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Electroanalysis of Diazepam on Nanosize Conducting Poly (3-Methylthiophene) Modified Glassy Carbon Electrode

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Abstract: *A glassy carbon electrode (GCE) was modified with nanosize poly (3-methylthiophene) (P3MT) and used for the sensitive voltammetric determination of diazepam. The cyclic voltammetric response of the bare GCE was compared with the P3MT modified electrode. Electrochemical impedance response of diazepam on modified GCE was studied by various concentrations of diazepam from 0.2 µM to 0.6 µM. The poly (3-methylthiophene) modified glassy carbon electrode (P3MT/GCE) can greatly enhance the peak currents and the detection sensitivity of diazepam under optimal conditions. The quantitative analysis of diazepam was made by the DPSV method. The experimental results showed that the peak current increased with the increase in concentration of diazepam. A calibration was made, which indicated the linear dependence of peak current with concentration (i_p = 13.31Conc. + 0.4359R2 = 0.9948) in the range od determination and ot was found to be good between 0.2 and 1.07 µg/L. The limit of detection was 0.1µg/mL. The reproducibility of the stripping signal was realized in terms of relative standard deviation for 6 identical measurements and was found to be 2.6%. The effect of interference of different cations and anions on the oxidation of diazepam was studied. Real sample analysis of diazepam was also studied through DPSV.*

Keywords: Nanosize poly(3-methylthiophene), Diazepam, Electrochemical impedance, Glassy carbon

1. INTRODUCTION

Electrochemical detection methods are very advantageous over other methods in terms of simplicity, sensitivity, selectivity and cost. However, these methods have not become as popular as other methods due to certain unavoidable problems such as electrode deactivation, with the necessity of frequent pretreatment and other procedures to reactivate the solid electrodes. Glassy carbon electrode (GCE), one of the widely used electrodes for electrochemical detection, due to its relatively wide potential window and low cost, is very susceptible to contamination and fouling. Therefore, a stable electrode material with sensitive detection capabilities is a prime requirement for wider application of electrochemical detectors. Polymer modified electrodes proved to be a potential candidate for being used as the modified electrode material [1-3]. Poly(3-methylthiophene) (P3MT) modified electrodes have been extensively reported and showed excellent electrocatalytic effect on phenolic compounds [4.5], neurotransmitter species [6-9], amino acids [10], dopamine [11], NADH [12], herbicide metamitron [13], Hg(II) [14], sulfonamides [15], epinephrine [16] and riboflavin [17]. Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-1,4-benzodiazepin-2(3*H*)-one) is a member of 1,4-benzodiazepine class of tranquilizers. It is a drug of potential abuse and can cause serious problems of addiction and as a result the drug was scheduled. It has been found as an adulterant in heroin also; hence the determination of diazepam becomes vital. An entire electrochemical study of diazepam, temazepam and oxazepam using modified carbon paste electrodes were reported [18]. In our present work we

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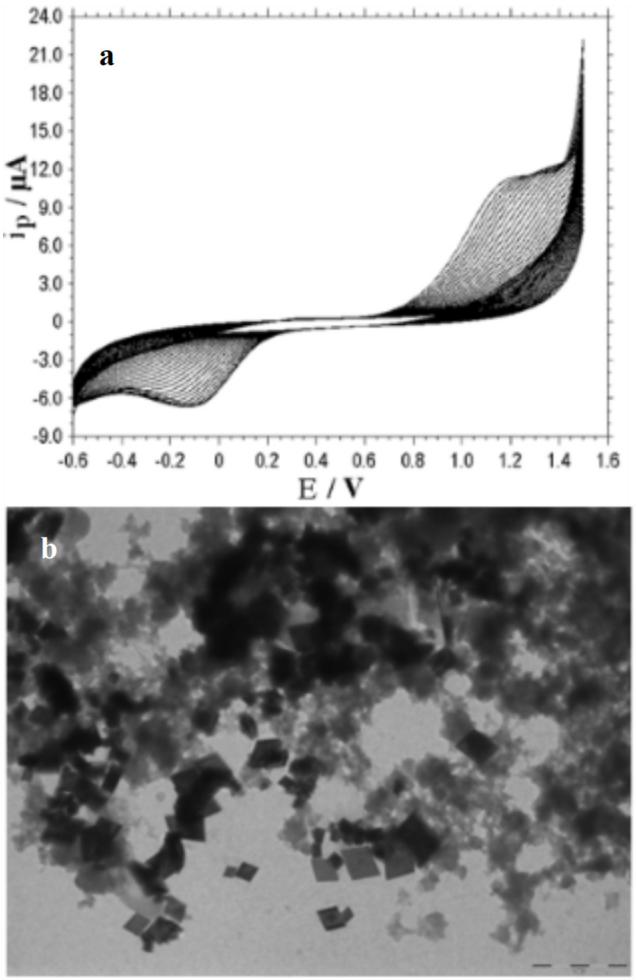

