows:

$$\log I_p\,(A) = 0.308 \log\upsilon\,(Vs^{-1}) + 0.3077,\ r=0.9517 \qquad (2)$$

The linear relationship between peak potential and logarithm of scan rate from Figure 5(C) can be expressed as,

$$Ep\,(V) = 0.08 \log\upsilon + 0.\ 1542,\quad r=0.9866 \qquad (3)$$

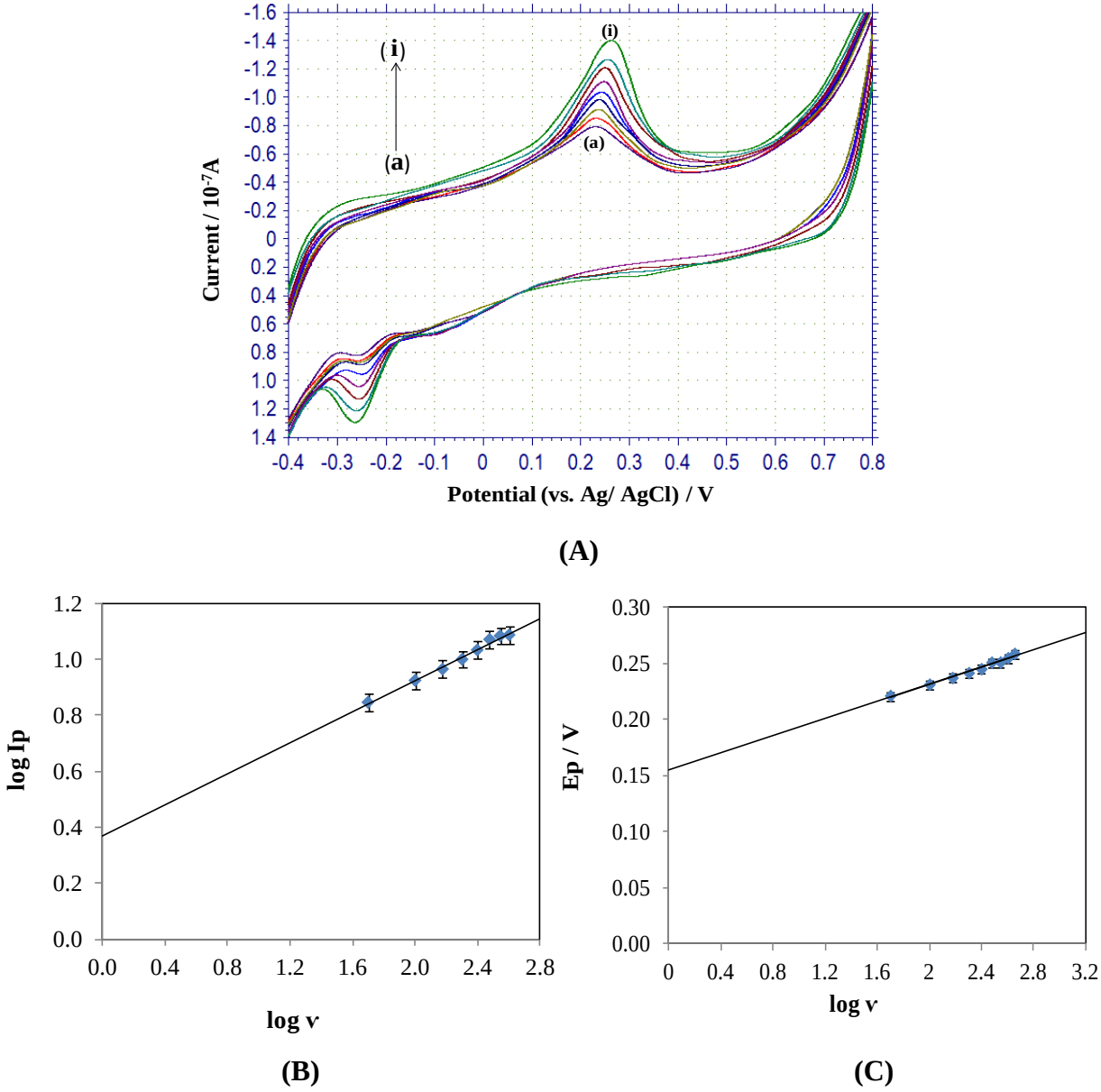


Fig. 5. (A) Cyclic voltammograms of 1.0 mM 2-TU in buffer solution of pH 6.0 at scan rates of (a) 50, (b) 100, (c) 150, (d) 200, (e) 250, (f) 300, (g) 350, (h) 400 and (i) 450 mV/s; **(B)** Relationship between logarithmic peak current and logarithmic scan rate; **(C)** Linear relationship between peak potential and logarithmic scan rate

