POLYMER MODIFIED CARBON AND TITANIUM: ELECTROCHEMICAL BEHAVIOR OF TEMAZEPAM

Inam-ul-Haque[a,b] and Kiran Bano[c]

Department of Chemistry, University of Engineering and Technology Lahore 54890 Pakistan

[a]J-263, Defence Housing Authority, Lahore-54792 Pakistan

[b]Present Address: Institut für Physikalische und Theoretische Chemie, Universität Bonn, 53117 Bonn, Germany

[c]Present Address: School of Chemistry, Monash University, Clayton, Victoria 3800, Australia

ABSTRACT: Carbon disc electrodes have been modified with poly-p-aminophenol and copolymer of p-aminophenol and p-phenylenediamine respectively. Likewise same copolymer was also obtained at titanium electrode, evident by an orange brown film over the titanium disc. Linear sweep, cyclic voltammetric and differential pulse voltammetric studies revealed electrochemical activity of the benzodiazepines at polymers coatings in mixture of nitric acid and solution of temazepam in dichloromethane and 2:3 water alcohol mixture. Electro-reduction of temazepam showed linear variation of current with change in concentration from 0.1 to 3.6 mM at copolymer; similar trend has been observed at poly-p-aminophenol for the concentration ranging from 0 to 0.3 mM.

INTRODUCTION

Organic compounds containing 1,4-benzodiazepine fragments are widely used in clinical practice as psychotropic agents [1]. Temazepam (Restoril) is a benzodiazepine commonly prescribed for insomnia and other sleep disorders. The determination of benzodiazepines in biological fluids is object of great interest for many scientists. In the daily medical practice, it is necessary to perform drug analytical monitorization in order to adequate the dose of these substances to the necessities of the patients, and to avoid toxic effects. In forensic toxicology, benzodiazepines are often found in fatal cases of drug intoxication, as well as in the blood of drivers involved in traffic accidents. These reasons justify the necessity of developing new analytical methods for their determination. A whole electrochemical study of diazepam, temazepam and oxazepam using modified carbon-paste electrodes is reported. Using the optimal conditions for the voltammetric measurements of these pharmaceuticals, diazepam was determined in plasma and oxazepam in urine. Due to the complexity of these biological matrices, a previous solid-phase extraction (SPE) procedure was necessary to separate the active principles before their determination. The best results were obtained for the determination of oxazepam in urine [2]. Modification of solid electrodes by polymerization of monomers have been of great interest now days. Our one of such interest involves the use of poly-p-aminophenol coated electrodes that have been used for the production of hydroxylamine [3]. Such type of electrodes have also been used for the production reduction of nitrate and their detection by differential pulse voltammetry [4]. The electrochemical quantitative analysis of many drugs has been carried out by polarographic techniques, some of them are benzodiazepines (bromazepam, chlordiazepoxide, clonazepam, diazepam, dipotassium chlorazepate, flunitrazepam; flurazepam, lorazepam, medazepam, midazolam, nitrazepam, oxazepam, pinazepam, prazepam, temazepam, triazolam, clobazam), meprobamate, phenothiazine- thioxantene- and butyrophenone-derivatives, sulpirid, reserpine, imipramine, thioridazine, amitriptyline, imipramine, opipramol, doxepine, chlorpromazine, levopromazine, perazine, prothipendyl, chlorprothixene, triperidol, methamphetamine [5]. The behaviour of oxazepam in adsorptive stripping voltammetry was studied taking into account those conditions which have an influence on the accumulation step (electrolyte, pH, time, potential, drop size and stirring rate), rest time and stripping step (pulse amplitude and scan rate). Oxazepam can be determined at a hanging mercury drop electrode by differential-pulse voltammetry in 0.008 M Britton-Robinson buffer at pH 2.0 with a -0.50 V accumulation potential [6]. Also, lorazepam was determined by adsorptive stripping voltammetry in urine sample [7].

