

Electrochemical determination of the antidepressive drug, Nitrazepam using Poly(3,4-ethylenedioxythiophene) modified glassy carbon electrode

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Abstract: - PEDOT was obtained by the electrooxidation of the 0.01M EDOT solution in 0.1M p-toluene sulphonic acid and used for the determination of nitrazepam. One well-defined reduction peak was observed at -1.04V in the cyclic voltammograms. Effect of pH was studied and pH 4.0 was found to be best for the determination of Nitrazepam. The two electron transfer reduction mechanism was proposed from Coulometric studies. Differential pulse stripping voltammetric studies was carried out and optimized conditions which yield maximum peak current were arrived. Determination studies were carried out in the range 83-178ppb and LOD was found to be 10ppb. The reproducibility of the stripping signal was also high. Hence this method can be an alternate to other spectrophotometric and chromatographic studies

INTRODUCTION

Nitrazepam (9-nitro-6-phenyl-2,5-diazabicyclo [5.4.0] undeca-5,8,10,12-tetraen-3-one) is a type of [benzodiazepine](#) drug. It is a hypnotic drug used in the treatment of insomnia, anxiety, amnesia and [neurological disorder](#). It is one of the largest classes of abused pharmaceutical. Hence its determination becomes essential. Analytical methods like spectrophotometry[1-5], HPLC[6,7], GC[8], flow injection analysis[9], gas-liquid chromatography[10], polarography [11,12,13], colorimetry[14] were already reported. 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 (GC), 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 better candidate for being used as the modified electrode material [15-18]. In this work we have electrochemically determined

Nitrazepam using poly(3,4-ethylenedioxythiophene) (PEDOT) modified GCE.

Experimental Apparatus and Reagents

Electrochemical workstation (CH instruments, USA) was employed for all electropolymerization studies presented in this work. The stock solutions were made up in ultra pure water (SG, International, Germany). Stock solution of 0.001M of Nitrazepam was prepared in ethanol. For the electrochemical studies, Buffer tablets (4,7,9,2, Merck) H₂SO₄ and NaOH were used as the medium for the analysis. 3,4-ethylenedioxythiophene (EDOT) (Aldrich) and p-toluenesulphonic acid (Sigma) were used for electropolymerization.

Preparation of PEDOT modified electrode

PEDOT was obtained by the electrooxidation of the 0.01M EDOT solution in 0.1M p-toluene sulphonic acid by cycling the potential between -0.6 and +1.0V (Versus Ag/AgCl) at the scan rate of 0.05Vs⁻¹. Thickness of the films was controlled by the number of segments. After polymerization was over the electrode has been repeatedly washed with ultrapure water and used for further studies.

Result and discussion

Electrochemical Studies

The cyclic voltammetric behaviour of Nitrazepam was studied at different scan rates, concentrations and pH using glassy

carbon electrode (GCE) and PEDOT modified GCE and the respective cyclic voltammograms were recorded. The response was more in PEDOT modified GCE than bare (Fig.1). Hence detailed studies were carried in PEDOT modified GCE.

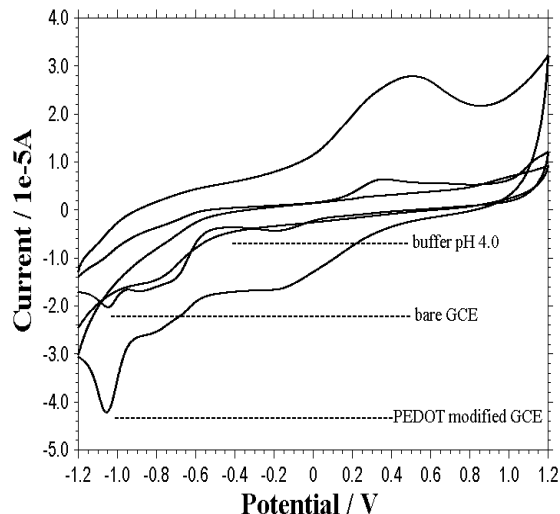


Fig.1. Cyclic voltammetric behaviour of 1mM Nitrazepam in pH 4 medium at a scan rate 100mV/S

