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Electrochemical Behavior of 2-Aminothiazole at Poly Glycine Modified Pencil Graphite Electrode

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Abstract- Electro analysis of 2-Aminothiazole (2-AT) by a low cost poly Glycine modified Pencil Graphite Electrode (poly Gly/PGE) was studied using of cyclic voltammetric (CV) and differential pulse voltammetric (DPV) techniques. The optimal experimental conditions to determine 2-AT was setup by the variation of the current with scan rate, concentration and pH. Electrochemical performance of the 2-AT at poly Gly/PGE was found adsorption-controlled reaction. A plausible electro oxidation mechanism was proposed. The anodic peak current of DPV was found to be linearly proportional to the 2-AT concentrations in the range from 1.0×10^{-6} to 12.0×10^{-6} M with a detection limit 7.29×10^{-8} M with better selectivity and sensitivity under the optimal conditions. Quantitative analysis of 2-AT levels in urine as real sample was carried out by applying this proposed method. Moreover, some common interfering substances were used to the study of interference. The clinical and quality control laboratories can employ this method to determine 2-AT.

Keywords- 2-Aminothiazole, Voltammetric, Glycine, Pencil Graphite Electrode, Oxidation, Polymerization

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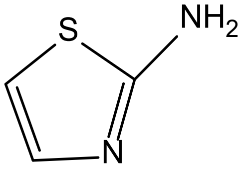
1. INTRODUCTION

A water soluble heterocyclic 2-Aminothiazole (2-AT) is a thaizole core, the structure of which is mentioned in Scheme 1. Biocides, Fungicides, Sulfur drugs, dyes were synthesized by using 2-AT as a starting material. Large numbers of 2-AT derivatives are used in pharmaceutical industries [1-3]. 2-AT is also used in the synthesis of different types of dyes for the production of synthetic fibers [4,5]. 2-AT has antibacterial characteristic property, in the treatment of hyperthyroidism. 2-AT is used as a thyroid inhibitor. Prion diseases were cured by therapeutic drug, 2-aminothiazole [6]. The derivatives of 2-AT were used for biological activities due to the use of them in biological systems; these compounds exhibited antimicrobial [7-9], antifungal, anti-inflammatory [10], anesthetic [11], antiviral [3], anti-leukemic [12], antiproliferative and [13] activities. Due to enormous industrial applications and biological importance of 2-AT, it is very important to analyze 2-AT in real and pharmaceutical samples.



Scheme 1. Structure of 2-Aminothiazole

In analytical studies the qualities such as easy to perform, cost-effective, responsive, fast, reproducible and consistent, are crucial for the determination of drug's ingredients, as quality control and medical laboratories are in search of such a versatile technique. Among various techniques, electrochemical method is an adaptable and sensitive analytical technique that provides better selectivity, precision, reproducible, reliability, wider range of linearity, accuracy and as well as relatively low-cost instrumentation. The electrochemical analytical applications are more often used in the field of industry [14], ecology [15] and moreover in the medicinal chemical study [16] in their prescription forms and particularly in bio-organic liquids.

In view of electrochemical and economical advantage of pencil graphite electrodes (PGEs), it has attracted the applicability to the analysis of various compounds in both organic and inorganic substances. Pencil graphite leads are of low cost and commercially available in market. Apart from low cost, it is easy to use, more convenient and sensitive, yields well-defined voltammetric peak and reproducible signals, saves the time by disposal of pencil graphite electrode instead of cleaning or washing the electrode. A wide range of samples can be studied by applying different voltammetric techniques, such as square wave voltammetry (SWV), linear wave voltammetry (LWV), differential pulse voltammetry (DPV) etc. The review and history of the applications of PGEs in the analysis of environmental samples is recently reported [17]. The uses of unmodified and modified pencil graphite electrodes for electrochemical oxidation were reported [18,19] from our research group.

The modified electrodes with polymer have attracted their use due to their greater sensitivity, reproducibility and stability character. Electro-polymerization is a very good access to immobilize polymers to modify the electrochemical specifications which can manage film thickness property, stability and transfer of electrons [20]. The poly Glycine (Gly) modified carbon paste electrodes [21,22] have attracted more attention in recent years. In the present study, the pencil graphite electrode has been modified by poly Glycine, because of its low cost component which exhibited various excellent electrochemical characteristic properties and very good electrochemical sensitivity and stability. Such remarkable properties displayed the poly Glycine modified pencil graphite electrode (poly Gly/PGE) to provide better reproducibility.

There is no report found in the literature survey particularly on electrochemical oxidation of 2-AT at poly glycine modified pencil graphite electrode (poly Gly/PGE) and the current experimental observations represented for the first time in the voltammetric study of 2-AT at poly Gly/PGE with different techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The literature survey revealed that there is no report related to analytical work except on oxidation of 2-Aminothiazole using bare pyrolytic graphite plate [23] describing only analysis of product, and not studied the effect of scan rate, response of electrode at wider range of 2-AT concentration, effect of interfering chemicals and application of method in real samples. The modified electrode (poly Gly/PGE) showed a very good enhancing catalytical property for the oxidation of 2-AT in micro quantity. The proposed method is highly selective with lower limit of detection, low cost, high sensitivity and reproducibility for the determination of 2-AT. This method was used in the determination of 2-AT in urine as a real sample.

