Int. J. Electrochem. Sci., 3 **(2008)** 711 - 720

International Journal of ELECTROCHEMICAL SCIENCE
www.electrochemsci.org

Electrochemical Studies of Buzepide Methiodide and their Analytical Applications

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Received: 23 February 2008 / *Accepted:* 28 March 2008 / *Online published:* 20 April 2008

The electrochemical behavior of buzepide methiodide (BZP) was investigated by cyclic, linear sweep and differential pulse voltammetric (DPV) techniques. The electrochemical studies were carried out on a glassy carbon electrode at room temperature. BZP showed a pair of quasireversible peaks. The effect of peak current and peak potential on scan rate, pH, concentration and different electrolytes was investigated. The oxidation and reduction currents were observed to be diffusion controlled between the scan rates of 5 to 500 mV s^{-1} and 5 to 50 mV s^{-1}, respectively. The oxidation and reduction currents of BZP were observed to be pH dependent. Bulk electrolysis was performed to obtain the oxidized product and the same was identified by IR spectroscopy. Probable mechanism was proposed for redox reaction of BZP. Considering the sensitivity, anodic peak was selected for analytical application. Highly resolved diffusion controlled oxidation peak was noticed in Britton-Robinson (BR) buffer of pH 2.5. An analytical method with adequate precision and accuracy was developed for the determination of BZP with the detection limit of 1.13 x 10^{-6}. A linear response of current-concentration was obtained in the range of 1.6 x 10^{-6} to 1.7 x 10^{-5} M for DPV with a correlation coefficient value of 0.9978. The proposed procedure was successfully applied to the determination of BZP in tablets.

Keywords: Buzepide methiodide; electrochemical studies; Differential pulse voltammetry; Pharmaceutical formulation

1. INTRODUCTION

BZP chemically known as (1-(4-amino-4-oxo-3,3-diphenylbutyl)hexahydro-1- methyl-1H-azepinium iodide) is an antidepressant [1]. It is also used in the treatment of functional intestinal disorders in combination with haloperidol [2]. In a metaanalysis of Randomezed Clinical Trial (RCT) it was concluded that antidepressants may be effective in reducing Irritable Bowel Syndrome (IBS) symptoms in about one-third of patients [3]. Since BZP is an antidepressant, attempts have been made

to assess its effect to treat the IBS [4]. The mechanism of action of mostly tricyclic antidepressants in the treatment of IBS was probably related to their central effect and the anticholinergic properties [5].

In recent years, the electrochemical techniques have led to the advancement in the field of analysis because of their sensitivity, low cost and relatively short analysis time when compared with other techniques. Electrochemical methods have proven to be useful for development of very sensitive and selective methods for the determination of organic molecules including drugs. Addition application of electroanalytical techniques includes the determination of electrode mechanisms. Redox properties of drugs can give insights into its metabolic fate or their *in vivo* redox processes or pharmaceutical activity [6-8].

Only two HPLC methods for the assay of BZP have been reported [9,10]. These methods work out at higher concentration of the drug. Critical literature survey revealed that no attempt has been made so far to investigate the electrochemical behavior of BZP. This prompted us to examine the electrochemical behavior of BZP on glassy carbon electrode by cyclic, linear sweep and DPV techniques and to develop an electroanalytical procedure for its determination in pharmaceutical formulations.

2. EXPERIMENTAL PART

2.1. Apparatus

Electrochemical studies were carried out using a CHI-1110a electrochemical Analyzer (CH Instruments Ltd. Co., USA, version 4.01) electrode system, including a glassy carbon electrode (3mm diameter) as the working electrode, a platinum wire as a counter electrode, and an Ag/AgCl (3 M KCl) as reference electrode. For improved sensitivity, resolution and reproducible results, the glassy carbon electrode was polished with aqueous slurry of alumina powder (0.3 micron) on a smooth polishing cloth before each measurement. All reported potentials are against Ag/AgCl (3 M KCl)

The pH measurements were made on a Schott Gerate pH meter CG 804.

The DPV conditions were: pulse amplitude, 50 mV; pulse width, 30 ms; scan rate, 20 mVs^{-1}.