Effect of Scan rate

As the scan rate was increased from 50 to 500 mV/S at a fixed concentration of nitrazepam the cathodic peak current and peak potential increased with increase in scan rate. Plots of peak current versus scan rate resulted in slightly curved line whereas the plot of peak current with square root of scan rate resulted in a straight line ($R^2 = 0.999$). This suggests diffusion controlled reduction reaction. The log peak current versus log scan rate plot also resulted in a straight line whose slope was found to be 0.3324. These factors confirm that the reduction of NITZ was a diffusion controlled cathodic reduction reaction. The absence of anodic peak in the forward scan along with the fractional αn value (0.4286) calculated from the slope of the straight-line in the plot of peak potential versus log scan rate indicate that the reduction is found to be irreversible.

Effect of pH

The influence of pH on the cyclic voltammetric behaviour of nitrazepam was studied at a scan rate of 100 mV/sec (Fig.2). Nitrazepam exhibits characteristic response in the acidic, neutral and basic pH media. Therefore the studies were carried out in the pH region 1.0 to 13.0. A well defined cathodic peak around -1.04V was obtained in all pHs. In the pH 1.0 additional one cathodic peak was obtained which was similar to the one reported by Rajeev et al., [13]. The intensity of the peak was very high for pH 4.0.

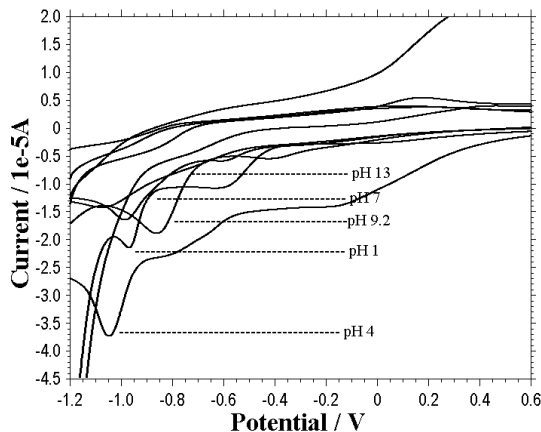
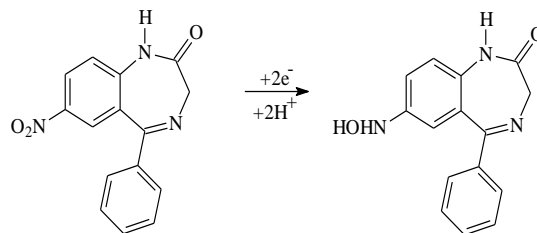


Fig 2. Effect of pH of 1mM Nitrazepam at a scan rate 100mV/S

Reaction mechanism

Chronocoulometric experiments were conducted for a concentration of 250ppb of NITZ in pH 4.0 on PEDOT modified GCE. Diffusion coefficient "D" was calculated from the plot of Q vs. $t^{1/2}$ (Fig.6.20) and it is around $2.378 \times 10^{-4} \text{ cm}^2/\text{s}$ at 25°C. Controlled potential coulometry was performed at pH 4.0. The coulometric 'n' value was determined after exhaustive electrolysis and it was found to be 2 for 250ppb NITZ solution. This indicates two-electron transfer in the electron reduction for nitrazepam. The polymer modification increases the peak current to certain extent. Hence, it is concluded that the PEDOT modification serves as a better electrode for analytical purpose. The above studies reveal that the reduction of nitrazepam is an irreversible, diffusion controlled, and two electron transfer reaction. On the basis of the above results, the following mechanism was proposed which was already reported [13,18].



Electroanalysis of Nitrazepam

Differential pulse stripping voltammetry (DPSV) is inherently one of the most sensitive electroanalytical techniques and has received a great deal of attention in recent years, primarily due to its lower limit of detection. Experiments were carried out to find the best accumulation parameters in the chosen pH medium 4.0 on PEDOT modified GCE. Upon observing some preconcentration stripping voltammograms performed for accumulation potentials (E_{acc}) varying from 0 to -600 on modified electrode at deposition time (DT) of 30s, it showed the best peak current for an accumulation potential -400mV. The deposition time was varied between 10 and 60 s.

