



Determination of tinidazole by voltammetric analysis using poly (L-arginine) modified carbon paste electrode

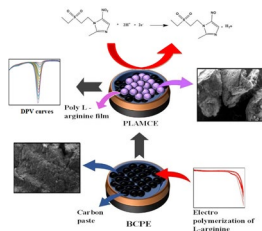
D. P. Prashanth^{1,2} · J. G. Manjunatha¹ · K. P. Moulya¹ · C. Raril³ · Samar A. Aldossari⁴ · Saikh Mohammad⁴

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Abstract

The current study presents the analysis of tinidazole with the aid of electrochemical analysis by utilizing 0.2 M of phosphate buffer saline and a modified carbon paste electrode. Poly(L-arginine) fabricated carbon paste electrode (PLAMCPE) was obtained by polymerizing L-arginine (L-agn) onto a bare carbon paste electrode (BCPE) by applying electropolymerisation method at pH 6.5. To examine the electrochemical response and characterization of the developed PLAMCPE, differential pulse voltammetry (DPV), cyclic voltammetry, electrochemical impedance spectroscopy, and scanning electron microscope techniques were implemented. The PLAMCPE shows significantly enhanced electrochemical sensitivity for the reduction of tinidazole compared to BCPE. At optimal working circumstances, the consequences of several parameters like scan rate, pH, concentration variation, and active surface area were analyzed. By examining the scan rate, the reaction was found to be diffusion controlled. The attained values of limit of detection was 0.0841 μM , and limit of quantification was 0.2803 μM at a linear range of 0.2 to 9.0 μM for DPV method. Moreover, PLAMCPE shows great selectivity and sensitivity at sensing tinidazole in the occurrence of other interfering organic dyes and metal ions. In addition, it demonstrates commendable repetitiveness, stability, and reproducibility. The developed electrode has a remarkable recovery rate indicating its applicability to the real sample.

Graphical abstract



Keywords Tinidazole · L-Arginine · Carbon paste electrode · Voltammetry · Dopamine

✉ J. G. Manjunatha
manju1853@gmail.com

¹ Department of Chemistry, FMKMC College, Mangalore University Constituent College, Madikeri 571201, Karnataka, India

² Department of Chemistry, Sri Jayachamarajendra College of Engineering, JSS Science and Technology University, Mysuru 570006, India

³ State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

⁴ Department of Chemistry, College of Science, King Saud University, P. O. Box 2455, 11451 Riyadh, Saudi Arabia

Introduction

Tinidazole (1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole, TNZ) is an anti-bacterial or anti-parasitic drug used to treat infections caused by bacteria and parasites. It is a derivative of nitroimidazole [1] that can cure diseases like trichomoniasis [2], giardiasis [3, 4], amebiasis [5], and bacterial vaginosis [6, 7]. Apart from the use of TNZ, it has some undesirable effects such as fatigue, constipation, dyspepsia, diarrhea, and dizziness [8, 9]. As a result of this,

it has gained researchers interest in designing a new method for the detection of TNZ in pharmacological samples.

The presence of TNZ can be determined by numerous methods, such as the chromatographic method [10–12], spectrophotometric method [13–15], potentiometry [16], polarography [17], and capillary electrophoresis [18]. Nevertheless, every method has specific advantages and disadvantages. Some of them are very expensive and time-consuming, and some samples need pre-processing as well as very complex instrumentation techniques. Contradictorily, a few other methods are inexpensive, require less time, and give effective results. One among them is electrochemical analysis (ECA) [19–21]. ECA methods are the most flexible and extensive method for sensing biologically active compounds due to their better responsiveness, high precision, less expensive, and easily attainable laboratory conditions [22–24]. In this method, several working electrodes like carbon paste electrodes (CPE) [25–27], carbon nanotube electrodes [28, 29], composite electrodes [30], glassy carbon electrodes [31, 32], graphene paste electrodes [33], graphene oxide [34], nanomaterials [35, 36], etc. are used for analysis. In the present work, we are using CPE which is modified for the analysis of the analyte, as the modification increases the sensitivity and efficiency of the electrode toward the electroactive molecule increases as reported in the previous works [37–39].

In the last few years, polymerized electrodes have been extensively used in voltammetric analysis for the detection of electroactive compounds, polymerization is generally done using amino acids and dyes. L-agn is one of the amino acids, it contains two functional groups, NH_2 and COOH . It commonly occurs in the zwitterionic form, which includes a positive charge dispersed over three nitrogen groups in the guanidyl group. This positive nature of L-agn involves it in the formation of hydrogen bonds with negatively charged species. Due to the multidentate nature of L-agn, it forms

long-range hydrogen bonds, and the electron flow between the electrode surface and the analyte increases. This added polymerized layer on the electrode shows exceptional electrochemical response [40, 41]. Hence L-agn was used as the modifier for the detection of TNZ.

In the proposed work we developed a PLAMCPE to determine TNZ using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) technique. The developed electrode was successfully employed to determine TNZ by studying different parameters. Also, TNZ was simultaneously detected with dopamine. The developed electrode gave high selectivity, repeatability, stability, and reproducibility and was applied to pharmaceutical samples. The literature survey revealed that no work has been reported to detect TNZ by PLAMCPE.