Figure 1. a. Cyclic voltammogram of 0.1M P3MT in 0.1M H_2SO_4 at a scan rate of 0.05 V/s, b. TEM image of P3MT

have determined diazepam on poly (3-methylthiophene) modified glassy carbon electrode by differential pulse stripping and electrochemical impedance method.

2. EXPERIMENTAL

2.1. Apparatus and Reagents

Electrochemical workstation CHI 650C (CH Instruments, USA) was employed for electrochemical and electroanalytical studies. Diazepam (Sigma) and 3-methylthiophene (Alfa Aeasar) were purchased and used as such. The stock solutions were made up in ultra pure water (SG International, Germany) and ethanol (Jiangsu Huaxi International, China). For the electrochemical studies, 0.1 M H_2SO_4 (pH 1.0), 0.01 M H_2SO_4 (pH 2.0), 0.001 M H_2SO_4 (pH 3.0), Buffer tablet (Merck) (pH 4.0), 2 M Sodium acetate / 2 M CH_3COOH (pH 5.0), 1 M NaOH / 0.1M KH_2PO_4 (pH 6.0), Buffer tablet (Merck) (pH 7.0), Buffer tablet (Merck) (pH9.2) and 0.1 M NaOH (pH 13.0) were used.

2.2. Procedure

Purging of nitrogen was done for analytic solution placed in the electrochemical cell of 15 mL capacity for 20 minutes under stirred conditions. Various voltammograms were recorded while nitrogen gas was blanketed. To get reproducible results, great care was taken in the electrode pretreatment. The glassy carbon electrode was pretreated in two ways: mechanical polishing over a velvet microcloth with an alumina suspension (1.0, 0.3, and 0.05 µm) and electrochemical treatment by applying at a potential of 1.5 V for 2 s.

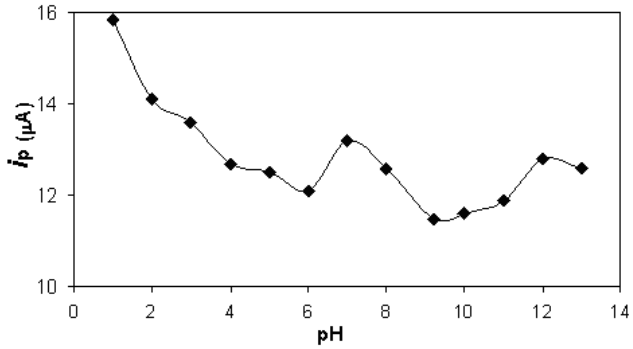

Figure 2. Plot of peak current vs pH

2.3. Preparation of Preparation of P(3-Methylthiophene) Coated Glassy carbon Electrode (P3MT/GCE)

Poly (3-Methylthiophene) films were deposited on GCE by the electrooxidation of 0.1 M 3-methylthiophene containing 0.1 M H_2SO_4 by cycling the potential between –0.6 to 1.5 V (versus Ag/AgCl); scan rate 50 mV/s. One anodic and one cathodic peak were observed around 1.2 V and -0.1 V respectively (fig. 1a). After the completion of 25 cycles a grey colour poly (3-methyl thiophene) film was formed on the electrode surface. The electrode was washed with distilled water and used for further studies. Coated polymer film wss stripped from glassy carbon electrode and dispersed in ultra pure water. Dispersed polymer film was characterized by TEM. A TEM (fig 1b) image of dispersed film is shown in nano size spongy like structure.