This behavior was consistent with the electrochemical nature of the reaction in which the electrode reaction is coupled with an irreversible follow-up chemical step [25]. As for an irreversible electrode process, according to Laviron, Ep is defined by the equation (4):

$$E_P = E^0 + \left(\frac{2.303RT}{\alpha nF}\right) log \left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) logv \qquad (4)$$

Where α is the transfer coefficient, k^0 is the standard heterogeneous rate constant of the reaction, n is the number of electrons transferred, υ is the scan rate and E^0 is the formal redox potential. Other symbols have their usual meanings. Thus, the value of αn can be easily calculated from slope of Ep versus log υ plot (equation 3). In this system, the slope was found to be 0.082. Taking T=298K, R=8.314$JK^{-1}mol^{-1}$ and F=96485$Cmol^{-1}$, αn was calculated to be 0.7392.

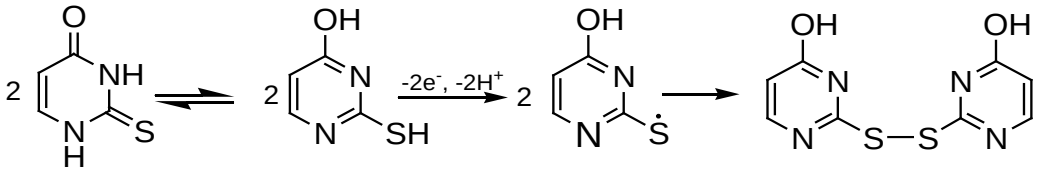
Again α was calculated using the Bard and Faulkner formula (equaion5) [26] in an irreversible electrode process.

$$\alpha = \frac{47.7}{E_{P-E_{P/2}}} mV \quad (5)$$

where $E_{p/2}$ is the potential where the current is at half the peak value. So, from this we got the value of α to be 0.354. Further, the number of electrons (n) transferred in electro-oxidation of 2-TU was calculated to be 2.254~2. 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 equation can be obtained from the intercept of Ep versus υ curve by extrapolating to the vertical axis at υ=0 [27] and E^0 obtained was 0.2217 V. In our system k^0 was calculated to be 2.878×10^3 s^{-1}.

3.6. Proposed mechanism and identification of product of electrolysis

Based on the voltammetric experiment the number of electrons transferred (n) was calculated to be 2.254~2. In most of the electrochemical oxidations of 2-TU, S–S linked dimer was obtained as the major product [28-29]. The electrolysis was carried out for 12 hrs for complete oxidation using 1.0 mM 2-TU and phosphate buffer as supporting electrolyte under hydrodynamic conditions in order to speed up the mass transport. Oxidized product was extracted from ether. Then the oxidation product was confirmed by (a) IR spectra (Nicolet model 6700 FT-IR spectrophotometer). The S-H stretching lies in range from 2600 to 2550 cm^{-1}; for 2-TU the S-H stretching absorbed at 2608 cm^{-1}. In the Figure 6, no absorption was observed in the S-H stretching vibration range. Usually the S-S stretching vibration is observed in the 500– 430 cm^{-1} range. The formation of the S-S bond is confirmed from the stretching absorption at 452 cm^{-1} in the IR spectra of the product (Figure 6). This indicates conversion of the S-H group of TU into the corresponding disulphide. (b)A UV-vis absorption study shows the decrease in the absorption peak after electrolysis due to the formation of product. (c) Iodine oxidizes 2-TU rapidly to form dimer, the composition of which has been established by Miller et al [30] and the dimer is 2-thiouracil disulfide. (d) According to Miller et al. [30], this dimer behaves as a strong acid and forms disodium salt which can be easily precipitated from the solution by acetone. The proposed mechanism is as shown in Scheme. 2.