Results and discussion

SEM characterization of the electrode materials

The investigation of external characteristics of both PLAMCPE and BCPE was done by scanning electron microscopy (SEM). We can observe the rough surface unevenly distributed on BCPE in Fig. 1a (BCPE), while Fig. 1b displays an even distribution of L-agn on the surface of PLAMCPE. The polymer film assists in enhancing the active surface area of the modified sensor. So, detecting TNZ was much more convenient in PLAMCPE than in BCPE.

Electrochemical polymerization of L-arginine on BCPE

The polymerization on BCPE was accomplished by using 1.0 mM L-agn in 6.5 pH of 0.2 M phosphate buffer saline (PBS) through the CV technique in the potential window of

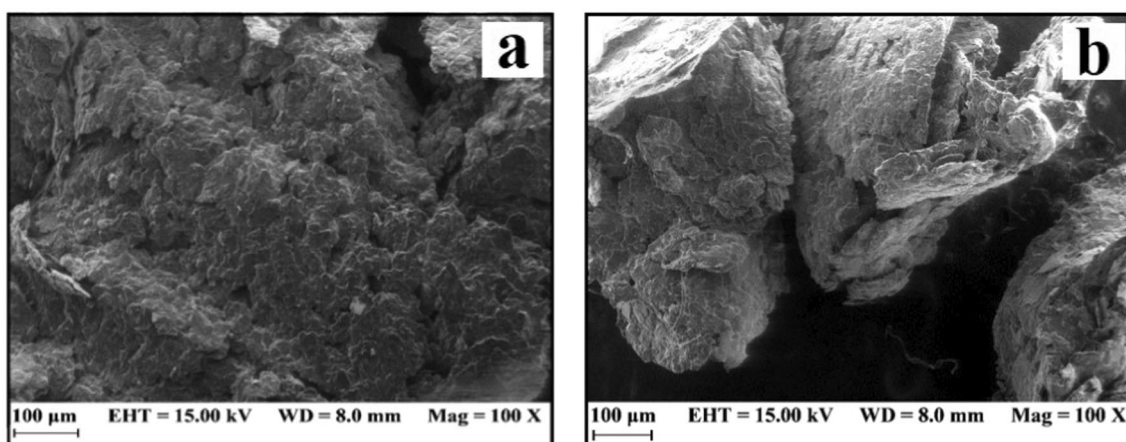


Fig. 1 SEM images displaying. **a** BCPE; **b** PLAMCPE

–0.3 to 1.6 V at a sweep rate of 0.1 V/s as seen in Fig. 2a, but the electrochemical response also depends on the number of cycles. To achieve the optimum number of cycles where the electrochemical response for detecting the TNZ is greater, the signals for each cycle of 5, 10, 15, and 20 were recorded. In which polymerization by 10 cycles exhibited the maximum response than the remaining cycles, as shown in Fig. 2b. Hence 10 cycles of polymerization were considered optimum and used throughout the experiment.

Active surface area and EIS study

The electrochemical sensing (ECS) capacity of both BCPE and PLAMCPE was examined by calculating the active surface area of the sensor. The CV was recorded

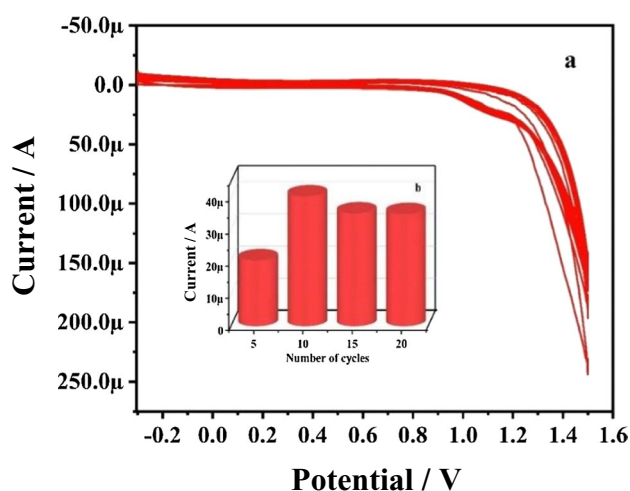
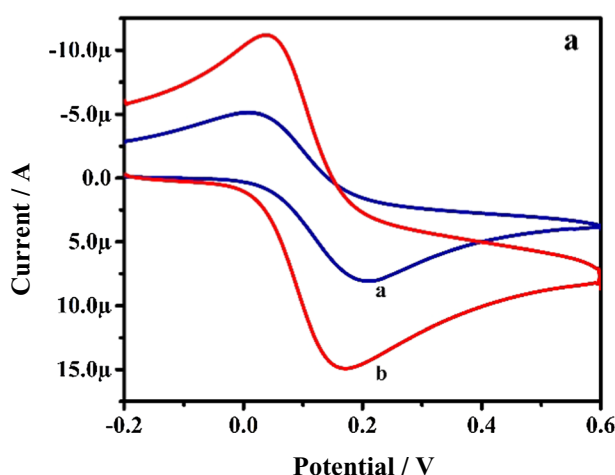