Alprazolam was identified by measuring their half-wave potentials and determined quantitatively by measuring the height of the polarographic peaks [8]. The electro oxidative behavior of loprazolam on a carbon-paste electrode was studied in the pH range 3.0-10.5 in aqueous solution using differential pulse, sampled direct current and cyclic voltammetry [9]. Direct square-wave voltammetry (SWV) and square-wave cathodic stripping voltammetry (SWCSV) at hanging mercury drop electrodes have been developed for determination of the psychoactive 1,4-benzodiazepine compounds clonazepam, bromazepam, midazolam, diazepam, medazepam, and flurazepam over a wide range of concentrations [10]. Diazepam and its major metabolites, nordazepam, temazepam and oxazepam, in human urine samples, were analyzed by liquid chromatography (LC)/tandem mass spectrometry (MS/MS) using a hydrophilic polymer column (MSpak GF-310 4B), which enables direct injection of crude biological samples. Matrix compounds in urine were eluted first from the column, while the target compounds were retained on the polymer stationary phase. The analytes retained on the column were then eluted into an acetonitrile-rich mobile phase using a gradient separation technique. All compounds showed base-peak ions due to [M+H]+ ions on LC/MS with positive ion electrospray ionization, and product ions were produced from each [M+H]+ ion by LC/MS/MS. Quantification was performed by selected reaction monitoring. All compounds spiked into urine showed method recoveries of 50.1-82.0%. The regression equations for all compounds showed excellent linearity in the range of 0.5-500 ng/mL of urine. The limits of detection and quantification for each compound were 0.1 and 0.5 ng/mL of urine, respectively. The intra- and inter-day coefficients of variation for all compounds in urine were not greater than 9.6%. The data obtained from actual determination of diazepam and its three metabolites, oxazepam, nordazepam and temazepam, in human urine after oral administration of diazepam, are also presented

[11]. A rapid CZE method was developed for the simultaneous determination of nine benzodiazepines in spiked beverages (nitrazepam oxazepam, alprazolam, flunitrazepam, temazepam, diazepam, 7-aminoflunitrazepam, 7-aminonitrazepam and 7-aminoclonazepam). The method employed a double-coated capillary coated with poly(diallyldimethylammonium chloride) and then dextran sulphate. The BGE conditions were 100 mM ammonium phosphate buffer, pH 2.5, which gave baseline resolution between consecutive peaks and a run time of less than 6.5 min. This method offers improvements in both resolution and run time, compared to those attained under analogous conditions with an uncoated capillary. The validated method was successfully applied to beverages that had been spiked with benzodiazepines at concentrations simulating

EXPERIMENTAL

Chemicals and Materials:

Following chemicals were used:

Temazepam, Novartis; Dichloromethane, Reidel-de-Haen; Nitric acid, BDH; Perchloric acid, Merck; Ethanol, Merck

Instrumentation:

Polymerization was carried out with eDAQ Echem v2, 0.9. Controls of the potentiostat and data acquisition were accomplished using eDAQ electrochemistry research software on a dedicated PIV microprocessor coupled to the potentiostat. Voltammetry was done with Versastat II (PARC) coupled to a PII personal computer for data acquisition. Three-electrode configuration was used including Ag/AgCl, Cl^{-1} as reference electrode and platinum spiral as auxiliary electrode, Carbon and titanium electrodes were used as working electrode. All experiments were carried out at room temperature (30±2 ºC)

Working electrode: Coated carbon electrode (0.1256 cm^2) and coated titanium disk electrode (0.05 cm^2)

Reference electrodes: Two types of reference electrodes were used:

Ag/AgCl, saturated KCl: Silver wire was coated with silver chloride by providing potential of 0.21 for 45 min in 10% HCl solution. Then the inner reference compartment with a finer sinter at its one end was filled with saturated KCl solution. The outer bridge also with a sinter was filled with back-ground electrolyte.

Calomel: Saturated Calomel Electrode was used for the experiments of titanium.

Counter electrode: Before introducing electrodes into the cell for experimentation, the platinum counter electrode was properly washed with nitric acid followed by distilled water and dried.

Electropolymerization

Polymerization of p-aminophenol

Carbon disk electrodes were coated with poly-p-aminophenol by reduction of 6.5 mM of the p-aminophenol solution in 1.0 M HNO_3 using 30 cycles of cyclic voltammetry. Potential cycling was done between -0.1 to -1.2 V at scan rate 100 mV/s.

Co-polymerization of p-aminophenol and p-phenylenediamine at carbon and titanium electrodes:

Carbon disk electrode was coated with poly-p-aminophenol by reduction of 100 mM each of the p-phenylenediamine and p-aminophenol solution in 1.0 M HNO_3 using 30 cycle cyclic voltammetry. Potential cycling was done between -0.1 to -1.2 V at scan rate 100 mV/s. Similarly, titanium electrodes were coated.