2. EXPERIMENTAL

2.1. Reagents and chemicals

2-AT was purchased from SPECTROCHEM PVT. LTD. MUMBAI (India) and other analytical grade chemicals were used. 1.0 mM 2-AT stock solution was prepared with double distilled water. The 0.04 M Glycine solution was prepared in 5 pH phosphate buffer solution for formation (electro-polymerization) of poly Glycine on the bare pencil graphite electrode (PGE). Phosphate buffer solutions (PBS) of concentration 0.20 M ranging from pH 3.0-8.0 were prepared in double distilled water [24]. The PGEs were Cello Fine lead, Cello Writing Aids Pvt. Ltd. 0.25 mm in radius and 60 mm in length.

2.2. Preparation of poly Gly/PGE

The modified electrode was prepared by running the potential scan in the midst of -0.5 V to 1.8 V in pH 5.0 PBS (0.2 M) containing 0.04 M Glycine for 3 cycles at scan rate of 0.10 Vs^{-1} [21]. Thus formed poly Glycine film was washed with double distilled water several times and utilized for the analysis of 2-AT. The formation of poly glycine on pencil graphite electrode is already reported in literature [25].

2.3. Instrumentation

The cyclic and differential pulse voltammetric curves at poly Gly/PGE was recorded using CHI630D Electrochemical Analyzer (CH Instruments Inc., USA). A system of three electrode cell consisting of a platinum (Pt) wire, the Ag/AgCl /saturated KCl solution and poly Gly/PGE as the auxiliary, reference and working electrode respectively, was used in a 10-ml single compartment to carry out the voltammetric measurements. To measure the pH values of the solutions, an Elico LI120 pH meter (Elico Ltd., India) was used.

2.4. Voltammetric measurements

Fig. 1 shows cyclic voltammogram of 0.04 M Glycine in 0.20 M PBS of pH 5.0 at pencil graphite electrode. Electropolymerisation [21] was achieved by the formation of film that grew between -500 mV and 1800 mV at a scan rate of 100 mV/s for three cycles using cyclic voltammetry as shown in Fig. 1. After polymerization, the poly Gly/PGE was sufficiently rinsed with double distilled water and utilized for quantitative analysis of 2-AT.

The modified electrode (poly Gly/PGE) was dipped in phosphate buffer solutions (PBS) of 0.20 M concentration with pH 5.0 containing a known quantity of 2-AT, in a system of three-electrode. Then, cyclic and DPV voltammograms were achieved. The general criteria for CV were at 0.10 Vs^{-1} scan rate. The specifications for DPV were starting potential E_{pa}: 400 mV; ending potential E_{pa}: 1200 mV; pulse period 0.5 **s**; quiet interval 2 **s**; sensitiveness: 1.0×10^{-4} AV^{-1}.

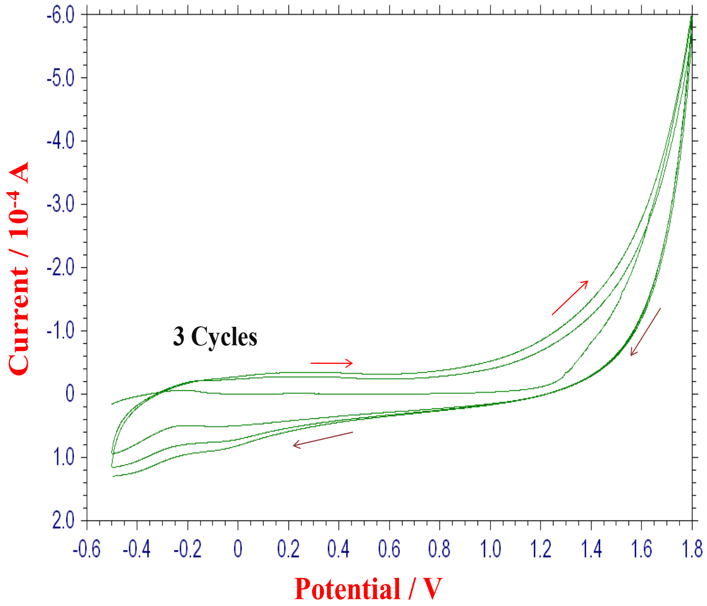


Fig. 1. Cyclic voltammogram of electropolymerisation of glycine on pencil graphite electrode at scan rate of 100 mVs^{-1}

2.5. Optimization of number of cycles

The response of modified electrode (poly Gly/PGE) was observed to be increased with the increase in the number of cycles until 3 cycles, later on decreased (Fig. 2). The highest peak current for the oxidation of 2-AT was observed by applying 3 cycled electro-polymerized PGE. Hence the standard number of cycles was 3 and selected for electro-polymerization.