2.2. Reagents

A stock solution of BZP (1mM) was prepared in methanol-water (10:90, v/v) and stored in a refrigerator at 4 ^{0}C. Standard solutions were prepared by diluting the stock solution with a selected supporting electrolyte. All the chemicals used were of Analar grade. In the present study, three different electrolytes were used namely sulphuric acid (pH 1-2). 0.2 M Acetate buffer (pH=3.5-5.6) and 0.04 M Britton-Robinson (pH=2.5-10) were prepared in doubly distilled water. All measurements were carried out at room temperature.

2.3. Assay of Tablets

The BZP tablets were obtained from local commercial sources. The amounts labelled of BZP are 1 mg per tablet. Ten tablets were weighed accurately and ground to a fine powder. A portion of

the powder equivalent to 1 mM BZP was transferred to a 100 ml volumetric flask and completed to volume with distilled water containing 10 % methanol and sonicated for 15 min to effect complete dissolution. The sample from the clear supernatant liquor was withdrawn and quantitatively diluted with the selected supporting electrolyte. The content of the drug in tablet was determined referring to the calibration graph or regression equation.

3. RESULTS AND DISCUSSION

3.1. Voltammetric Behavior of BZP

Fig. 1 shows the molecular structure of BZP. Fig. 2a represents a cyclic voltammogram of 1.2 x 10^{-5} M BZP in BR buffer of pH 2.5 using glassy carbon electrode in the potential range of -0.1 to +1.0 V, with a potential sweep rate of 10 mV s^{-1}. BZP exhibited a pair of quasireversible peaks, with the oxidation peak potential of E_{pa} = 0.540 V and reduction peak potential at around E_{pc} = 0.306 V. Fig. 2b shows linear sweep voltammogram of BZP at a scan rate 100 mV s^{-1}.

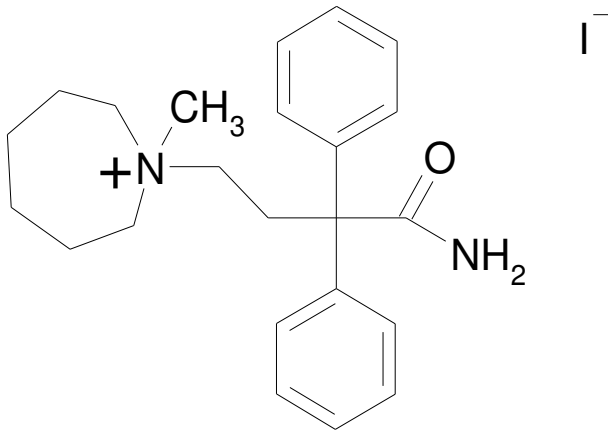

Figure 1. The molecular structure of BZP.

The effect of scan rate (ν) on peak currents (i_{pa} and i_{pc}) and peak potential (E_{pa} and E_{pc}) of BZP was studied. A linear relationship was observed between the oxidation peak current and the square root of the scan rate with a significant correlation coefficient of 0.9975 indicating thereby that the electrode process is diffusion-controlled between the scan rate of 5 and 500 mV s^{-1}. The anodic peak current becomes adsorption controlled after the scan rate of 500 mV s^{-1} [11]. The corresponding equation is,

$$i_{pa}\ (\mu A) = 0.363\ \nu^{1/2}\ (mV\ s^{-1})^{1/2} - 1.65\ ;\ R^2 = 0.9975$$

Cyclic voltammograms of 1.2 x 10^{-5} M BZP at different scan rates were recorded and are shown in Fig. 3. The reduction peak of BZP was observed to be diffusion controlled only between the scan rates of 5 and 50 mV s^{-1}. Further, it was noticed that the reduction peak became broader and

almost disappeared at higher scan rates. The plot of log of peak current (log i_{pa}) *versus* log of scan rate (log ν) yielded a slope value of 0.45 in the scan rate range of 5-500 mV s^{-1}. This was close to the theoretical value of 0.5 reported for an ideal reaction for the diffusion-controlled electrode process [12]. The corresponding equation could be shown as