Extraction of temazepam:

From dichloromethane: Restoril capsules contents (0.5 g) were weighed and dissolved in dichloromethane and volume was made upto 25 mL.

From ethanol: Restoril capsules contents (500 mg) were weighed and dissolved in 3:2 ethanol:water mixture 25 mL.

Voltammetric experiment:

Different voltammetric experiments like linear sweep voltammetry, cyclic voltammetry, differential pulse voltammetry were carried out.

RESULT AND DISCUSSION

Electrodeposition of copolymer at carbon was achieved by running 80 consecutive cycles in mixture of p-aminophenol and p-phenylenediamine in cathodic direction over the potential range -0.1 V to -1.0 V. Electroreduction of the mixture was found to be reversible as characterized by an anodic wave at around -0.36 V and its anodic counter part appearing at -0.24 V. There was a decrease in cathodic peak and anodic peak currents in successive cycles, thereby depicting formation of a film over the electrode surface, Figure 1.

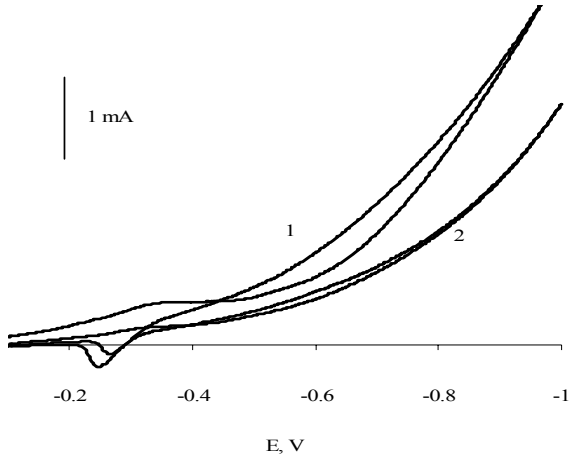

Fig. 1: Cyclic voltammetric curves at carbon cathodes from polymerization mixture, 100mM each of *p*-aminophenol and *p*-phenylenediamine in 1.0 M HNO_3, 1st cycle (1) 80th cycle (2); Scan rate, 100 mV. Potential vs. Ag / AgCl, Cl^{-1}.

The over-potential for the hydrogen evolution at modified carbon disc electrodes was investigated by running a polarization curve in 1.0 M nitric acid; parallel experiments were carried out using bare carbon disc for comparative studies as revealed by Figure 2. Hydrogen evolution at coated electrodes was found to occur at high over potential compared to that at bare carbon which indicates copolymer coatings to be better cathode material.

Electro-reduction of temazepam at copolymer modified carbon electrode

Reduction of temazepam at bare carbon electrode showed no redox response; however noticeable reductive and anodic waves were observed at polymer modified carbon electrode as depicted in Figures 3 and 4, respectively.

Redox studies were carried out by varying the potential sweep rate from 0.05 V/s to 0.35 V/s. Scan rate dependence of cathodic and anodic currents on scan rate is represented as Table 1. Variation of current function with natural log of sweep rate is shown as Fig. 5, which features two linear regions. There is initial rapid fall in the current function consistent with a rapid follow-up reaction subsequent to electro-reduction of temazepam. The second approximately linear region has a somewhat smaller slope compared to the corresponding value for the first linear region.

However, Figure 6 shows linear variation of cathodic current with increasing concentration of temazepam ranging from 0.17 mM to 3.60 mM; with linear regression value, R^2 = 0.9574.

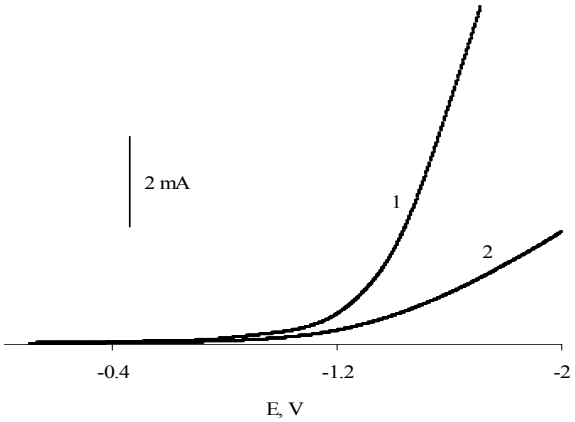

Fig. 2: Linear sweep voltammetric response in 1.0 M HNO_3 at (1) bare carbon, and at films of poly-*p*-aminophenol and *p*-phenylenediamine electrodeposited on carbon by (2) eighty successive cyclic voltammetric scans, Scan rate, 100 mV/s, Potential vs. Saturated Calomel electrode.