Fig. 2 a Electropolymerization of 1.0 mM L-agn in 6.5 pH of 0.2 M PBS on BCPE; b Graph of number of cycles vs current



for $[K_4Fe(CN)_6]$ (1.0 mM) as an analyte and KCl (0.1 M) as the supporting electrolyte at a sweep rate of 0.1 V/s. Figure 3a reveals that the ECS of PLAMCPE (curvature b) has the highest peak current than the BCPE (curvature a), so it can be concluded that the PLAMCPE has a more active site for the transfer of electrons. The active site area was calculated by utilizing the following equation [42]:

$$I_{pa} = 2.69 \times 10^5 n^{3/2} AD^{1/2} v^{1/2} C \quad (1)$$

where I_{pa} symbolizes peak current, n signifies the number of electrons engaged in the redox reaction, D represents diffusion coefficient ($7.6 \times 10^{-6} \text{ cm}^2/\text{s}$) [43], A denotes active surface area, v signifies sweep rate, and C represents the concentration of $[K_4Fe(CN)_6]$. By substituting all the values in the Eq. (1), the active surface area of 0.3449 and 0.6279 cm^2 were obtained for BCPE and PLAMCPE, respectively. The active surface area of PLAMCPE was relatively more than that of BCPE. Hence, PLAMCPE has a higher ECS.

The EIS study was performed to gain knowledge about the conductivity and charge transfer resistance of the electrode. Nyquist plot of BCPE (curvature a) and PLAMCPE (curvature b) in KCl of 0.1 M buffer comprising 1.0 mM $[K_4Fe(CN)_6]$ was plotted. The graph of both electrodes shows a semicircle and a linear curve, as represented in Fig. 3b. Here, (curvature a) belongs to BCPE, and (curvature b) belongs to PLAMCPE. The EIS study showed that the PLAMCPE depicts good conductivity and good charge transfer for electrochemical sensing.

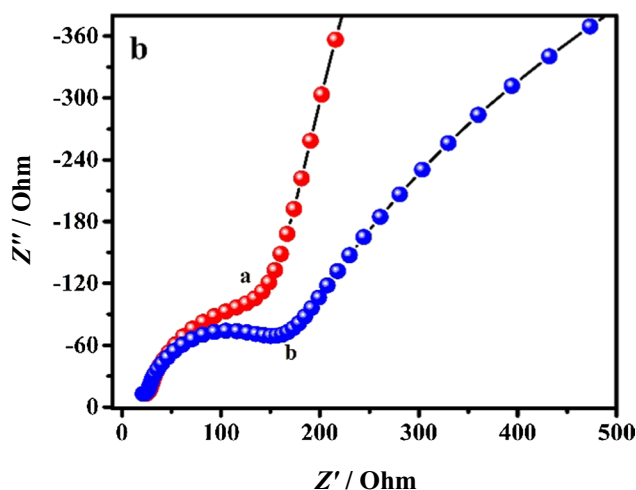


Fig. 3 a CVs of $[K_4Fe(CN)_6]$ (1.0 mM) in KCl (0.1 M) at BCPE (curvature a) and PLAMCPE (curvature b) at a sweep rate of 0.1 V/s; b Nyquist plot of $[K_4Fe(CN)_6]$ (1.0 mM) in KCl (0.1 M) at BCPE (curvature a) and PLAMCPE (curvature b)

Electrochemical behaviour of TNZ at the surface of BCPE and PLAMCPE

The electrochemical behaviour of 0.1 mM TNZ in 4.5 pH of 0.2 M PBS was analyzed by applying CV technique at the surface of BCPE and PLAMCPE in the potential range of 0.0 to -1.0 V at 0.1 V/s sweep rate obtained results are displayed in Fig. 4. From the CV responses, we can conclude that BCPE (curve b) shows lesser electrochemical response with weak reduction peak current of $19.44 \mu\text{A}$ for TNZ, while PLAMCPE (curve c) exhibits improved reduction peak current with a greater electrochemical response of $40.19 \mu\text{A}$. By analyzing the obtained peak current for both electrodes, it can be seen that the PLAMCPE improves the peak current response by 2 times more than the BCPE. Due to the absence of an oxidation peak at the surface of electrodes, the reaction is claimed to be irreversible. Moreover, the CV recorded for blank (containing only 4.5 pH of PBS 0.2 M) does not show any electrochemical response (curve a) at PLAMCPE.

Influence of pH

pH is one of the parameters that influences the analyte. To study its impact, CV was taken for a pH range of 3.0–5.5 using 0.2 M PBS containing 0.1 mM TNZ across a potential range of -0.25 to -1.0 V at 0.1 V/s scan rate which is evident in Fig. 5a. As portrayed in the plot, the peak current changes for every different pH and peak potential has been heading towards its negative side as the pH increases. Initially, from pH 3.0 to 4.5, the peak current increases, and as the pH continues to rise further from 5.0 to 5.5, a decrease in peak current was witnessed, which can be seen in Fig. 5a

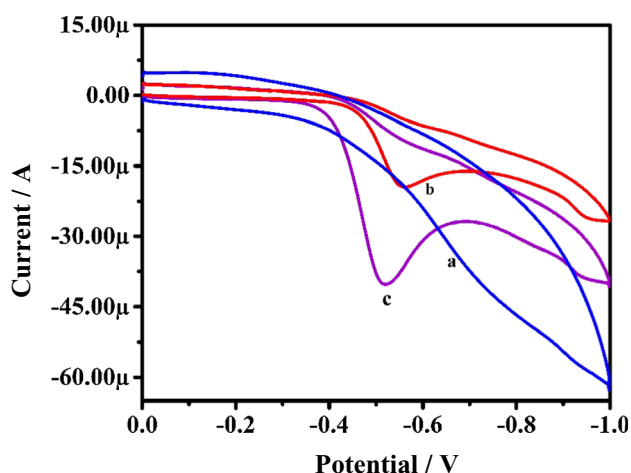


Fig. 4 CVs for 0.1 mM TNZ at BCPE (curve b) and PLAMCPE (curve c) in pH 4.5 of 0.2 M PBS and in the absence of TNZ (curve a) at 0.1 V/s sweep rate

and b. Thus, the pH 4.5 was considered as optimum and the further analysis was carried out using pH 4.5. The graph of E_{pa} vs. pH (Fig. 5c) shows great linearity, which can be represented by a linear regression equation as follows:

$$E_{pa} (\text{V}) = -0.0905 - 0.0977 \text{ V/pH} \quad (R^2 = 0.9788) \quad (2)$$