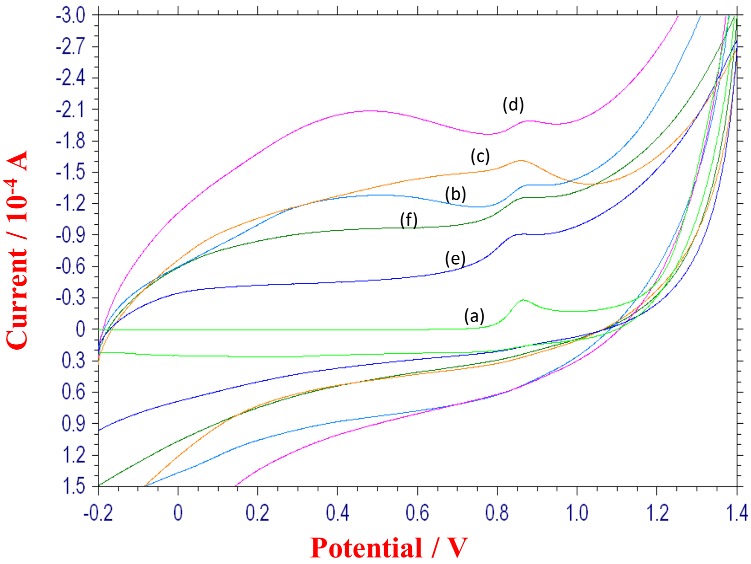


Fig. 2. Cyclic voltammogram of 0.1 mM 2-AT at poly Gly/PGE with different number of scans in 0.04 M Glycine in PBS (pH 5.0) at the scan rate 100 mV s^{-1}, (a) 0 (bare), (b) 1, (c) 2, (d) 3, (e) 4 and (f) 5 cycles.

2.6. Electrochemical response of $K_3[Fe(CN)_6]$ at poly Gly/PGE

Electrochemical characteristics of poly Gly/PGE (Fig. 3) was investigated by using coordinate complex ($K_3[Fe(CN)_6]$) as the electrochemical redox probe. The modified electrode (poly Gly/PGE) observed the increased redox current peak (curve b) in comparison with the bare PGE (curve a). The cyclic voltammogram of 1 mM $K_3[Fe(CN)_6]$ at poly Gly/PGE (curve b) showed the oxidation peak potential at 306 mV and reduction peak potential at 183 mV in 0.1 M KCl solution with 100 mVs^{-1} scan rate. The cyclic voltammogram of 1mM $K_3[Fe(CN)_6]$ at bare PGE observed the oxidation peak potential at 321 mV and reduction peak potential at 183 mV in 0.1 M KCl solution with 0.10 Vs^{-1} scan rate. The anodic peak currents of 1mM $K_3[Fe(CN)_6]$ were found -6.306×10^{-5} A and -2.732×10^{-5} A (curve b and a) for poly Gly/PGE and bare PGE respectively. The shift in peak potential (15 mV) and peak current (3.574×10^{-5} A) showed excellent catalytic activity of poly Gly/PGE.

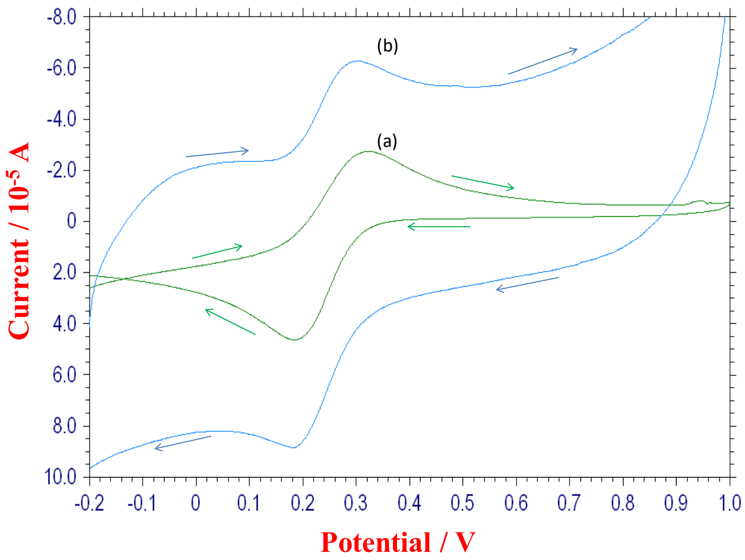


Fig. 3. Cyclic voltammograms of (a) bare PGE and (b) poly Gly/PGE in 1.0 mM $K_3[Fe(CN)_6]$ in 0.1 M KCl solution. Scan rate100 mVs^{-1}

2.7. Surface areas of electrodes

The 1.0 mM K_3 $[Fe(CN)_6]$ solution used as a probe at different scan rates was utilized to determine the electrode surface area, using the Randles-Sevcik formula (eq. 1) [25]:

$$i_{pa} = (2.69x10^5)\ n^{3/2}\ A_o\ D_o^{1/2}\ C_o\ \upsilon^{1/2} \qquad (1)$$

where 'i_{pa}' assigns the oxidation peak current, 'A_o' is the surface area of electrode, 'n' is the no. of electrons involved, 'D_o' is the diffusion coefficient, 'υ' is the rate of scan, and 'C_o' is the concentration of potassium ferricyanide. For 1.0×10^{-3} M $K_3[Fe(CN)_6]$ in 0.1 M KCl electrolyte, $D_o=7.6\times10^{-6}$ cm^2s^{-1} and n=1. The plot of 'i_{pa}' as a function of '$\upsilon^{1/2}$' produced slope from which the surface area of the electrode was determined. The surface areas were found to be 0.116 cm^2 and 0.018 cm^2 for ploy Gly/PGE and for PGE respectively.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetric response of 2-Aminothiazole

The cyclic voltammetric response of 2-AT at poly Gly/PGE electrode was studied in supporting electrolyte 0.20 M PBS of pH 5.0 (Fig. 4), which showed the electro-catalytic result of poly Gly/PGE. The poly Gly/PGE showed no electrochemical action in blank supporting electrolyte buffer solution (Fig. 4 curve a), where as in Fig. 4 (curve b), (curve c) point out the cyclic voltammetric report of 0.10 mM 2-AT at bare PGE and poly Gly/PGE respectively.