Table 1: Variation of cathodic and anodic current at 740 mV and 400 mV respectively with scan rate at poly-*p*-aminophenol-*p*-phenylenediamine electrodeposited on carbon in 2.3 mM temazepam's hydroalcoholic solution in 1.0 M HNO_3.

υ mV/s	Log (υ) (mV/s)	$\upsilon^{-1/2}$ $(mV/s)^{-1/2}$	Ic mA	Ia mA
50	1.698	7.07	254.1	64.15
75	1.875	8.66	243.7	50.13
100	2.00	10.00	214.8	66.29
125	2.096	11.18	230.6	47.00
150	2.17	12.24	235.7	46.73
200	2.30	14.12	246.8	51.66
250	2.39	15.81	1469.2	66.08
300	2.477	17.32	1458.7	43.34
350	2.44	18.70	1442.4	47.50

Electrode coatings obtained after 30 scans between -0.1 and -1.2 V in 100 mM each of *p*-aminophenol and *p*-phenylenediamine, Potential vs. Saturated Calomel electrode.

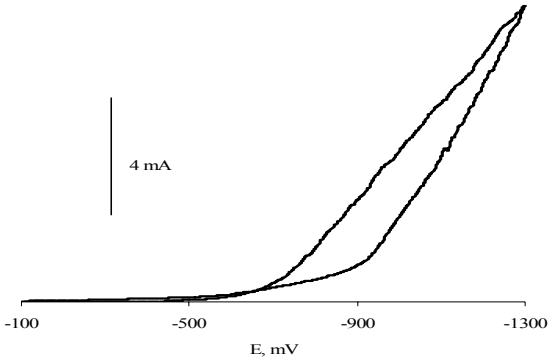

Fig. 3: Cyclic voltammogram at bare carbon in 0.17 mM hydroalcoholic solution of temazepam in 1.0 M HNO_3. Electrode coatings obtained after 30 scans between -0.1 and -1.2 V in 100 mM each of *p*-aminophenol and *p*-phenylenediamine, Scan rate 100 mV/s, Potential vs. Saturated Calomel electrode.

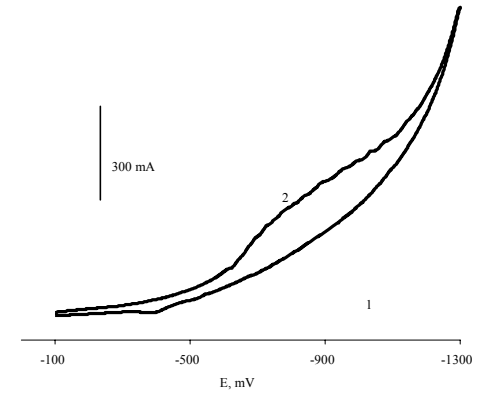

Fig. 4: Cyclic voltammogram in 2.3 mM temazepam's hydroalcoholic solution in 1.0 M HNO_3; at poly-*p*-aminophenol-*p*-phenylenediamine electrodeposited on carbon. Electrode coatings obtained after 30 scans between -0.1 and -1.2 V in 100 mM each of *p*-aminophenol and *p*-phenylenediamine, Scan rate 100 mV/s, Potential vs. Saturated Calomel electrode. Curve 2 only is shown. Background curve 1 is not shown.

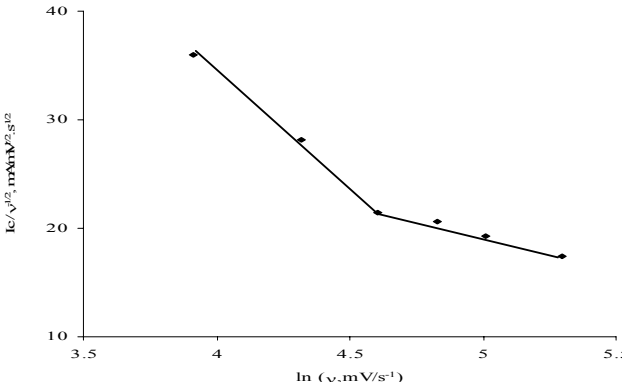

Fig. 5: Current function versus natural log of sweep rate plot for the cathodic linear sweep scan of 2.3 mm temazepam's hydroalcoholic solution in 1.0 m nitric acid. Electrode coatings obtained after 30 scans between -0.1 and -1.2 v in 100 mm each of *p*-aminophenol and *p*-phenylenediamine, scan rate 100 mv/s, potential vs. Saturated calomel electrode. Line is drawn to aid the vision, and is not a fit.