Effect of scan rate

The scan rate is a significant attribute that offers data about the number of electrons, rate of reaction, and mechanism of reaction. The variation of sweep rate on TNZ of 0.1 mM in 0.2 M PBS of 4.5 pH affects its electrochemical behaviour. The scan rate was varied from 0.025 to 0.400 V/s, which was achieved by CV (Fig. 6a), the reduction peak of TNZ increases as the scan rate increases. The graph plotted between the square root of scan rate and current shows linearity with $R^2 = 0.9976$, as displayed in Fig. 6b. So, the process between TNZ and PLAMCPE is known to be a diffusion controlled. The corresponding linear regression equation can be expressed as

$$I_{pa} (\text{A}) = 8.5625 \times 10^{-8} + 1.8833 \times 10^{-4} \vartheta^{1/2} (\text{V/s}) \quad (R^2 = 0.9976) \quad (3)$$

The graph of \log scan rate vs. $\log I_{pa}$ shows excellent linearity, as seen in Fig. 6c, and the slope of this graph was found to be 0.5159. Thus, this result confirms that the electrochemical reaction of TNZ was diffusion-controlled process and the corresponding equation is represented as

$$\log I_{pa} (\text{A}) = -3.7140 + 0.5159 \log \vartheta (\text{V/s}) \quad (R^2 = 0.9952) \quad (4)$$

To determine the number of electrons involved in the reaction, the graph of \log scan rate V/s potential was plotted as shown in Fig. 6d, and the correlated equation is as follows:

$$E_{pa} (\text{V}) = -0.5786 - 0.0599 \log \vartheta (\text{V/s}) \quad (R^2 = 0.9847) \quad (5)$$

By using this slope and Laviron's expression for the irreversible reaction, the number of electrons was calculated [44].

$$E_{pa} (\text{V}) = E^0 + \frac{2.3RT}{anF} \log \frac{RTk}{anF} + \frac{2.3RT}{anF} \log \vartheta \quad (6)$$

$$\text{Slope} = \frac{2.3RT}{anF} \quad (7)$$

where α denotes the electron transfer co-efficient for the irreversible process ($\alpha = 0.50$) [45], E^0 implies standard