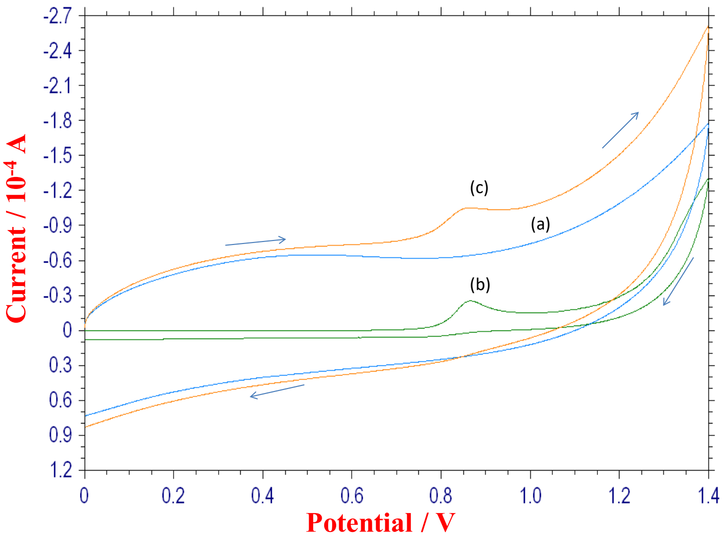


Fig. 4. (a) Cyclic voltammograms of poly Gly/PGE in 0.2 M PBS (pH 5.0) only. Cyclic voltammograms of 0.1 mM 2-AT in pH 5.0 PBS at (b) bare PGE and (c) Poly Gly/PGE. Scan rate of 100 mVs^{-1}

At the 0.8684 V, a well-resolved and intense peak with a strong increased peak current (1.051×10^{-4} A) was observed for poly Gly/PGE (Fig. 4 (curve c)) in the range of potential from 0.0 V to 1.4 V. In this range, at potential 0.8667 V, a less intense oxidation peak current (2.512×10^{-5} A), was observed for bare PGE (Fig. 4(Curve b)). The oxidation peak potential of poly Gly/PGE moved to lower potential by 0.0017 V, and remarkable increase in the peak current by 4.183×10^{-4} A for poly Gly/PGE certainly testify the electro catalytic performance of the poly Gly/PGE. This specified the improved sensitivity on its remarkable structure and properties such as larger area of surface, subtle electronic property [26]. In the reverse scan, no cathodic peak was found, indicating irreversible electrode reaction in the oxidation of 2-AT [27].

3.2. Effect of pH

The electrochemical behavior of 2-AT with various pH at poly Gly/PGE was investigated using phosphate buffer solutions, in pH range 3.0 to 8.0 (Fig. 5A). For 2-AT, as pH increased the peak potentials shifted towards negative potentials. This remarkable change affirmed that the supporting electrolyte's pH indicated a compelling control on the electro oxidation of 2-AT at the poly Gly/PGE. The graph of anodic peak potential versus pH was plotted (Fig. 5B) and a linear equation yielded (Eq. 2):

$$E_{pa}\,(V) = 1.172 - 0.061\ pH \quad (r^2 = 0.973) \qquad (2)$$

The slope 61 mV pH^{-1} was obtained from Fig. 5B which is the plot of the E_{pa} *vs.* pH. This was very close to the standard value of 60 mV pH^{-1}. This indicated equal number, i.e 3 electrons and 3 protons transfer [28] that involved in the electrode reaction. In order to select suitable pH for the electro analysis of 2-AT, pH 5.0 gave the best response in view of the highest peak current and peak shape (Fig. 5C). The pH 5.0 was used to carry out further experimental determinations of 2-AT.