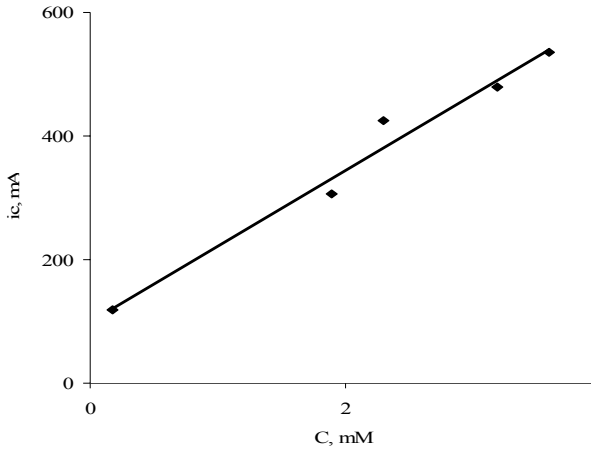

Fig. 6: Variation of cathodic current with concentration of temazepam's hydroalcoholic solution in 1.0 M HNO_3; at poly-*p*-aminophenol-*p*-phenylenediamine electrodeposited on carbon in 1.0 M HNO_3. Electrode coatings obtained after 30 scans between -0.1 and -1.2 V in 100 mM each of *p*-aminophenol and *p*-phenylenediamine, Potential vs. Saturated Calomel electrode, y = 122x + 100.54, R^2 = 0.9748

Electrocopolymerization at titanium electrodes

Electrocopolymerization of *p*-aminophenol and *p*-phenylenediamine at titanium cathode after 50 cycles leads to the formation of an orange brown film of copolymer at titanium cathode. Figure 7 shows the 1st cycle (1) at titanium electrodes and after 50 cycles (2). Gradual decrease of current from 1st to 50th cycles thereby depicts the formation of a co-polymer film over the surface of electrode. Titanium surface before and after the polymerization can also be seen as the insets in Figure 7.

Polarization curve was run at polymer coated electrode and compared with that of bare titanium electrode in Figure 8. It can be observed from the figure that at bare titanium there was a considerable noise after -1.2 V, indicating damage of electrode surface at potential more than -1.2 V, whereas in case of copolymer-coated titanium electrode the enhanced surface stability is observed even at higher potential up to -1.7 V.

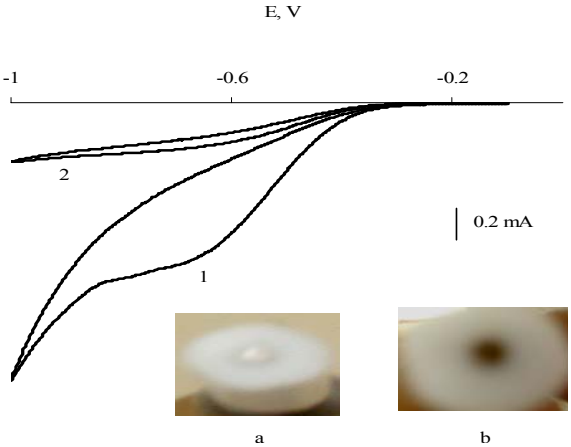

Fig. 7: Cyclic voltammetric curves at titanium cathodes from polymerization mixture, 100mM each of *p*-aminophenol and *p*-phenylenediamine in 1.0 M HNO_3, 1st cycle (1) 50th cycle (2); Electrode surface is shown as inset before (a) and after (b) electropolymerization, Scan rate, 100 mV. Potential vs. Ag / AgCl, Cl^{-1}.