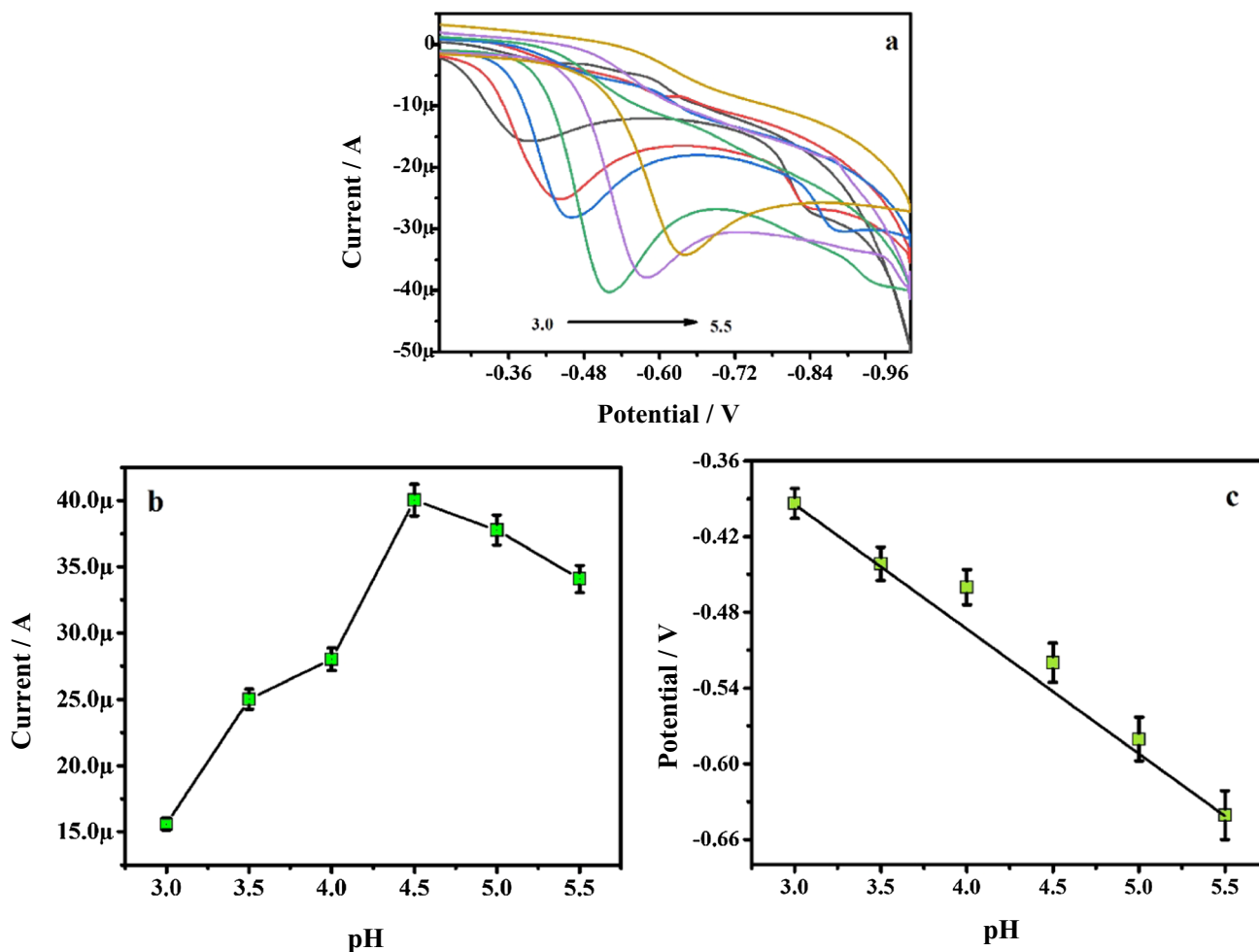


Fig. 5 **a** CV graph of 0.1 mM TNZ in PBS of 0.2 M at different pH buffers varying from 3.0 to 5.5 at the surface of PLAMCPE; **b** graph of pH vs. current; **c** graph of pH vs. potential

electrode potential, k represents the rate constant of the reaction, F represents the Faraday's constant ($96.485 \text{ C mol}^{-1}$), T indicates the temperature (298 K), R implies universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$). By utilizing Eq. (7) and other constants, the calculated number of electrons was found to be 1.96, which is approximately equal to 2. Thus, 2 electrons were involved during the reduction reaction of TNZ. The possible mechanism scheme can be seen in Scheme 1 [46].

Study of concentration variation

To study the sensitivity and detection limit of the developed electrode, the DPV technique was employed. This was done by varying the concentration of TNZ from 0.2 to $9.0 \mu\text{M}$ in pH 4.5 of 0.2 M PBS at 0.1 V/s scan rate and the DPV curves corresponding to this can be seen in Fig. 7a. It can be observed that the current response increases

linearly with the increase in concentration of TNZ which can be seen in the graph of I_{pa} vs. [TNZ] (Fig. 7b). Then the corresponding linear regression equation to this can be represented as

$$I_{pa} (\text{A}) = 6.0009 \times 10^{-6} + 1.8666 [\text{TNZ}] (\text{M}) (R^2 = 0.9891) \quad (8)$$

Based on this linear equation, the LOQ and LOD were calculated by using the subsequent expressions: $\text{LOQ} = 10 \times \text{standard deviation of the blank/slope of the calibration curve}$, and $\text{LOD} = 3 \times \text{standard deviation of the blank/slope of the calibration curve}$ [47]. The evaluated values of LOQ and LOD were found to be 0.2803 and $0.0841 \mu\text{M}$, respectively. The comparison of the LOD of the developed sensor with the already reported sensors has been tabulated in Table 1. The comparison table concludes that the developed electrode has a better sensitivity than the reported works.