$$\log i_{pa}\ (\mu A) = 0.457 \log \nu\ (mV\ s^{-1}) - 0.097,\ R^2 = 0.996,$$

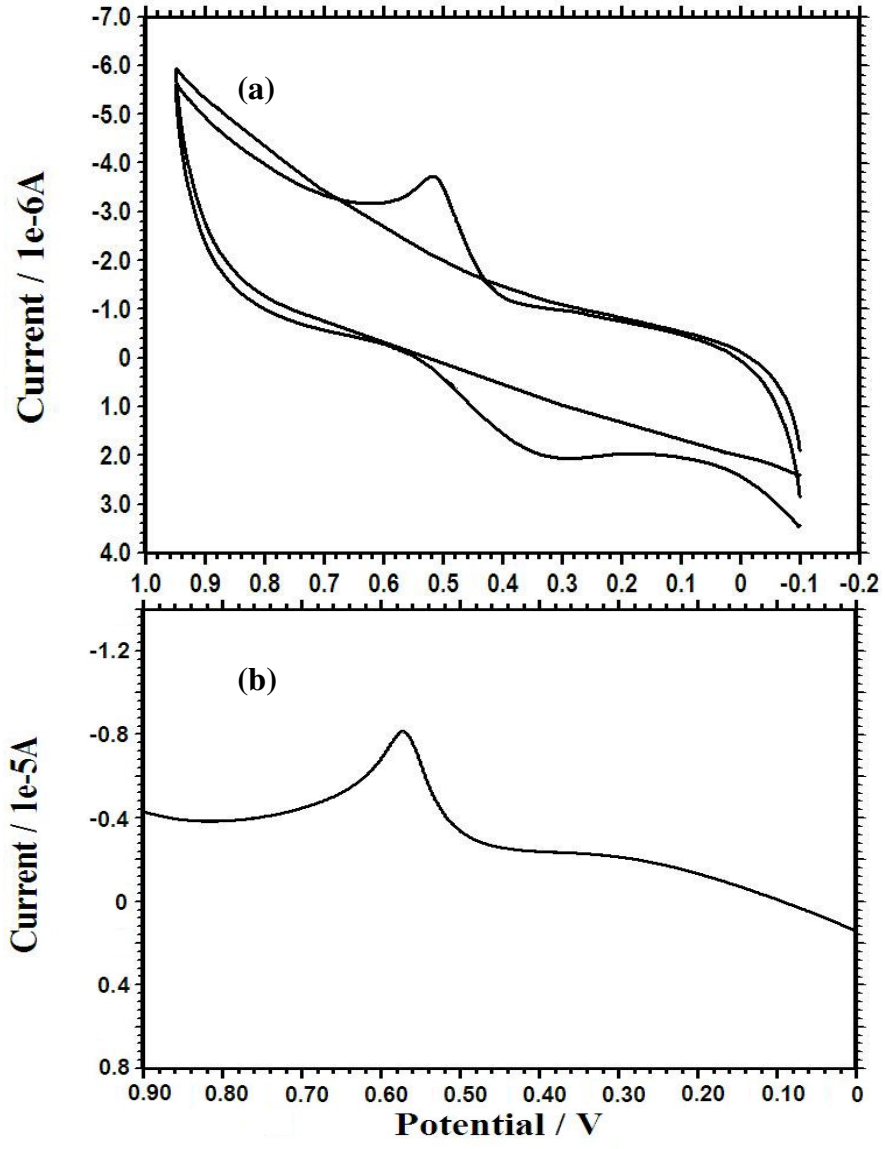

Figure 2. (a) Cyclic voltammograms of 1.2 x 10^{-5} M BZP in BR buffer at pH 2.5 and a scan rate 10 mV s^{-1} (1) and blank (2). (b) Linear sweep voltammogram of BZP at a scan rate 100 mV s^{-1}.