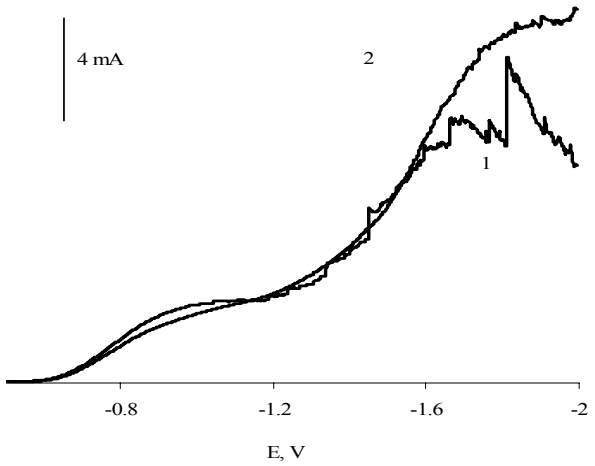

Fig. 8: Linear sweep voltammetric response in 1.0 M HNO_3 at (1) bare carbon, and at films of poly-*p*-aminophenol and *p*-phenylenediamine (2). Films electrodeposited on carbon by fifty successive cyclic voltammetric scans between -0.1 to -1.0 V, Scan rate, 100 mV/s, Potential vs. Saturated Calomel electrode.

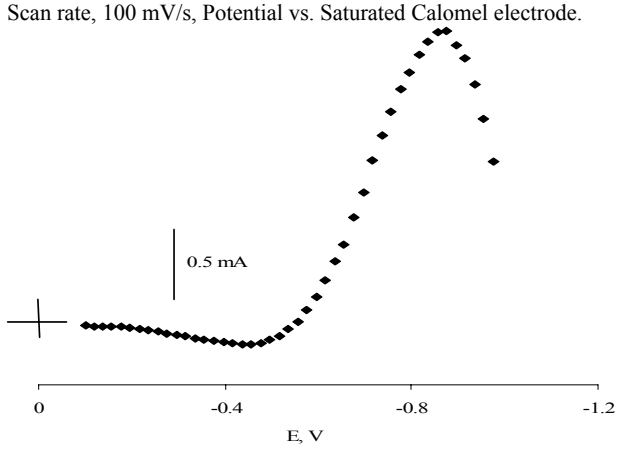

Fig. 9: Background subtracted linear scan current potential curve at poly-p-aminophenol coated carbon electrodes for the reduction of 0.19 mM temazepam in 1.0 M $HClO_4$ + 20 mM HNO_3. Electrode coatings were obtained after 30 scans between -0.1 and -1.2 V in 6.5 mM p-aminophenol in 1.0 M HNO3 at scan rate 100 mV/s. Potential vs. Ag / AgCl, Cl^{-}.[1]

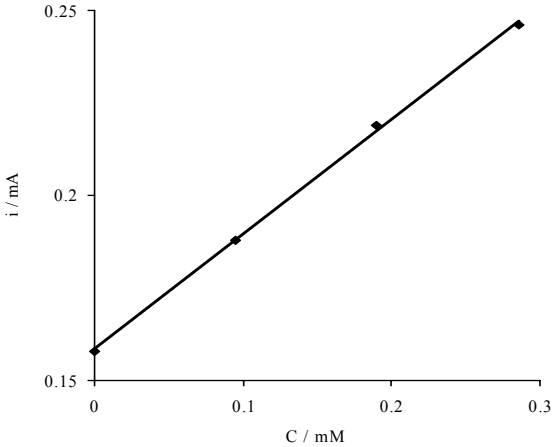

Fig. 10: Linear sweep voltammetric current at poly-*p*-aminophenol coated carbon electrodes at -0.65 V vs. concentration of temazepam in 1.0 M $HClO_4$ + 20 mM HNO_3. y = 0.0295x + 0.1584, R^2 = 0.9992. Coating conditions as per figure 8.