Scheme 2. Proposed mechanism of oxidation of 2-thiouracil

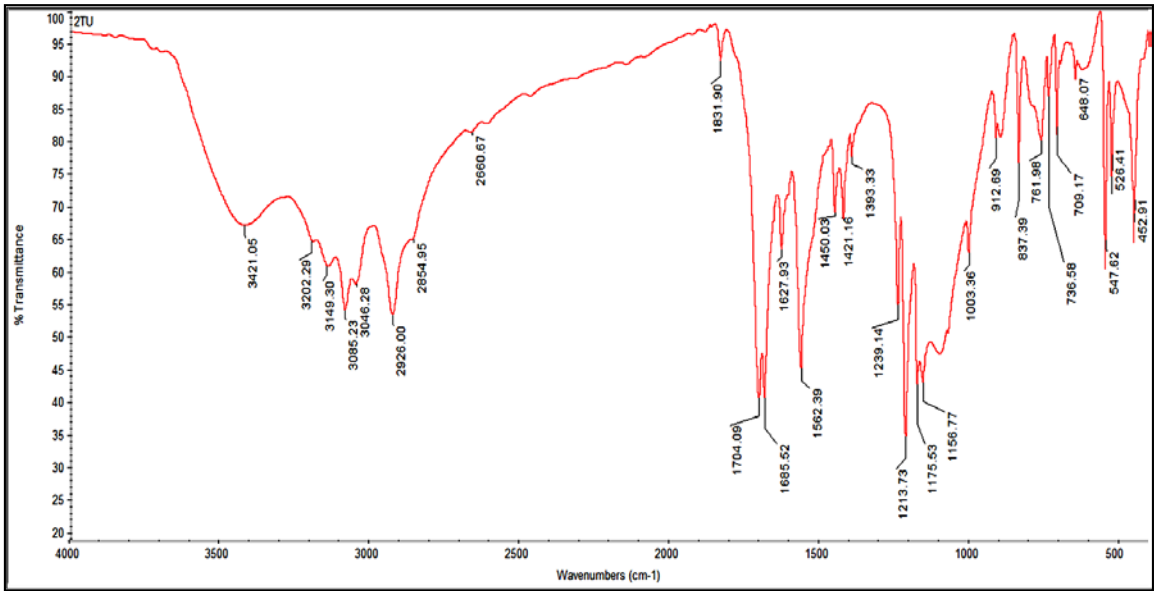


Fig. 6. FT-IR spectrum of the electrolyzed product

3.7. Analytical applications

In order to develop a rapid and sensitive voltammetric method for determining the 2-TU, the DPV and SWV techniques were employed, because the peaks were sharper and better defined at lower concentration of 2-TU than those obtained by CV, with low background current, resulting in improved resolution. From the obtained results, it was possible to apply these techniques to the quantitative trace analysis of 2-TU. The phosphate buffer solution of pH 6.0 was selected as the supporting electrolyte for the quantification of 2-TU as it gave maximum peak current at pH 6.0. Differential pulse and square wave voltammograms obtained with increasing amount of 2-TU showed that the peak current increased linearly with increasing concentration, as shown in Figure 7A, 7B and Figure 8A, 8B respectively. It was found that the plot of I_{pa} versus concentrations showed linearity over the drug concentration range of 10 to 100.0 10^{-8} M for DPV and SWV. Using the optimum conditions described above, the linear equations obtained were

$$I_p\ (\mu A) = 0.0084C\ (10^{-8}M) + 0.1927 \qquad (r = 0.9891) \quad (DPV) \qquad (6)$$

$$I_p\ (\mu A) = 0.0052C\ (10^{-8}M) + 0.3031 \qquad (r = 0.9977) \quad (SWV) \qquad (7)$$