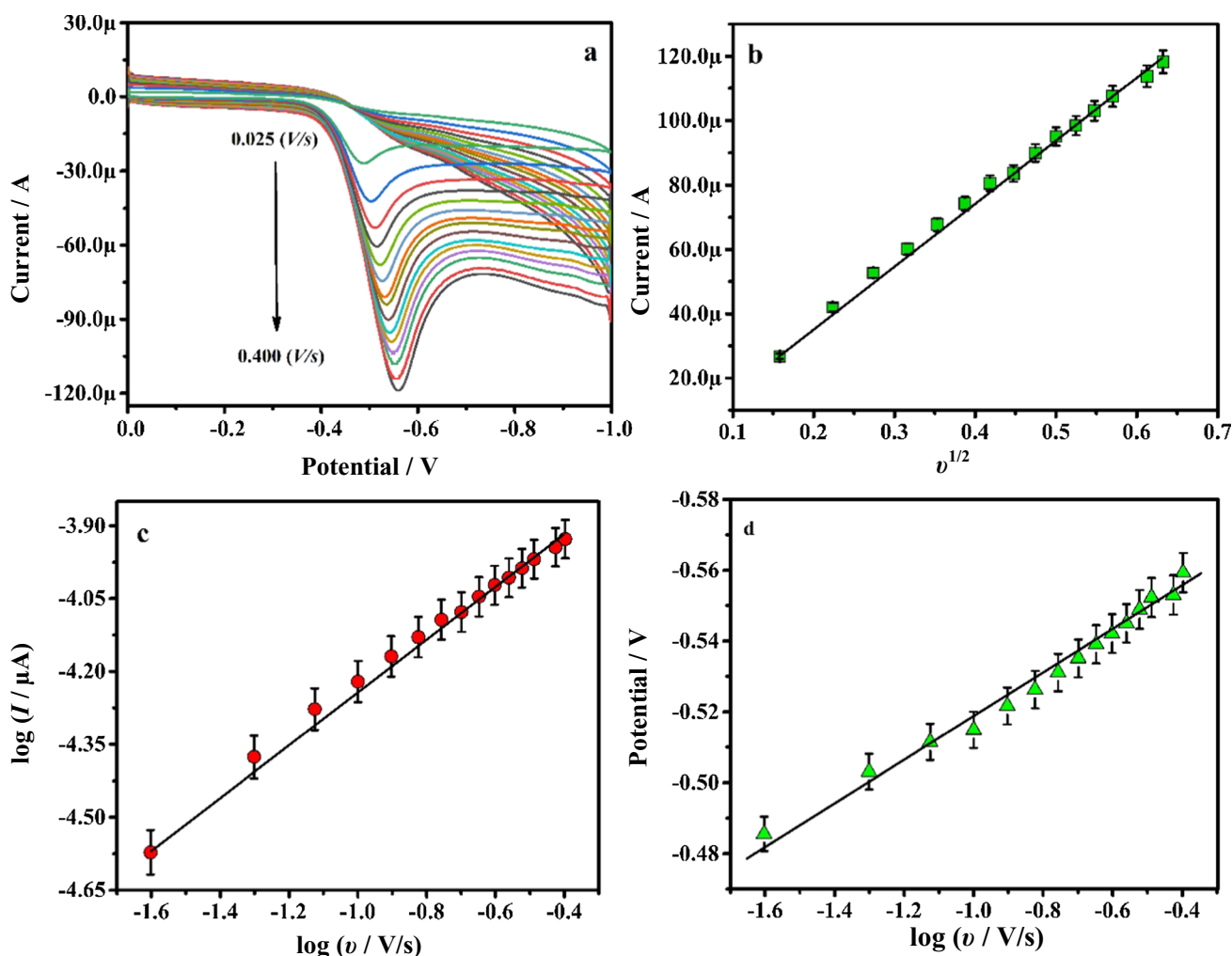
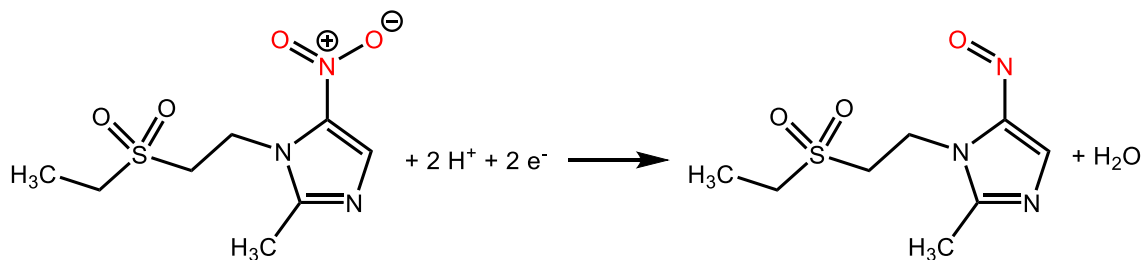


Fig. 6 a CV plot of 0.1 mM TNZ in 4.5 pH of 0.2 M PBS at different sweep rates ranging from 0.025 to 0.400 V/s at PLAMCPE; b graph of $v^{1/2}$ vs. current; c plot of $\log v$ vs. \log current; d graph of $\log v$ vs. potential

Scheme 1



Interference analysis

To analyze the selectivity of PLAMCPE towards TNZ for the interference of metal ions like cadmium (Ca²⁺), arsenic (As³⁺), cobalt (Co³⁺), lead (Pb²⁺), potassium (K⁺), cadmium (Cd²⁺), sodium (Na⁺) and organic dyes such as methyl orange (MO) and tartrazine (TR) was studied in

pH 4.5 of PBS 0.2 M containing TNZ (0.1 mM) using CV technique. The obtained results can be seen in Fig. 8, the current obtained for the ions and dyes shows faint variation, which was below $\pm 5\%$ and was in the tolerable limit. Hence, PLAMCPE can be admitted as an excellent appliance for reliable selectivity of TNZ and can be stated as an interference-free electrochemical sensor.