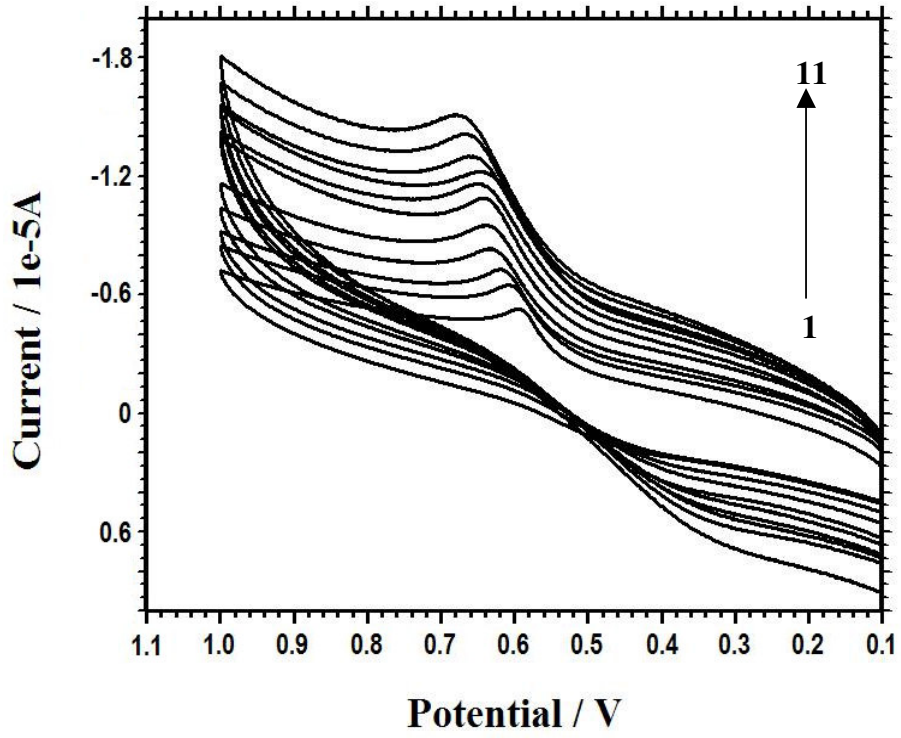

Figure 3. Cyclic voltammograms of 1.2 x 10^{-5} M BZP in BR buffer of pH 2.5 at different scan rates: 50 (1) 80 (2) 100 (3) 150 (4) 200 (5) 250 (6) 300 (7) 350 (8) 400 (9) 450 (10) and 500 mV s^{-1} (11).

The electrochemical behavior of a drug may depend on pH of the medium. Hence, the electrochemical behavior of BZP was investigated over a pH range of 1-10. It was observed that the BZP was oxidized in the pH range of 1-7.4 and no oxidation peak was observed beyond pH 7.4. Further, the anodic peak potential was shifted towards more positive potential (Fig. 4) with increase in pH indicating thereby that the pH of the supporting electrolyte exerts a significant influence on electrooxidation of BZP at the glassy carbon electrode. A good linear relationship between E_{pa} and pH of the medium at glassy carbon electrode was noticed and the same is represented as

$$E_{pa} = 1044.3 - 58.2 \text{ pH mV}^{-1}, \ R^2 = 0.991$$

The slope value of 58.2 mV per pH, being close to the expected value of 59 mV per pH indicates that the number of protons and electrons involved in the oxidation of BZP is equal [13].

The cathodic peak was observed in the pH range of 1-4.5. It was almost disappeared beyond pH 4.5 thereby revealing that the reduction of the oxidized product of BZP was possible only in strong acidic condition. Therefore, the oxidation of BZP becomes irreversible at pH above 4.5. The redox behavior of BZP in different electrolytes like H_2SO_4, BR, phosphate and acetate buffer was examined. For anodic peak, dramatically decreased current responses with peak broadening were observed in electrolytes other than BR. The cathodic peak was noticed to be very broad and undetectable in H_2SO_4, phosphate buffer and in acetate buffer. Therefore, we selected BR buffer as a suitable

electrolyte for electrochemical studies. The influence of concentration of BZP on peak currents at glassy carbon electrode was also studied. It was found that the plot of i_{pa} *versus* concentration showed linearity over the drug concentration range of 6 x 10^{-6} M - 1.6 x 10^{-5} M (from CV) suggesting further that the electrode process was diffusion controlled [14,15]. The number of electrons involved in the redox reaction was calculated to be 2 using the equation shown below [16].

$$i_{pa} = (2.69 \times 10^5)\, n^{2/3}\, A\, D_o^{1/2}\, \nu^{1/2}\, C_o^*$$

where, n = number of electrons, A = area of the electrode, D_o = diffusion coefficient, ν = scan rate, C_o^* = concentration of electroactive species. This was useful to predict the mechanism of redox process of BZP.