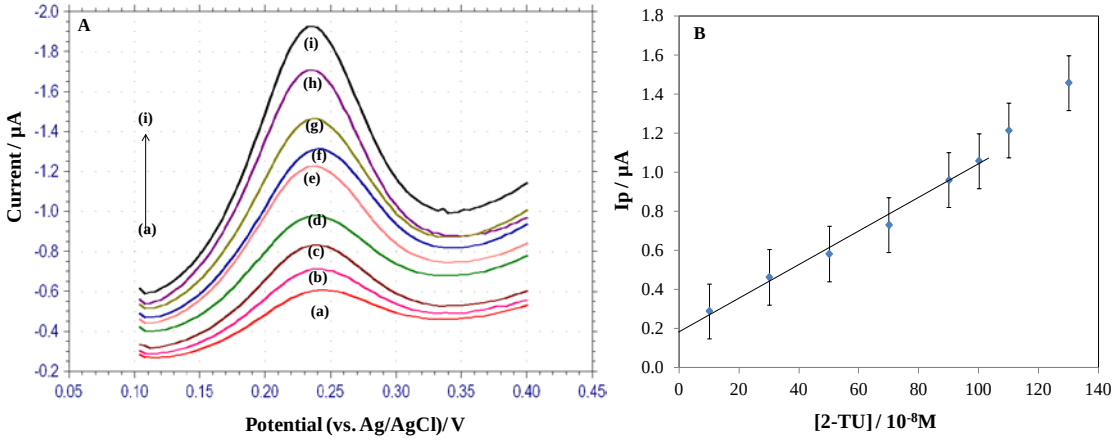


Fig. 7. (A) Differential pulse voltammograms at NSCPE at different concentration of 2-TU solution: (a) 10, (b) 30, (c) 50, (d) 70, (e) 90, (f) 100, (g) 110, (h) 130 and (i) 150×10^{-8}M; **(B)** Plot of peak current against the concentration of 2-TU

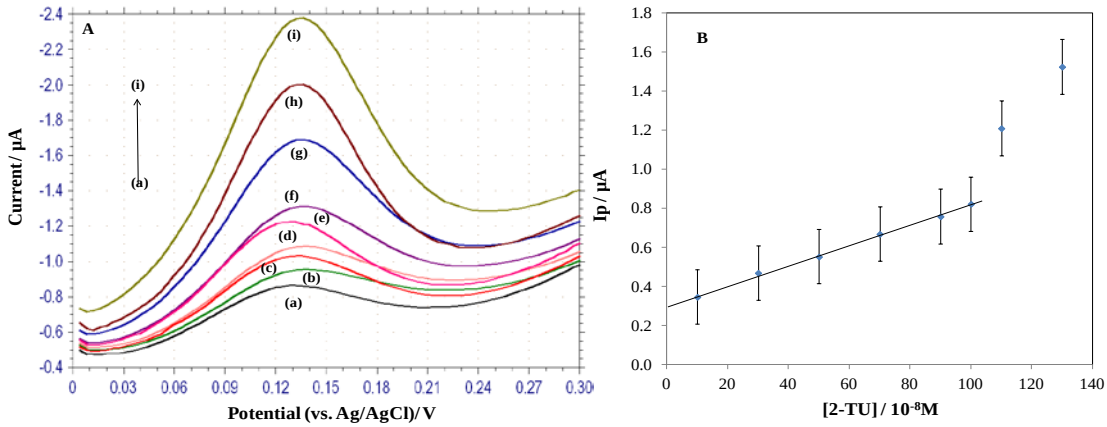


Fig. 8. (A) Square wave voltammograms at NSCPE at different concentration of 2-TU solution: (a) 10, (b) 30, (c) 50, (d) 70, (e) 90, (f) 100, (g) 110, (h) 130 and (i) 150x10^{-8} M; **(B)** Plot of peak current against the concentration of 2-TU

Deviation from linearity was observed for higher concentrated solutions, due to the adsorption of oxidation product on the electrode surface. The DPV presents a good linear response as compared to SWV; DPV was selected as suitable technique for further experimental procedures in view of less intercept of calibration graph (Figure 7B). Related statistical data of the calibration curve was obtained from five different observations. The limit of detection (LOD) and quantification (LOQ) for DPV were 2.1×10^{-9} M and 6.9×10^{-9} M respectively. The LOD and LOQ were calculated using the following equations:

$$LOD=3s/m, \quad LOQ=10s/m \tag{8}$$

Where, *s* is the standard deviation of the peak currents of the blank (five runs) and *m* is the slope of the calibration curve [31]. Comparison of earlier methods with present method, showed the present method was better for the determination of 2-TU in view of low LOD (Table 1).

Table 1. Comparison of some methods for the determination of 2-TU with the proposed method

Analytical method	Linearity range(mM)	Detection limit(nM)	Ref.
Liquid Chromatography	(0.5 - 4.0)	(429)	[20]
Carbon-Paste Electrode Modified with CoPc	(0.7 - 800)	(4000)	[32]
Glassy Carbon Electrode	(0.001–0.020)	(160)	[33]
Carbon-Paste Electrode Modified with $NiSO_4$	(0.0001-0.001)	(2.1)	This work

3.8. Stability and reproducibility

In order to depict the stability and reproducibility of the electrode, a 10 μM 2-thiouracil solution were measured with the same electrode (renewed every time) for every several hours within day; the RSD of the peak current was 0.72% (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 constant which could be attributed to the excellent stability and reproducibility of NSCPE.