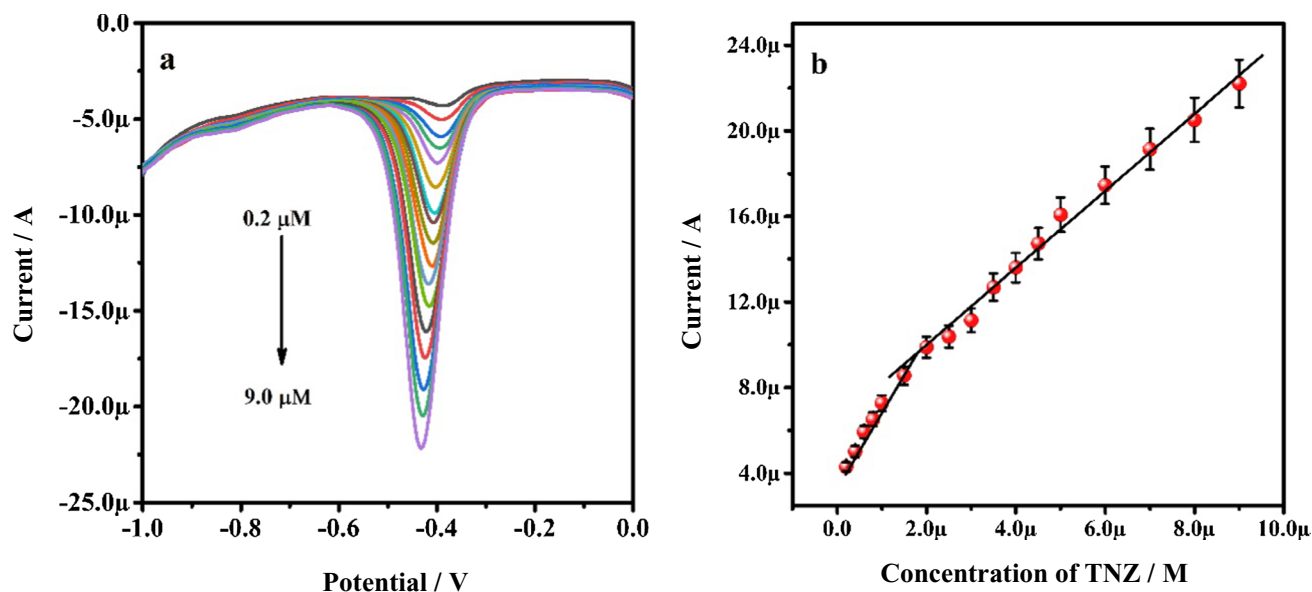


Fig. 7 **a** DPVs for the different concentrations of TNZ ranging from 0.2 to 9.0 μM in pH 4.5 of 0.2 M PBS at the sweep rate of 0.1 V/s at PLAMCPE; **b** graph of concentration of TNZ vs. current

Table 1 Assessment of LOD of the developed sensor with the LOD of other works

Electrode	Technique	Linear range/μM	LOD/μM	References
CPE	DPV	5–20	0.51	[48]
SG/CPE ^a	DPV	1–10	0.024	[49]
Poly(L-Cys)/GCE ^b	SWV ^c	0.7–10	0.073	[50]
Poly(EBT)/GCE ^d	CV	10–600	1.60	[51]
Ag-Co ₃ O ₄ NPs/GCE ^e	AMP ^f	0.5–388.8	0.035	[52]
Poly(carmin) MGCE ^g	LSV ^h	0.1–50.0	0.05	[53]
PTRMCNTMGPE ⁱ	DPV	2.0–35	0.20	[54]
PLAMCPE	DPV	0.2–9	0.0841	Present work

^aSilicon gel

^bPoly(L-cytosine)

^cSquare wave voltammetry

^dEriochrome black T modified glass carbon electrode

^eNoble metal silver incorporated carrom coin structured cobalt oxide

^fAmperometric technique

^gPolycarmine film modified glassy carbon electrode

^hLinear sweep voltammograms

ⁱPoly-threonine-modified carbon nanotube mixed graphene paste electrode

Reproducibility, stability, and repeatability of PLAMCPE

The reproducibility, stability, and repeatability of the PLAMCPE can be inspected using 4.5 pH containing 0.1 mM TNZ with the aid of the CV technique. The calculated RSD of the peak current generated was 1.73% through maintaining the same analyte (TNZ) for five independent electrodes, reproducibility was assessed.

The stability of the PLAMCPE was verified by scanning the TNZ for 30 cycles, and the estimated degradation of current was found to be 91.77%, which conveys that the developed electrode is stable. The repeatability was evaluated by keeping a fixed electrode for five repeated solutions of the same concentration. The obtained RSD was 1.35%. Therefore, the modified electrode shows commendable repeatability.

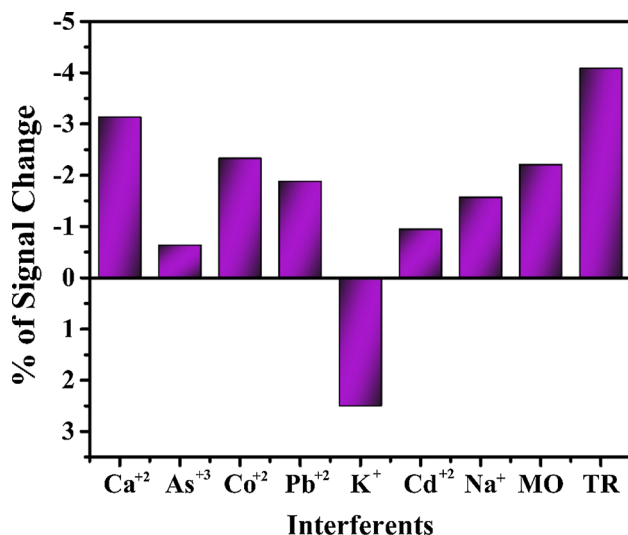


Fig. 8 Plot of various interferents vs. percentage of signal change

Simultaneous study of TNZ and DP

The simultaneous study was accomplished using the CV technique for TNZ and DP by both PLAMCPE and BCPE (Fig. 9) to investigate the selectivity of the developed electrode. PLAMCPE shows two distinct peaks (curve a) free from intervening with each other in the potential range of -1.0 to 0.4 V with high sensitivity, but the peak obtained by BCPE (curve b) was indiscernible. By this evidence, it can be determined that the developed sensor shows fine selectivity.