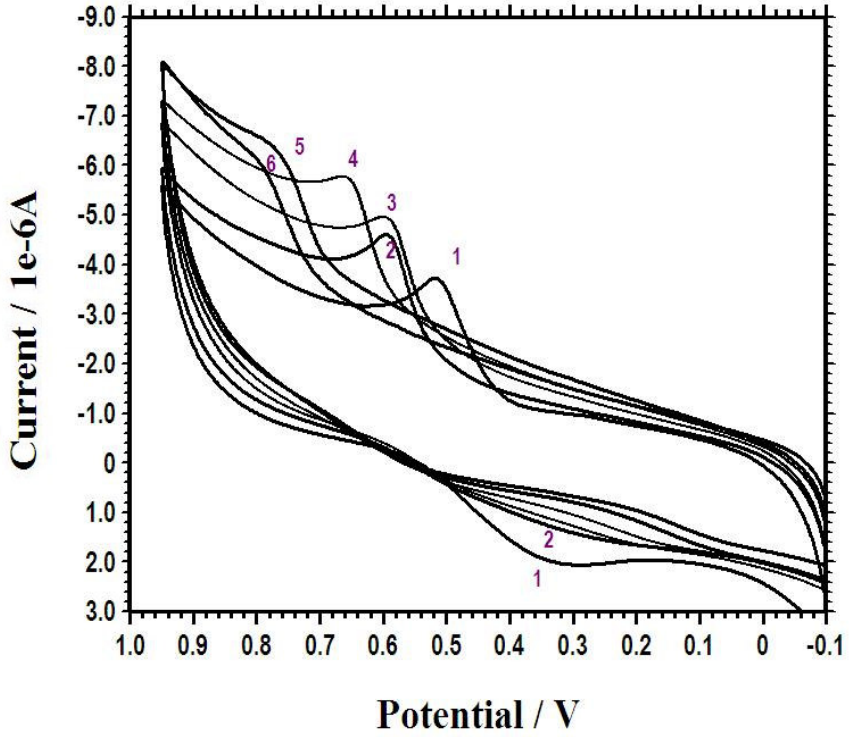

Figure 4. Cyclic voltammogram of 1.2 x 10^{-5} M of BZP in BR buffer of pH 2.5 (1), 3.5 (2), 4.5 (3), 5.5 (4), 6.5, (5) and 7.4 (6) at a scan rate 10 mV s^{-1}.

In the reaction condition, the amide functional group can be easily oxidized to carboxylic acid. The -$CONH_2$ present in BZP was found to be oxidized to -COOH. Bulk electrolysis of 1.2 x 10^{-5} M BZP in BR buffer of pH 2.5 was performed at glassy carbon working electrode with a larger surface area. For this, the electrolysis potential was fixed at 0.70 V and a total charge of 2.965 C was passed for 5 h. The completion of the reaction was examined by taking the DPV of the solution after 5 h. No oxidation peak was obtained after the bulk electrolysis indicating that almost all the BZP has been oxidized. All measurements were carried out at ambient temperature (25 ^{0}C). Oxidized products were isolated and separated in a column. The oxidized product was further identified by IR spectral data.

The obtained IR spectral results were in good agreement with that of the proposed oxidized product. A medium intensity broad band observed in the region of 3238-3285 cm^{-1} was attributed to ν (NH) vibrations in BZP and the same band was disappeared in the oxidized product with the formation of a new broad peak at 3430 cm^{-1} due to intramolecular H-bonded-OH of carboxylic group. This confirmed the replacement of amine group by O-H in the product. A medium intensity sharp band observed at 1620 cm^{-1} due to C=O in BZP was noticed to be shifted to higher region (1690 cm^{-1}) due to O-H group in the oxidized product. Further, a medium intensity broad band of CH_3 group was noticed at 2935 cm^{-1} in BZP and oxidized product. Based on foregoing discussion, the probable reaction mechanism as shown in Fig. 5 was proposed.