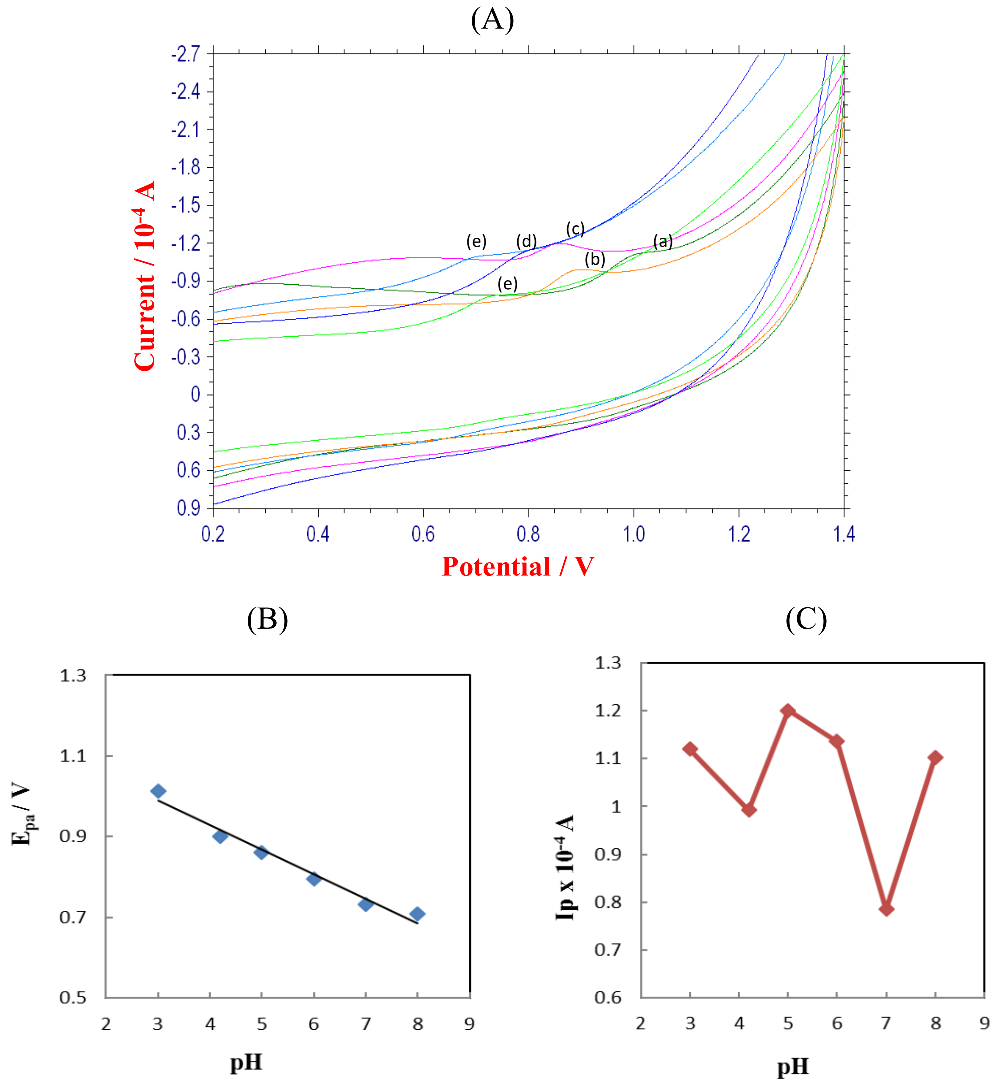


Fig. 5. (A) Influence of pH on the shape of the anodic peak current at pH: (a) 3.0; (b) 4.2; (c) 5.0; (d) 6.0; (e) 7.0; (f) 8.0, with scan rate 100 mVs^{-1}; (B) The plot of peak potential E_{pa} (V) *vs.* pH; (C) The plot of peak current I_{pa} (10^{-4}A) *vs.* pH.

3.3. Effect of scan rate

The electrochemical behavior of 2-AT at modified electrode (Poly Gly/PGE) at different scan rates was studied using cyclic voltammetry. The superimposed voltammograms (Fig. 6A) were obtained for varying scan rate from 0.05 to 0.45 Vs^{-1}. It was observed that as the scan rate increased, the peak current (I_{pa}) for oxidation of 2-AT also increased. An expression of linear regression equation was given as below (Fig. 6B).

$I_p(10^{-5}\ A)=57.21\ v^{1/2}\ (V^{1/2}s^{-1/2}) - 9.536\ (r^2=0.971)$

A straight line with slope 0.807 was obtained from the logarithm of anodic peak current versus logarithm of scan rate (Fig. 6C), which indicates that slope is nearly equal to theoretical value of 1.0. Hence, it was predicted the reaction as adsorption controlled process [29]. The Eq. 3 was obtained from Fig. 6C.