3.8.1. Selectivity of NSCPE

2-TU bearing chemically active group –SH, a weak acid with pKa value of 9.84 [34], can be deprotonated by transition metal complexes ($NiSO_4$. $6H_2O$). Hence, NSCPE has affinity for the -SH proton of 2-TU. Thus, NSCPE can be electro catalyzed for the redox behaviour of 2-TU.

3.9. Effect of interferents

2-TU was formulated in single as well as multi-component tablets. The oxidation peaks of interferents should not appear where the peak corresponds to 2-TU oxidation peak. So in

order to investigate the effect of co-formulated substances such as glucose, starch, sucrose etc., on the voltammetric response of 2-TU, was carried out. Differential- pulse voltammetric experiments were carried out for 1.0 µM 2-TU in the presence of 1.0 mM of each of the interferents. The results are listed in Table 2. It was observed that 1000 folds of citric acid, dextrose, D-glucose, gum acacia, oxalic acid, starch and sucrose did not interfere. Therefore, the proposed method can be used as a selective method.

Table 2. Influence of potential interferents on the voltammetric response of 1.0 µM 2-TU

Interferents	Concentration(mM)	Signal Change (%)
Citric acid	1.019	0.18
Dextrose	1.002	0.93
D-Glucose	1.0650	-4.37
Gum acacia	0.9860	3.97
Oxalic acid	1.013	2.56
Starch	1.018	2.51
Sucrose	1.062	-3.06

3.10. Tablet analysis and recovery test

In order to evaluate the applicability of the proposed method, the commercial medicinal sample containing 2-thiouracil from Propylthiouracil®**,** India, was used. The procedure for sample preparation is described in sample preparation section. Differential pulse voltammograms were then recorded under exactly similar conditions that were employed for plotting calibration plot.

Table 3. Results of the assay and the recovery test of 2-TU in pharmaceutical preparations using differential pulse voltammetry

	Propylthiouracil tablet
Labeled claim (mg)	500.0
Amount found (mg)[(a)]	492.0
RSD (%)	0.65
Added (mg)	5.00
Found (mg)[(a)]	4.92
Recovered (%)	98.4
RSD (%)	0.38
Calculated F	1.24
Calculated t	2.16

(a)Mean of five determinations

It was found that 2-TU concentrations determined using this method is in good agreement with the reported values (Table 3). The F and Student t-tests were carried out on the data using calibration curve and are given in the Table 3.

3.11. Determination of 2-thiouracil in urine samples

The applicability of the DPV to the determination of 2-TU in spiked urine was also investigated. The recoveries from urine were measured by spiking drug free urine with known amounts of 2 -TU. The urine samples were diluted 100 times with the phosphate buffer solution before analysis without further pre-treatment. A quantitative determination can be carried out by adding the standard solution of 2-TU into the detect system of urine sample. The calibration graph was used for the determination of spiked 2-TU in urine samples. The results of four urine samples were obtained and listed in Table 4. The recovery determined was in the range from 98.26% to 99.67% and the R.S.D. was 0.34%. Thus, satisfactory recoveries of the analyte from the real samples and a good agreement between spiked and detected concentrations in the urine samples when treated with the drug, make the developed method applicable in clinical analysis.

Table 4. Determination of 2-TU in urine samples

Urine	Spiked(μM)	Detected[(a)] (μM)	Recovery (%)	SD (μM) ±RSD (%)
Sample 1	2	1.9805	99.02	0.0118±0.596
Sample 2	4	3.9792	99.48	0.0126±0.317
Sample 3	6	5.9802	99.67	0.0148±0.248
Sample 4	8	7.8610	98.26	0.0136±0.173

(a)Mean of five determinations

4. CONCLUSION

Present study has indicated that the NSCPE exhibits good electro catalytic activity towards 2-TU oxidation. The electrochemical process undergoes 2 electrons–2 protons change and is diffusion- controlled. Under the optimal conditions, there was a good linear relationship between peak currents and concentrations of 2-TU which helps in voltammetric determination of selected analyte as low as 1.0×10^{-7} M and can be used successfully to assay the drug in pharmaceuticals and urine samples. High percentage of recovery showed that the method is free from interferences commonly used excipients in the formulations of drug. This method is suitable for quality control laboratories as well as pharmacokinetic studies with satisfactory results.

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