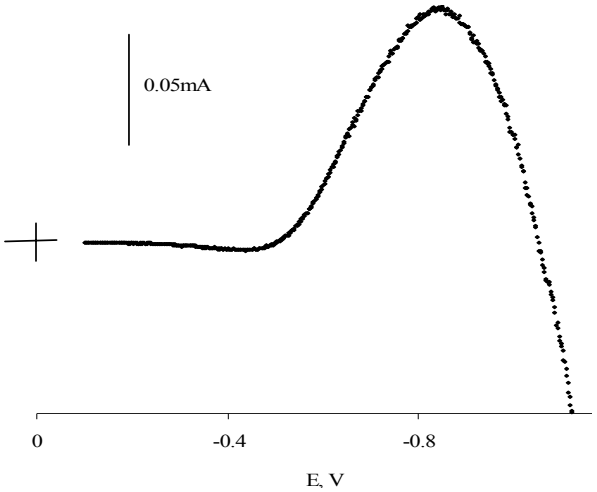

Fig. 11: Background subtracted differential pulse voltammograms at poly-*p*-aminophenol coated carbon electrodes for the reduction of 0.19 mM temazepam in 1.0 M $HClO_4$ + 20 mM HNO_3. Potential vs. Ag/AgCl, Cl^{-1} at scan rate 5 mV/s, pulse height 70 mV, step time 0.4 s, pulse width 50 ms. Coating conditions as per figure 8.

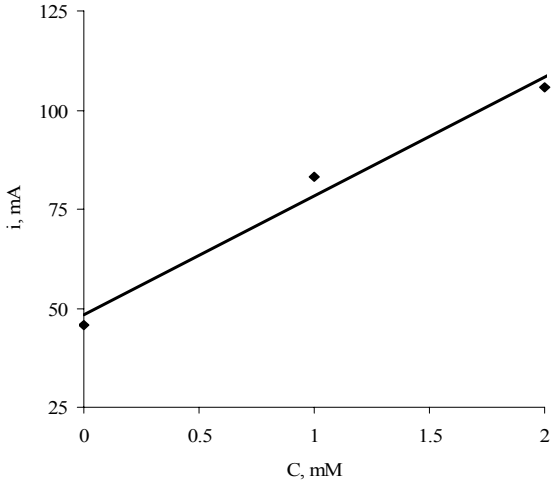

Fig. 12: Differential pulse voltammetric currents vs. concentration of temazepam in 1.0 M $HClO_4$ + 20 mM HNO_3 at *p*-aminophenol modified carbon electrodes, Potential vs. Ag/AgCl, Cl^- at scan rate 5 mV/s, pulse height 70 mV, step time 0.4 s, pulse width 50 ms. Coating conditions as per figure 8. $y = 30.07x + 48.207$, $R^2 = 0.9805$

ACKNOWLEDGMENTS

Facilitation of this work by University of Engineering and Technology, Lahore-Pakistan is gratefully acknowledged. Authors are indebted to Japan International Co-operation Agency (JICA) for the gift of the potentiostat. IUH acknowledges gracious hospitality of Prof Dr Helmut Baltruschat, Institut für Physikalische und Theoretische Chemie, Universität Bonn, Germany.

REFERENCES

1. E.I. Korotkova, E.A. Mamaeva, N.V. Bashkatova and A.A. Bakibaev, *Pharm. Chem. J.*, **38**, 52 (2004).
2. M.E.L.- Chaves, J.M.P.- Santander, L.M.C.- Aguilera, I.N.- Rodríguez and J.L.H.-H.-d.-Cisneros, *Sensors Actuators B*, **115,** 575 (2006).
3. L. Halaoui, H. Sharifian and A.J. Bard, *J. Electrochem. Soc.*, **148**, 386-393 (2001).
4. I. U. Haque and K. Bano, *Electrochem. Soc. Trans.*, **6**, 57 (2007).
5. H. Oelschläger, *Bioelectrochem. Bioenerg*., **10,** 25 (1983).
6. A. Zapardiel, J.A.P. López, , E. Bermejo, L. Hernández and M. Chicharro, *Anal. Chim. Acta*, **244**, 49 (1991).
7. P. Rivera, E. Bermejo, A. Zapardiel, J.A.P.- Lopez and L. Hernandez, *Electroanalysis*, **3**, 399 (1991).
8. A.H.R. Habeeb, J.R. Procopio and L. Harndez, *Microchem. J*., **40**, 341 (1989).
9. M.J. Arenaza, B. Gallo, L.A Berrueta and F. Vicente, *Anal. Chim. Acta*, **305**, 91 (1995).
10. M.M.C.D. Santos, V. Famila and M.L.S. Gonçalves, *Anal. Bioanal. Chem.*, **374**, 1074 (2002).
11. H. Umezawa, X.P. Lee, Y. Arima, C. Hasegawa, A. Marumo, T. Kumazawa and K. Sato, *Rapid Commun. Mass Spectrom*., 22, 2333 (2008).
12. R. Webb, P. Doble and M. Dawson, *Electrophoresis*, **28**, 3553 (2007).