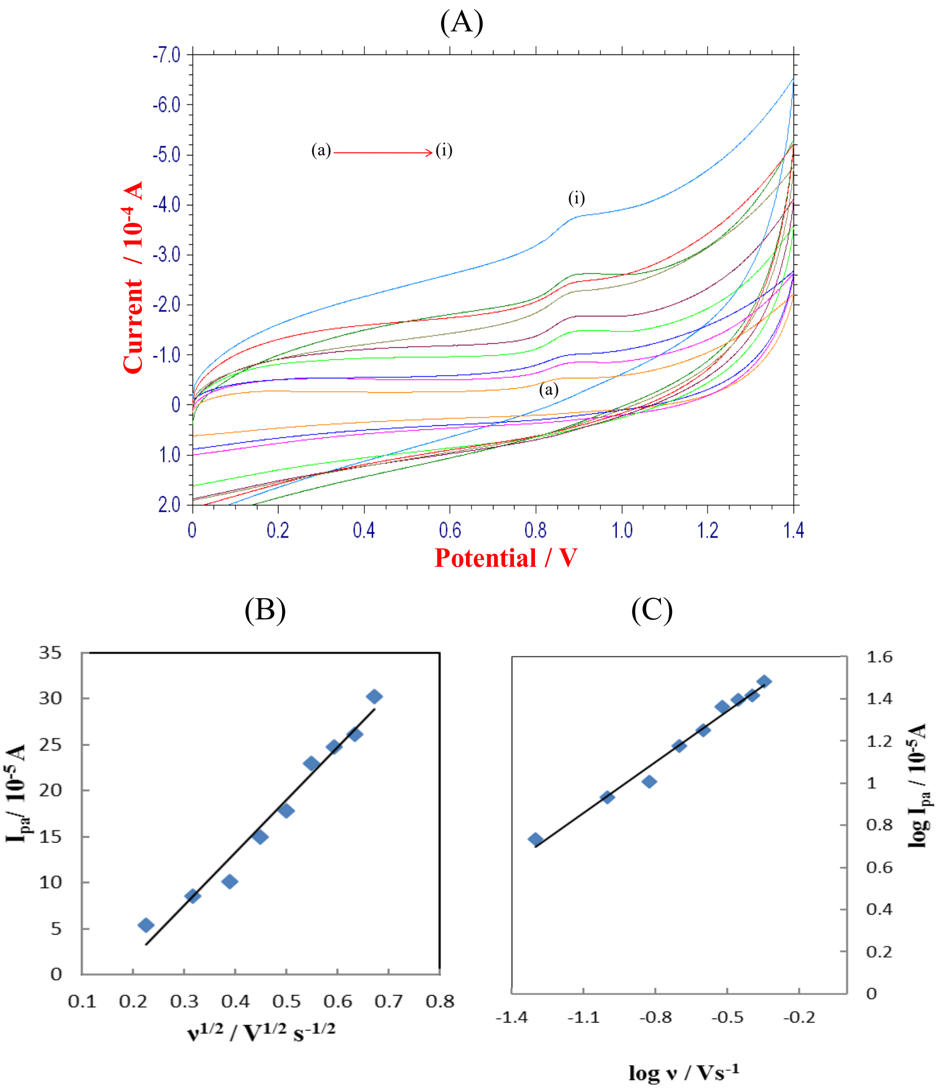


Fig. 6. (A) Cyclic voltammograms obtained for 0.1 mM 2-AT in 0.2 MPBs of pH 5.0 at scan rates of (a) 0.05, (b) 0.1, (c) 0.15, (d) 0.2, (e) 0.25, (f) 0.3, (g) 0.35, (h) 0.4 and (i) 0.45 Vs^{-1}; (B) The plot of I_{pa} *vs.* $v^{1/2}$; (C) The plot of log I_{pa} *vs.* log v

$$\log I_{pa}\ (10^{-5}\ A)=0.807\ \log v\ (V\ s^{-1})+1.746,\ (r^2=0.982) \qquad (3)$$

Further, increasing the scan rate (v), the anodic peak potential E_{pa} at modified electrode, was shifted positively. It was also observed that peak potential increased linearly with increase in logarithm of scan rate as shown in the Fig. 7A. This indicated an irreversible electrochemical reaction. The equation obtained was:

$$E_{pa}\ (\mathrm{V}) = 0.039 \log \nu\ (\mathrm{V\ s^{-1}}) + 0.948, \quad (r^2 = 0.948) \tag{4}$$

According to the Laviron equation (5) [30], E_{pa} is expressed for an irreversible electrode process as:

$$E_{pa} = E^0 + \left(\frac{2.303RT}{\alpha nF}\right)\log\left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right)\log \nu \tag{5}$$

where, 'n' The no. of electrons involved in oxidation of 2-AT process, 'α' is the transfer coefficient, '*v*' is the scan rate, 'k^0' is rate constant of the heterogeneous reaction and 'E^0' is the formal standard redox potential. Additional symbols have their normal meaning. Thus from the slope of the graph E_{pa} versus log *v* (Fig. 7A), αn was calculated as 1.516 by taking T=298 K, R=8.314 $JK^{-1}mol^{-1}$, and F=96480 C mol^{-1}. The coefficient of transfer 'α' value is 0.5 for an irreversible electrode reactions [22], the value of 'n' was found to 3.03≈3 by knowing the 'αn' and 'α' values. From the intercept of Fig. 7A and knowing the 'E^0' value, k^0 value was determined as 1.0×10^3 s^{-1}. From the intercept of the graph of peak potential versus scan rate (Fig. 7B) 'E^0' value was obtained, by extrapolating to *v*=0 [31].