3. RESULT AND DISCUSSION

3.1. pH Variation

The cyclic voltammetric studies at 1.0 to 13.0 pHs revealed electroactive nature of diazepam on poly (3-Methylthiophene) electrode at 50 mV/s. The cathodic peak current was correlated with pH in order to understand the influence of pH. Fig. 2 shows the variation of peak current with pH and this resulted in mixed curve. Maximum cathodic peak current was observed at pH 1.0. This may be due to faster electron transfer rate at acidic pH and this indicates that the rate of the reaction is controlled only by electron transfers. Detailed studies like cyclic voltammetry, chronoamperometry, chronocoulometry, differential pulse stripping and electrochemical impedance spectroscopy were carried out at pH1.0.

3.2. Cyclic Voltammetric Behaviors

The response of the bare and P3MT modified glassy carbon electrode was investigated at pH1.0 as supporting electrolytes. Fig. 3 shows the cyclic voltammetric response of the bare GCE compared

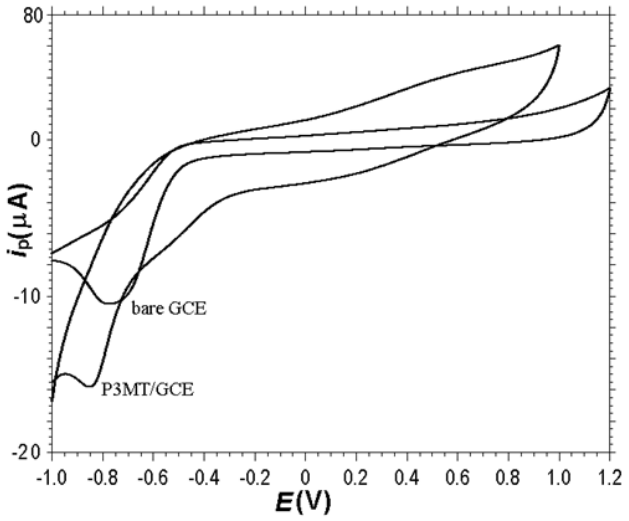

Figure 3. Cyclic voltammogram of 0.2 µM diazepam on bare and P3MT modified GCE at pH1.0; scan rate 0.05 V/s

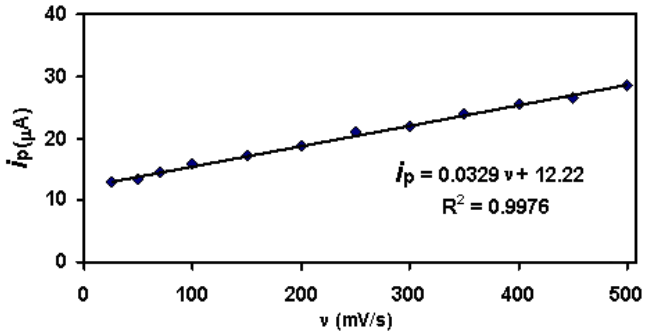

Figure 4. Plot of peak current vs scan rate

to the P3MT modified electrode. Upon modification of the electrode a catalytic response for the reduction of diazepam was observed with increase in current from 10.0 to 15.7 µA at peak potential of -0.850 V. The catalytic behavior of the modified electrode may be attributed to the electrostatic interaction that exists between the thiophene backbone and the drug. The response of the modified electrode shows that P3MT can be successfully used in the determination of diazepam.

3.3. Effect of Scan Rate

The effect of scan rate was studied by varying the scan rate in the range 0.25-0.5 Vs^{-1} in pH1.0. A well-defined cathodic peak was seen in all sweep rates at around -0.85 V. As the scan rate was increased the peak current also increased linearly (fig. 4) and the peak potential shifted anodically. The peak becomes broader as the scan rate increased. Peak currents were correlated with the square root of scan rates. A straight line was observed (fig. 5). Logarithmic values of peak currents were correlated with the logarithmic values of scan rate and it resulted in a straight line as in fig. 6. These facts reveal that the reduction of diazepam was controlled by adsorption process.