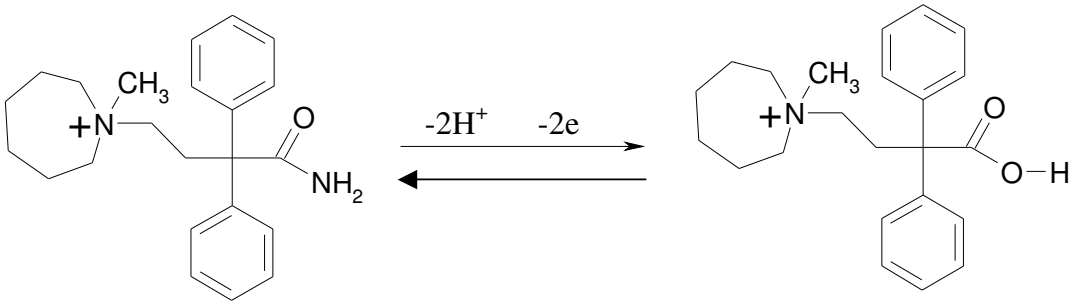

Figure 5. Probable reaction mechanism.

3.2. Optimization of measurement conditions

The influence of several electrolytes (H_2SO_4, BR, phosphate and acetate buffers) on analytical signal was studied using different electroanalytical techniques. Considerably improved sensitivity could be achieved with the application of DPV. The peak height and sensitivity of the anodic peak was observed to be comparatively better than the cathodic peak. Hence, the anodic peak was chosen for analytical applications. Further, broad and ill-defined peaks were observed with positive shift in peak potential with increase in pH. However, sharper and better-defined anodic curves were obtained in BR buffer of pH 2.5. Hence, we have carried out the studies at pH 2.5 in subsequent work.

The measurement conditions were optimized by observing the variation of the peak current with pulse amplitude, pulse width and scan rate. The best peak was observed with 50 mV pulse amplitude, 30 ms pulse width and 20 mV s^{-1} scan rate. With increasing pulse amplitude from 25 to 90 mV, the peak current was observed to be increased while the peak became broader and ill defined. However, the peak current decreased as the pulse width increased from 30 to 90 ms. The peak current increased linearly with the scan increment up to 20 mV s^{-1}.

3.3. Validation of the analytical procedure

An analytical method was developed involving DPV for the determination of the drug. For this, the variation of peak current (i_{pa}) with the concentration of BZP was investigated. The DPV of

different concentrations of BZP are shown in Fig. 6. Under the optimized experimental conditions, a linear relationship between the peak current of BZP and concentration was noticed in the range of 1.6 x 10^{-6} - 1.7 x 10^{-5} M. In this concentration range, the response was diffusion controlled. Above this concentration, loss of linearity was noticed and this was probably due to the adsorption of BZP on the electrode surface. The analytical characteristics of the calibration plot are summarized in Table 1. Validation of the optimized procedure for the quantitative assay of BZP was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), accuracy, precision and recovery. LOD and LOQ were calculated based on the peak current using the following equations shown below [17,18].

$$LOD = 3\ s/m;\ LOQ = 10\ s/m$$

where s is the standard deviation of the peak current (five runs) and m is the slope of the calibration curve. The LOD and LOQ values were calculated to be 1.13 x 10^{-6} M and LOQ 1.5 x 10^{-6} M, respectively. Low values of both LOD and LOQ values confirmed the sensitivity of the proposed method. The process of validation within-day variations was studied by analyzing six replicates of 3.0 x 10^{-6} M, 6.0 x 10^{-6} M and 1.1 x 10^{-5} M BZP. The values of relative standard deviations were calculated to be 1.09, 1.73 and 1.94 % respectively. The RSD values for intra-day assay for 5 x 10^{-6} M and 1.2 x 10^{-5} M solutions (n = 6) were found to be 1.21 and 1.43 % indicating good reproducibility of the method. The corresponding results are shown in Table 1.