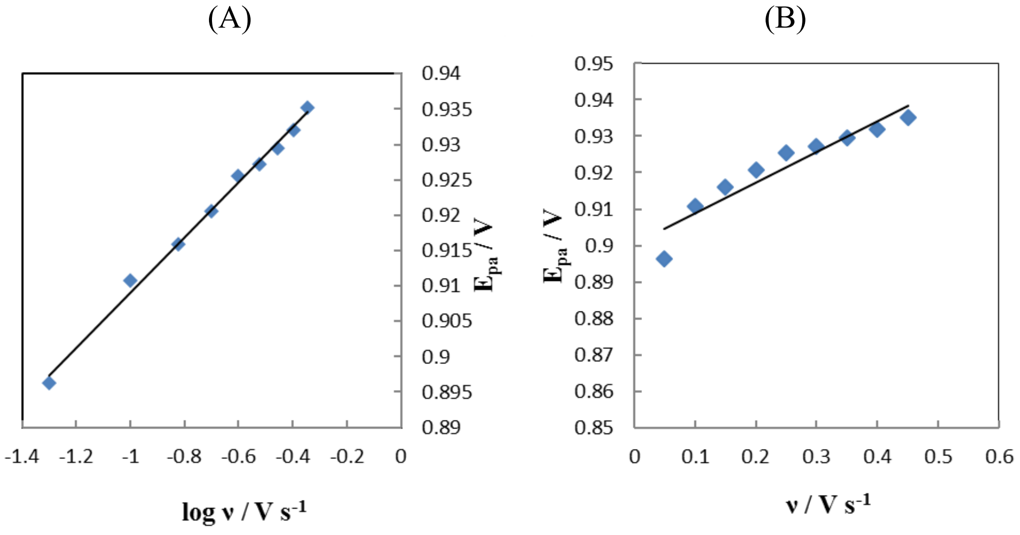
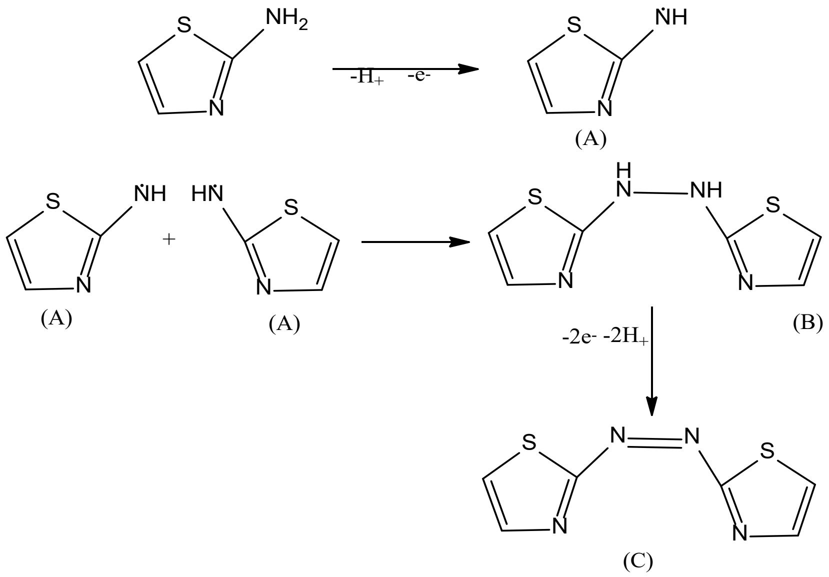


Fig. 7. (A) The plot of E_{pa} *vs.* log v; (B) The plot of E_{pa} *vs.* v

Based on cyclic voltammetric experiment, transfer of equal number of electrons and protons was observed. The following mechanism was proposed as shown in Scheme 2. The 2-AT free radical (A) was formed on electrochemical oxidation of 2-AT with a loss of 1 proton and 1 electron. This free radical combined with one more free radical to from hydrazo compound (B). The hydrazo group of (B) readily lost 2 electrons and 2 protons by electro oxidation to form the product, 1,2-di(thiazol-2-yl)diazene (C). This observation was in accordance with earlier report [24].



Scheme 2. Electro-oxidation of 2-AT at poly Gly/PGE electrode

3.4. Calibration curve and detection limit

To develop a rapid and sensitive voltammetric method for determining the 2-AT, the sharper and well defined peaks were found in the DPV technique at lower concentrations of 2-AT than CV. Hence DPV technique has been employed for quantitative analysis of 2-AT.

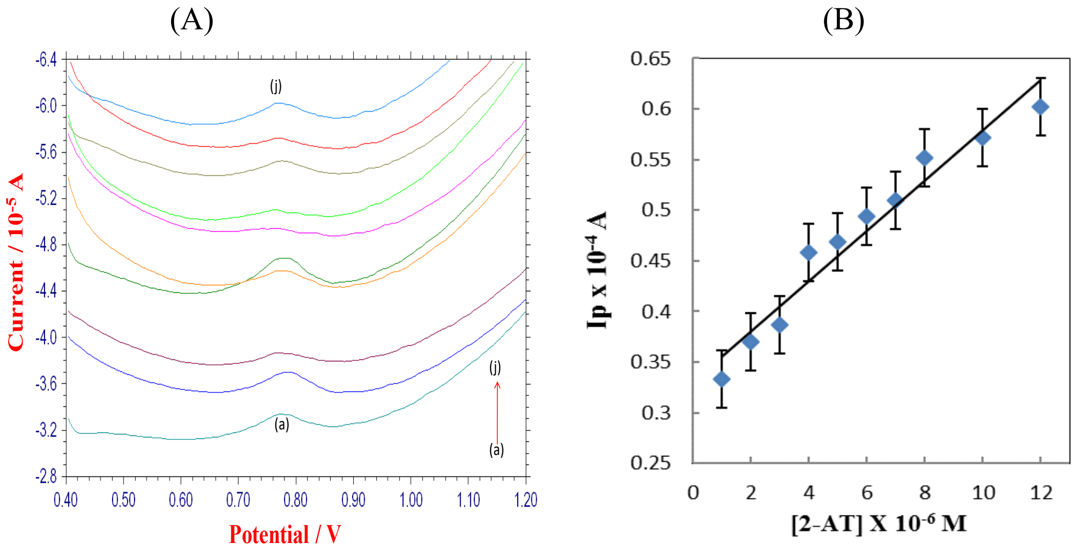


Fig. 8. (A) Differential Pulse Voltammograms of 2-AT with different concentrations of (a) 1.0, (b) 2.0, (c) 3.0, (d) 4.0, (e) 5.0, (f) 6.0, (g) 7.0, (h) 8.0, (i) 10.0, (j) 12.0/10^{-6} M in 0.2 M phosphate buffer solution at poly Gly/PGE; (B) Plot of peak current versus concentration of 2-AT