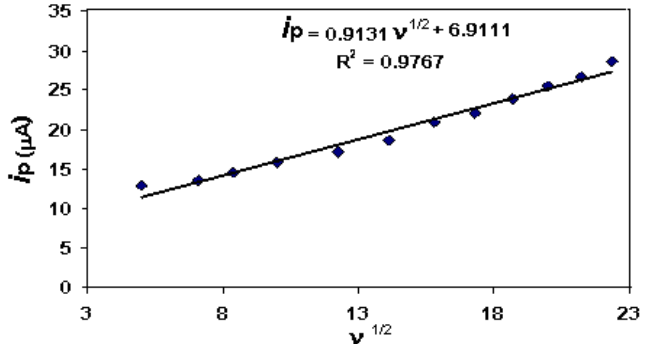

Figure 5. Plot of peak current vs log of scan rate

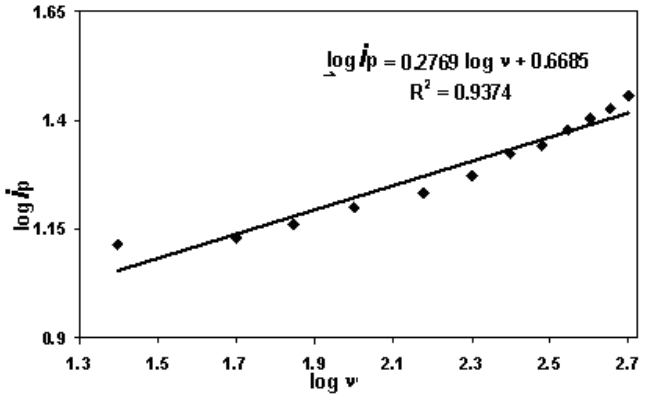

Figure 6. Plot of log peak current vs log of scan rate

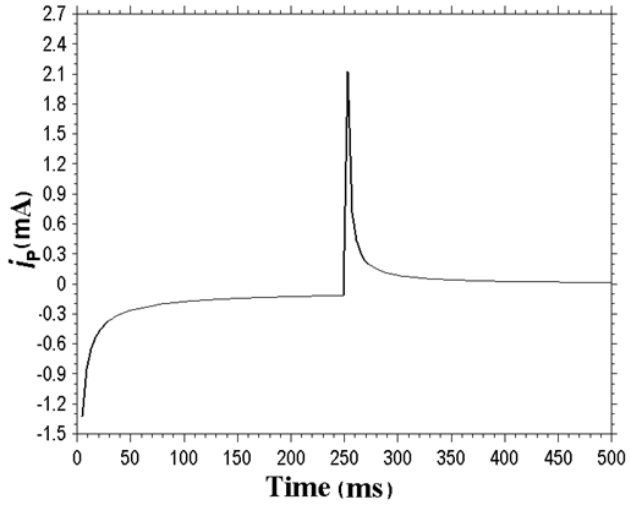

Figure 7. Chronoamperometric response of 0.2 µM of diazepam

3.4. Chronoamperometry and Chronocoulometry Studies

Chronoamperometric study was used for the determination of diffusion coefficient, *D*, for the reduction of diazepam. The experimental plots of current versus time with 0.2 mM concentrations of diazepam have been given in fig. 7. From the slope of the curve and

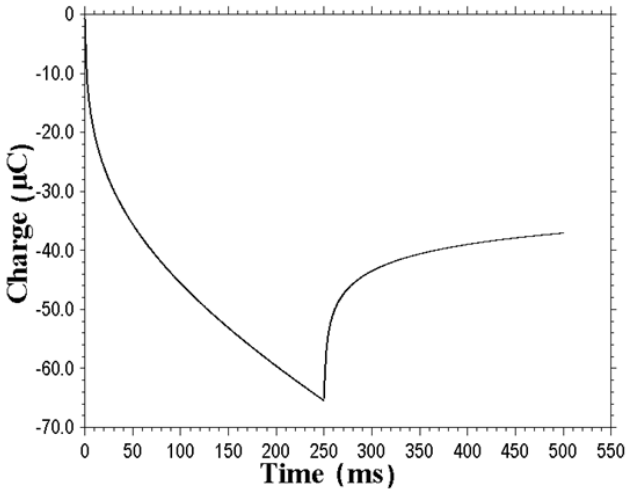

Figure 8. Chronocoulometric response of 0.2 μM of diazepam during the detection of 0.2 μM diazepam on bare and P3MT/GCE by various concentrations of diazepam from 0.2 to 0.6 μM, the chosen pH was 1.0 with solution containing 0.2 μM of diazepam