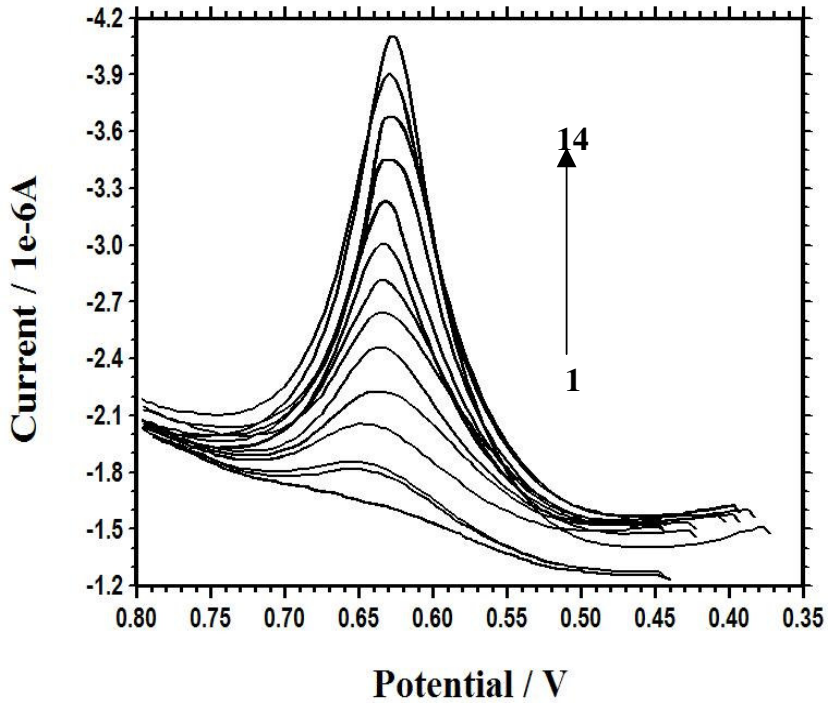

Figure 6. DPV for the increasing concentrations of BZP in BR buffer at pH 2.5. Scan rate, 20 mV s^{-1}; pulse amplitude, 50 mV and pulse width, 30 ms. Blank (1); BZP concentration : 1.6 x 10^{-6} (2), 2.0 x 10^{-6} (3), 3.0 x 10^{-6} (4), 4.0 x 10^{-6} (5), 5.0 x 10^{-6} (6), 6.0 x 10^{-6} (7), 7.0 x 10^{-6} (8), 8.0 x 10^{-6} (9), 9.0 x 10^{-6} (10), 1.0 x 10^{-5} (11), 1.1 x 10^{-5} (12), 1.2 x 10^{-5} (13), and 1.3 x 10^{-5} M(14).

Table 1. Characteristics of BZP calibration plot

	DPV
Linearity range (M)	1.6×10^{-6} to 1.7×10^{-5}
Slope of calibration graph (μAM^{-1})	1.98×10^{5}
Intercept (μA)	0.0276
Correlation Coefficient(r)	0.9978
RSD of slope	1.01
RSD of intercept	0.98
LOD (M)	1.13×10^{-6}
LOQ (M)	1.5×10^{-6}
Repeatability (RSD %)	1.21
Reproducibility (RSD %)	1.09

Table 2. Analysis of BZP in tablet by DPV and recovery studies

	[1]Denoral Tablet
Labeled claim (mg)	1.0
Amount found (mg)*	0.984
RSD (%)	1.04
Added (mg)	5.0
Found (mg)*	4.92
Recovered (%)	98.4
RSD (%)	1.21

* Average of 6 determinations

3.4. Determination of BZP in pharmaceutical dosages

The developed DPV method was applied successfully for the assay of BZP in tablets. The results of analysis of BZP are recorded in Table 2. In order to validate and to obtain the precision and accuracy of the developed method, recovery studies have been carried out at different concentration levels of drug. These studies were carried out by standard addition method. For this, known quantities of pure BZP were mixed with definite amounts of pre-analyzed formulations and the mixtures were analyzed as before. The total amount of the drug was then determined and the amount of the added drug was calculated by difference. The average percent recoveries obtained were found to be quantitative 98.4% indicating good recovery of the drug.