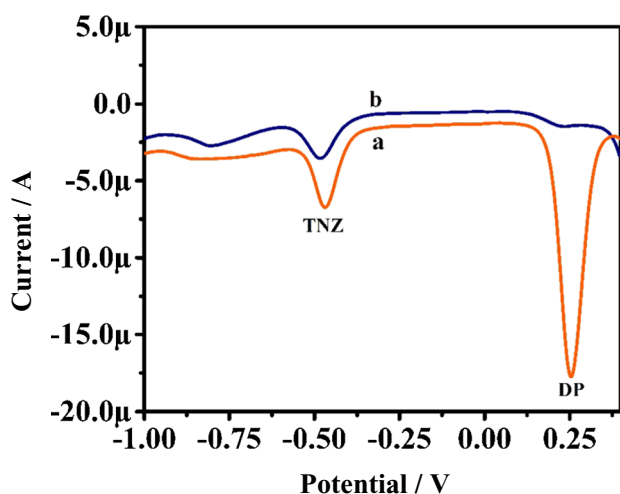


Fig. 9 DPVs of TNZ and DP in PBS 0.2 M of pH 4.5 at a sweep rate of 0.1 V/s in the potential window of -1.0 to 0.4 at the surface of BCPE (curve b) and PLAMCPE (curve a)

Table 2 Recovery rate of TNZ in pharmaceutical sample

Sample	Added/ μM	Found/ μM	% of Recovery
Tablet	100	98.32	98.32
	150	190.5	95.28
	200	346.39	98.97
	250	399.6	99.90

Real sample analysis

The real sample was analyzed using a pharmaceutical tablet to verify the versatility and adaptability of PLAMCPE for the determination of TNZ by implementing the DPV technique. The real sample was analyzed by varying the concentration of the tablet sample in the range of 100 to 250 μM in 4.5 pH of 0.2 M PBS, the data secured from the DPV technique was evaluated and the recovery rate was calculated which was found to be in the range of 95.28 to 98.32%. This is mentioned in Table 2, and from this, we can conclude that the prepared electrode is excellent in analyzing TNZ in the tablet sample.

Conclusion

The data obtained in the current work displays a better electrochemical technique for the reduction of TNZ on the surface of PLAMCPE than on the surface of BCPE. The PLAMCPE was easily prepared by polymerizing the L-agn on the surface of BCPE using the CV technique, due to the polymerisation an increase in the active surface area was observed which increased the current response of PLAMCPE. The difference in the active surface area of PLAMCPE and BCPE was confirmed using SEM and EIS. The enhancement in the electrochemical response of TNZ was obtained at the pH of 4.5. It was found that the reduction reaction of TNZ involves the transfer of two electrons and it was a diffusion-controlled process. The developed electrode shows a linear increase in its reduction peak with an increase in the concentration of TNZ in the linear range of 0.2 to 9.0 μM and also with the increase in the scan rate of 0.025 to 0.400 V/s. The lower LOD of 0.0841 μM was obtained from the detection limit and LOQ was found to be 0.2803 μM . The process of constructing the PLAMCPE is very affordable and simple, and it also portrays good stability, reproducibility, and repeatability. The proposed electrode has great sensitivity, as well as selectivity in sensing TNZ in the occurrence of other interfering ions, and organic molecules. All these factors make the developed electrode a helpful tool for analyzing TNZ in medicinal samples.

Experimental

The electrochemical measurements were done using the CHI-6038E (Electrochemical analyzer, USA) instrument. It works based on three electrode systems, which are connected to the analyzer. The platinum wire was practiced as the auxiliary electrode, the saturated calomel electrode was practiced as the reference electrode, and BCPE and PLAMCPE were used as the working sensors. Different pH solutions were prepared through the EQ-610 digital pH instrument. SEM images were assessed to study the outer surface layer of PLAMCPE and BCPE, these data were received from Vignan Bhavan, University of Mysore.

TNZ (98% purity), dopamine hydrochloride (DP), DMSO (98% purity) and L-agn (98.5–101.5% purity) were procured from Molychem (India). Silicon oil (90% purity), KCl (99.5% purity), and carbon powder (94% purity) were purchased from Nice Chemical Pvt. Ltd (India). Monosodium phosphate (99% purity), disodium phosphate (99.5% purity), and $[K_4Fe(CN)_6]$ (98.5% purity) were brought from Himedia Chemicals Pvt. Ltd (India). The acidic pH solutions in the range of 3.0 to 5.5 was prepared using the EQ-610 pH instrument. The TNZ stock solution of 0.1 mM concentration was prepared by dissolving TNZ in DMSO, and then diluting with water and L-agn was formulated using double distilled water. Every single analytical standardized reagent was used without any additional purification.

Real sample preparation

The pharmaceutical tablet, was brought from a nearby medical store of Madikeri, was crushed to powder, weighed, dissolved in DMSO, filtered by using Whatman filter paper a clear solution was obtained and then diluted using distilled water. Thereafter, the obtained stock solution was used for further analysis.

Preparation of working electrodes

Carbon paste was gained by hand mixing graphite powder and silicon oil in a ratio of 70:30 on mortar and pestle until a consistent uniform paste was formed. Then, BCPE was created by packing carbon paste into the terminal end of the Teflon tube, which consists of an internal diameter of 3 mm, and there is copper wire at the top connected to the Teflon tube, which provides electrical contact. The PLAMCPE was constructed by polymerizing BCPE for 10 cycles in 0.2 M PBS of pH 6.5 containing 0.1 mM of L-agn.

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Data availability Data will be made available on reasonable request.

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