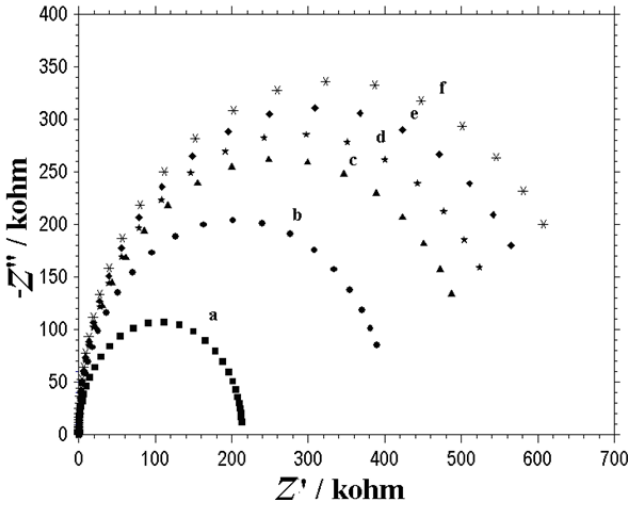

Figure 9. Impedance responses obtained at P3MT/GCE in the presence of various concentrations (a-0.2 μM, b-3.3 μM, c-4.29 μM, d-5.0 μM, e-5.5 μM, f-6.0 μM) of diazepam. Amplitude: 0.005 V, frequency: 100 mHz to 100 kHz

using the Cottrell equation [19], diffusion coefficient (D) of diazepam can be calculated, where n is number of electrons per molecule (eq/mol), F is Faraday's constant (96500 C/q), A is the electrode area (cm2), Cb represents the concentration of the electroactive species (mol/cm3) and t is time in second.

$$I = nFAD^{1/2} C_b \pi^{-1/2} t^{-1/2}$$

The diffusion coefficient of bare GCE in 0.2 μM diazepam was found to be 3.08 X 10^{-5} cm^2 s^{-1}, where as for P3MT modified GCE it was 8.62 X 10^{-5} cm^2 s^{-1}. The decrease in diffusion coefficient of P3MT/GCE clearly indicates the reduction of diazepam on the electrode surface. It was purely adsorption-controlled reaction, which is in good agreement with cyclic voltammetric studies. The charge density of the bare and modified electrode can be calculated from the plot of charge vs. time (fig. 8). The total charge consumed during the detection of 0.2 μM diazepam on bare and P3MT/GCE were $2.94x10^{-3}$ C and $8.81x10^{-2}$ C respectively. Only less charge has been consumed for the reduction of diazepam on P3MT/GCE.

3.5. Impedimetric Response of Diazepam

Impedance response of diazepam on modified GCE was studied by various concentration of diazepam from 0.2 μM to 0.6 μM. The obtained Nyquist plots are shown in fig. 9. The semicircles from inner to outer were at P3MT/GCE for diazepam concentration additions. It is clear that the semicircle diameter is increasing with increase in concentration. Similar results have been reported at other impedimetric sensors developed for glutamate [20], uranyl ion [21] and hydrazine [22] determinations, in which they observed an increase in semicircle diameter while increasing glutamate, uranyl, and hydrazine concentrations, respectively. The reason for the increase in semicircle diameter with increase in diazepam concentrations would be due to the insulation layer formed by the adsorbed diazepam on the electrode surface, which slows down the charge transfer for the redox probe.

3.6. Electroanalysis of Diazepam by Differential Pulse Stripping Voltammetry

Cyclic voltammetric results revealed the electroactive nature of diazepam in the P3MT modified glassy carbon electrode at pH 1.0. Hence, differential pulse stripping voltammetric study was carried out and performed well in the determination of diazepam. Experiments were carried out to find out the best accumulation potential in the chosen pH 1.0 with solution containing 0.2 μM of diazepam. Pre-concentration stripping voltammograms were performed by varying the accumulation potentials (*Eacc*) from 0.2 to 0.8 V at accumulation time (*DT*) of 15 s, maximum peak current was found at 0.2 V *Eacc*. Maximum peak current was observed only at 15 s accumulation time and above this the peak current was found to decrease. The optimum *Eacc* 0.2 V and an accumulation time 15 s was fixed and the stripping parameters such as initial scan potential, pulse height, pulse width, scan increment and pulse period were varied between –0.2 to 0.4 V, 0.01 to 0.05 V, 0.1 to 0.5 s, .004 to .01 V, 0.2 to 0.6 s respectively. The optimum conditions

Table 1. Optimum experimental conditions for the reduction of diazepam by differential pulse stripping voltammetry