The effects of excipients *viz*., talc, glucose, starch, lactose, dextrose, gum acacia and magnesium stearate on voltammetric assay of BZP were investigated. It was noticed that none of these interfered in the determination at the levels normally found in dosage forms. The results shown in Table 2 revealed that the proposed method is selective and accurate and hence, could be adopted for quality assurance.

4. CONCLUSIONS

The electrochemical behavior of BZP on glassy carbon electrode was studied for the first time. The electrochemical process is proved to be quasireversible and pH dependent. A two-electron two-

proton mechanism for the redox reaction of BZP was observed. The evidence for the proposed mechanism was obtained from the analysis of the oxidized product after bulk electrolysis. By selecting the anodic peak of BZP, the DPV procedure was developed for its assay in tablets. The proposed method is rapid, requiring less than 3 min to run a sample and does not include time-consuming steps. By the proposed method, as low as 1.1 x 10^{-6} M of BZP can be accurately determined with sufficient precision and accuracy. The simplicity, sensitivity, selectivity and low cost of analysis are the main advantages of proposed method of determination of BZP.

ACKNOWLEDGEMENTS
We are thankful to the authorities of the Karnatak University, Dharwad, for providing the necessary facilities.

References

1. R.E. Clouse, P.J. Lustman, R.A. Geisman, D.H. Alpers, *Aliment Pharmacol Ther*., 8 (1994) 409
2. J.P Barbier, G. Dorf, J, Gordin, F. Krainik, D. Neveu, H. Parlier, P. Richard, J. Vitaux, B. Fraitag, *Ann Gastroenterol Hepatol (Paris)*, 25 (1989) 123
3. Jackson J.L., O'Malley P.G., Tomkins G., Balden E., Santoro J., Kroenke K., *Am. J. Med.*, 108 (2000) 65
4. G. Bassotti, F. Chistolini, F. Sietchiping Nezpa, G. de Roberto & A. Morelli, *Scand. J. Gastroenterol*, 38 (2003) 1013
5. R. E. Clouse, *Dig. Dis. Sci*., 39 (1994) 2352
6. J.M. Kauffmann, J.C. Vire, *Anal. Chim. Acta*, 273 (1993) 329
7. J. Wang (Ed.), *Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine*, VCH, New York, 1988
8. P.T. Kissinger, W.R. Heineman, *Laboratory Techniques in Electroanalytical Chemistry*, 2nd Edition, Marcel Dekker, New York, 1996
9. G. Cavazzutti, L. Gagliardi, D. De orsi, D. Tonelli, *J. liquid chromatography*, 18 (1995) 227
10. W. Bialecka, A. Kulik, T. Kaniewska, *Acta Poloniae Pharmaceutica,* 59 (2002) 9
11. R. Greef, R. Peat, L.M. Pletcher, J. Robinson, *Instrumental Methods in Electrochemistry*, Ellis Horwood, Chichester, 1985, pp. 185
12. E. Laviron, *J. Electroanal. Chem*., 112 (1980) 11
13. R.N. Goyal, N. Bachheti, A. Tyagi, A.K. Pandey, *Analytica Chimica Acta*, 605 (2007) 34
14. R.N. Nicholson, I. Shain, *Anal. Chem*., 36 (1964) 722
15. W.J. Moore, *Physical chemistry*, 5th ed, Orient Longman Pvt. Ltd., New Delhi, 2004, pp. 502
16. A.J. Bard, L.R. Faulkner, *Electrochemical methods Fundamentals and applications,* Wiley, New York 1980
17. C.M. Riley, T.W. Rosanske, *Development and Validation of Analytical Methods*, Elsevier Science Ltd., New York, 1996
18. M.E. Swatz, I.S. Krull, *Analytical Method Development and Validation,* Marcel Dekker, New York, 1997