The pH 5.0 PBS (0.20 M) was taken as supporting electrolyte for the determination of 2-AT, since highest peak current was observed at pH 5.0. The Fig 8A, showed differential pulse voltammograms at different concentrations, as concentration of 2-AT increased anodic peak current also increased linearly. In the concentration range from 1.0×10^{-6} to 1.2×10^{-5} M, a linear calibration plot was observed for DPV Fig. 8B under setup condition described. The linear equation (Eq. 6) was

$$I_{pa}\,(10^{-4}\text{ A}) = 0.0248C\,(10^{-6}\text{ M}) + 0.330 \quad (r^2 = 0.952) \tag{6}$$

For more concentrated solutions, deviation from linearity was observed; at electrode surface, adsorption of 2-AT or its oxidation products was resulted. The average of five different determinants for each calibration curve was followed. The limit of detection (LOD) 7.29×10^{-8} M and limit of quantification (LOQ) 2.43×10^{-7} M were obtained. The Eq. (7) was used to calculate the LOD and LOQ.

$$\text{LOD} = \frac{3s}{m} \qquad \text{and} \qquad \text{LOQ} = \frac{10s}{m} \tag{7}$$

where‘**s**’ represented standard deviation of the peak current of the blank (five runs) and ‘**m**’ represented slope of the calibration curve.

3.5. Reproducibility and selectivity

The poly Gly/PGE within a day and inter-day were tested in 0.10 mM 2-AT solution. Relatively same current was produced. The relative standard deviation (RSD) was 2.65%, indicating that this method allows good reproducibility. Sample solutions recorded after ‘48 h’ did not show any appreciable change in the assay values.

3.6. Effect of interferents

The different interfering molecules with 2-AT were used to study the selectivity of modified electrode (poly Gly/PGE). The voltammetric behavior of poly Gly/PGE was studied in the presence of citric acid, lactose, glucose, oxalic acid, tartaric acid, maleic acid, sucrose, KCl, NaCl and $MgSO_4$ (Hundred fold excess) as interfering substance. The differential pulse voltammograms were obtained for 2-AT (µM) oxidation after the addition of different concentration of each interferent. The obtained potential was found 0.7759 V in the absence of any interferent with 2-AT [32] for poly Gly/PGE. The results obtained are shown in Table 1. There was no serious interference in the presence of foreign compounds on the electro oxidation of 2-AT (Table 1). So, 2-Aminothiazole need not be removed from these capsule supplements prior to its analysis in the tablets; it seemed to be unique.

Table 1. Influence of potential interference on the voltammetric response of 2-AT (µM)

Interference	Peak potential(V)	Signal change [a] (%)
2-AT	0.7759	-
Citric acid + 2-AT	0.8002	3.036741
Lactose + 2-AT	0.7723	-0.46614
Glucose + 2-AT	0.7685	-0.96291
Oxalic acid + 2-AT	0.7731	-0.36218
Tartaric acid + 2-AT	0.7881	1.548027
Maleic acid + 2-AT	0.7918	2.008083
Sucrose + 2-AT	0.7713	-0.5964
KCl + 2-AT	0.7723	-0.46614
NaCl + 2-AT	0.7825	0.84345
$MgSO_4$ + 2-AT	0.7881	1.548027

[a] Average of five replicates

3.8. Real sample analysis

The appropriateness of proposed method was studied by quantitative determination of 2-AT in spiked urine samples using differential pulse voltammetric technique. The urine samples were diluted 100 times with PBS pH 5.0 as supporting electrolyte. The urine samples were investigated with the same concentration as in the calibration. The calibration graph was used in the analysis of spiked 2-AT in the urine samples with recovery ranges 92.0 to 99.6%. The Table 2 shows the results of three urine samples investigated.

Table 2. Determination of 2-AT in urine samples

Urine	Spiked (µM)	Detected (a) (µM)	Recovery (%)	SD±RSD (%)
Sample 1	1	0.92	92	0.0178±1.907
Sample 2	3	2.94	98	0.0230±0.779
Sample 3	5	4.98	99.6	0.0228±0.460

(a) Average of five replicates

4. CONCLUSION

The medicinally important compound, 2-AT was determined using proposed electrochemical sensor poly Gly/PGE as electrode in the present work. The electrochemical response of the 2-AT at poly Gly/PGE was an adsorption-controlled process. A probable electro oxidation mechanism was proposed for the oxidation of 2-AT. The proposed method has also been successfully utilized for analysis of urine samples, with better recoveries obtained at the levels tested. The method is sensitive, rapid, and less time consuming. Hence, this method can very well be used for the analysis of 2-AT in medicinal, in quality control laboratories and in pharmaceutical studies where economy and time are essential considerations.

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