Significant peak current was observed at 15 s. Above this deposition time, the peak current decreases due less electron transfer on the electrode surface. Thus, the accumulation potential and deposition time were chosen as optimum for further studies. The initial scan potential was varied from -100 to 300mV and the stripping peak current was measured. Higher peak current was observed at the initial scan potential of 0mV. Hence, it was chosen as the optimised IP for further studies. The pulse height was increased from 10 to 150 mV, the peak current was found to increase initially and then decrease. The maximum peak current was obtained at 50 mV. Hence it was chosen as the optimum condition. The effect of pulse width was studied from 10 to 110 ms. The maximum peak current was observed at 75 ms. Varying it from 2 to 10 mV potential scan increment was optimized. The best potential scan increment was 6mV. This was chosen as the optimum condition. All experimental results are presented in table 1.

Table1. Optimum experimental condition for the reduction of nitrazepam by DPSV

Parameters	Range studied	Optimum value
pH	1.0 to 13.0	4.0
Accumulation potential (mV)	0 to -600	-400
Accumulation time (Sec)	10 to 60	15
Initial scan potential (mV)	300 to -100	0
Pulse Height (PH) (mV)	10 to 150	50
Pulse width (PW) mSec	10 to 110	75
Pulse period (sec)	2 to 10	2
Scan Increment (SI) mV	2 to 10	6

The optimum conditions that resulted in good peak response were used to study the effect of analyte concentration (Fig.3).The stripping signal, under optimum experimental condition is influenced by the concentration of drug. As the concentration increased the stripping peak current also increased linearly. A calibration graph between the concentration of drug and peak current was made. The linear dependence of peak current with concentration was

understood from the straight-line with good correlation (Fig. 4).

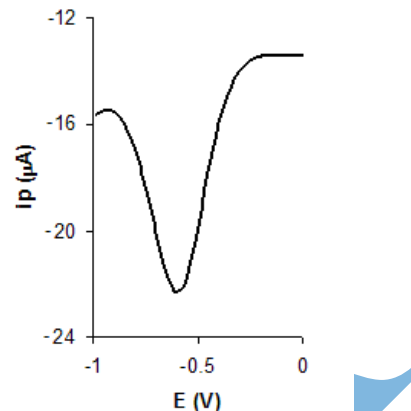


Fig.3 DPSV behaviour of 50ppb of NITZ on PEDOT

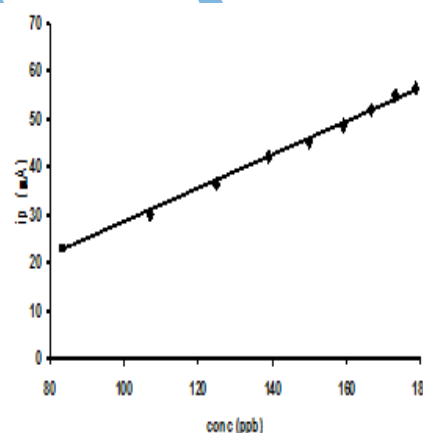


Fig.4. Calibration plot of effect of concentration on DPSV current

The range of determination and LOD was found and presented in table 2. The reproducibility of the stripping signal was understood from the relative standard deviation calculated for five identical measurements.

Table 2. Concentration range studied and LOD of NITZ on PEDOT /GCE

polymer	Range studied (ppb)	LOD (ppb)	% of RSD
PEDOT	83-178	10	2.5

CONCLUSION

In this work we have activated the electrode surface with PEDOT and used for the determination of antidepressant

Nitrazepam. The effect of pH and scan rate was studied. pH 4.0 was found to be best for the determination of Nitrazepam. From Chronocoulometric experiment the diffusion coefficient was calculated. The coulometric 'n' value was determined after exhaustive electrolysis and it was found to be 2. A systematic study of the experimental parameters that affect the differential pulse stripping voltammetric response was carried out and optimized conditions which yield maximum peak current were arrived. The range of determination and LOD was found 83-178ppb and 10ppb respectively. The reproducibility of the stripping signal was high. Hence the proposed method can replace tedious spectrophotometric or chromatographic methods.

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