Variables	Range examined	Optimum value
Accumulation potential, V	0.2 to1.0	0.2
Deposition time, s	15 to 60	15
Initial scanning potential, V	-0.2 to 0.4	0
Pulse height, V	0.01 to 0.05	0.05
Pulse width, s	0.1 to 0.5	0.2
Scan increment, V	0.004 to 0 .01	0.004
Pulse period, s	0.2 to 0.6	0.5
Rest period, s	-	2

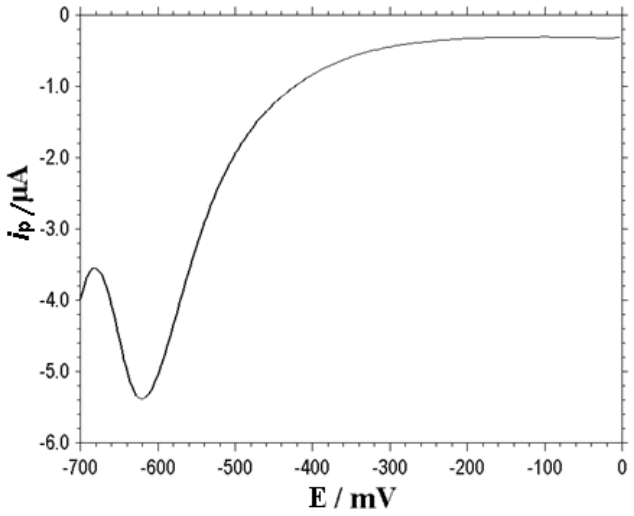

Figure 10. DPSV behaviour of diazepam under optimum experimental conditions

yielding maximum peak current were identified and are presented in table 1. A representative differential pulse stripping voltammogram is given in fig. 10.

Under these optimum experimental conditions, the influence of diazepam concentration on the stripping signal was studied. The experimental results showed that the peak current increased with the increase in concentration of diazepam. A calibration was made, which indicated the linear dependence of peak current with concentration (i_p = 13.31 Conc. + 0.4359; R^2 = 0.9948) in the range of determination and it was found to be good in between 0.2 and 1.07 μg/L. The limit of detection was 0.1 μg/mL. The reproducibility of the stripping signal was realized in terms of relative standard deviation for 6 identical measurements carried out at a concentration level of 10 μg/mL and was found to be 2.6%.

3.7. Interference of Other Ions

The effect of interference of different cations and anions on the oxidation of diazepam was studied and it was found that with Na^+, Mg^{2+}, K^+, Zn^{2+}, Pb^{2+}, PO_4^{3-}, Cl^-, I^-, Br^-, $S_2O_3^{2-}$, SO_4^{2-}, oxalate, citrate, tartrate, salicylate, acetate, nitroprusside, gluconate, pyrobrate, hydrogentartrate, metvanadate, sucrose, lactose, dextrose, glutamine, glycine and L-aspragine do not interfere upto 200 μg/mL. On the other hand, Ba^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, CN^-, NO^{2-}, SCN^-, MnO^{4-}, $Cr_2O_7^{2-}$ and EDTA interfered above 100μg/mL.

3.8. Real Sample

For the measurement of drugs in urine samples collected after 8 hours from administration of the drug, 1.0 mL of the urine was mixed with 0.1 M H_2So_4 solution and the pH was adjusted to 1.0 DPSV and was carried out under the optimum experimental conditions. This experiment was repeated 5 times and the average weight of drugs in 1.0 mL of urine sample was 0.7 ng. There was no appreciable interference due to the presence of other compounds present in the urine. There was no degradation of the drug in solution during the experiments.

4. CONCLUSIONS

The antidepressant drug of diazepam was cathodically reduced on nanosize P3MT modified glassy carbon electrode in the pH range 1.0–13.0; reduction of diazepam was controlled by adsorption reaction. From an analytical point of view, pH 1.0 was found to be suitable for differential pulse stripping voltammetric and electrochemical impedance studies. Optimum accumulation and stripping conditions were arrived and the calibrations were made. Lower limit of detection and percentage of RSD were observed. The developed method can be used to determine diazepam in real samples. This technique is simple and easy to carry out. Lower detection limit was obtained from this study showing that the proposed method is better than the available methods.

5. ACKNOWLEDGEMENT

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