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DIFFERENTIAL PULSE VOLTAMMETRIC DETERMINATION OF AMFEPRAMONE IN PHARMACEUTICAL FORMULATIONS AND SERUM SAMPLES USING GLASSY CARBON ELECTRODE

Sri Vidya D, Vishnu Priya M and NY Sreedhar*
Electroanalytical Lab, Department of Chemistry, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India

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Abstract: The electrochemical reduction of amfepramone (AFPN) was investigated systematically by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). A simple DPV technique was proposed for the direct quantitative determination of amfepramone (AFPN) in pharmaceutical formulations and serum samples for the first time. The reduction potential was -1.0V with glassy carbon electrode in phosphate buffer as supporting electrolyte. The dependence of the intensities of currents and potentials on pH, concentration, scan rate, deposition time, and nature of the supporting electrolyte was investigated. In the present investigation, the achieved limit of detection (LOD) was $3.2x10^{-8}$ M and a limit of quantification (LOQ) was $6.2x10^{-9}$M respectively. Excipients did not interfere with the determination of amfepramone (AFPN) in pharmaceutical formulations and serum samples. Precision and accuracy of the developed method were checked by recovery studies in pharmaceutical formulations and blood samples.

Key words: Amfepramone; Cyclic Voltammetry; Differential Pulse Voltammetry; Phosphate Buffer

INTRODUCTION

The abusive use of anorexics has grown lately, mainly because of its use in the treatment of obesity. The consumption of anorexics in Brazil is approximately 23.6 tons per year, surpassed only by Chile and Germany. [1] Furthermore, herbal formulations with claimed slimming activity, which are assumed to improve the effectiveness of food diets, have been in increasing use in Brazil. Obesity is a major risk factor for morbity and mortality [2,3] and its therapeutic treatment include anorexic agents such as amfepramone. It is chemically 2-(diethylamino)-1-phenylpropan-1-one. It belongs to the class benzene and substituted derivatives. Its chemical formula is $C_{13}H_{19}NO$. Chemical structure of AFPN was shown in Figure 1.

The administration of amfepramone can typically lead to the increase of locomotor activity, euphoria [4], nervousness, irritability, insomnia and hyperkinesis. [5] It may also induce to a schizophrenia-like psychosis if it is administered at high doses or for a long time. [6-8] To the best of our knowledge, there is still no official method for the determination of amfepramone. However, it has been determined mainly by thin layer and liquid chromatography [9,10], ^{1}H NMR spectroscopy [11] and voltammetry.

Some of these methods are time consuming and involve high instrumentation costs if compared to voltammetric methods. The voltammetric method published by Tan and coworkers [12] describes the determination of amfepramone by linear sweep voltammetry. However, a systematic investigation of the amfepramone determination in pharmaceutical formulations was not yet described in the literature.

In this context, the voltammetry offers the possibility for a rapid and sensitive determination of amfepramone in pharmaceutical formulations. Besides, solutions resulting from solubilization of tablets or capsules do not need to be filtered exhaustively before voltammetric determinations, which can be performed in the presence of particulate matter. [13] This is a further advantage of voltammetric methods compared with either spectroscopic or liquid chromatographic procedures, where the samples must be filtered until the complete separation of the insoluble excipients.

No voltammetric analysis using glassy carbon electrode was done till to date. This work describes a systematic study of the voltammetric behavior of amfepramone at the glassy carbon electrode (GCE) by cyclic (CV) and differential pulse voltammetric (DPV) methods. These studies illustrated the reduction behavior of amfepramone at GCE and were performed in supporting electrolyte. The voltammetric peaks obtained at –1.0 V for amfepramone was characterized by irreversible reduction processes.

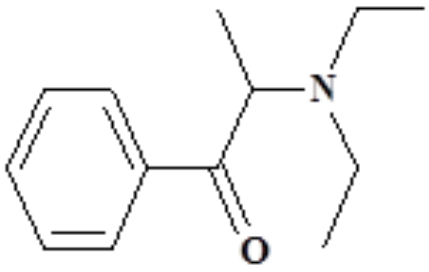

Figure 1: Structure of amfepramone.

***Corresponding Author:**
Dr. NY Sreedhar,
Electroanalytical Lab, Department of Chemistry
Sri Venkateswara University,
Tirupati-517502, Andhra Pradesh, India.

MATERIALS AND METHODS

Apparatus and procedure

The details of the instrumentation used for most of the electroreduction techniques were performed using an Autolab PG STAT101 supplied by Metrohm Autolab B.V., The Netherlands. In all the experiments a three electrode system comprising of a glassy carbon electrode as a working electrode, saturated Ag/AgCl/KCl as a reference electrode and Pt wire as a counter electrode were employed. An Elico LI-120 pH meter supplied by Elico Ltd, Hyderabad, India was used for pH measurements.

In the voltammetric experiments, 9 mL of the buffer solution in the cell was purged with oxygen free nitrogen gas for 3 min. A $1.0x10^{-3}$ M stock solution of the investigated compound in methanol was added to the buffer to reach a final concentration of $1x10^{-5}$ M. The mixture was purged for further 30 sec. and the voltammograms were recorded.

Procedure for sample

The contents of 10 capsules were mixed well and pulverized. A weighed quantity of the powder equivalent to 20 mg of the studied compounds was transferred into a small flask and extracted with methanol. The extract was filtered into a 100 mL volumetric flask. The conical flask was washed with few mL of methanol. The washings were passed into the same conical flask and completed to the mark with the same solvent. Aliquot volumes covering the working concentration range were transferred into 25 mL volumetric flasks. The volume was completed with phosphate buffer of pH 6.0. The whole contents of the flask were poured into the electrolytic cell.

RESULTS AND DISCUSSIONS

Cyclic voltammetric study

The cyclic voltammetric behavior of amfepramone at GCE in phosphate buffer at pH 6.0 was shown in Fig.2. It exhibits irreversible peaks at GCE, given a well-defined cathodic peak at –1.0 V. However, when the accumulation step of 120 s is used before the potential scan, the peak currents at –5.0 µA, was essentially a linear function of the square root of scan rate ($n^{½}$) in the range from 50 to 150 mV s^{-1}, indicating that the reduction of amfepramone. The linear regression value is r = 0.995. From these results, one can conclude that the cathodic peak current obtained in the irreversible reduction of amfepramone at the GCE in phosphate buffer.

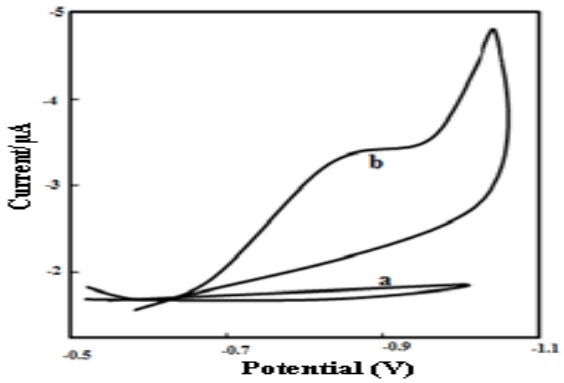

Figure 2: Cyclic voltammograms for $2.25x10^{-7}$M amfepramone at pH 6.0 in phosphate buffer solution at glassy carbon electrode at different scan rates (50 mV/s-150 mV/s).

Effect of pH

The effect of buffer pH on the electrochemical behavior of amfepramone was investigated over the range of pH 2.0 -8.0 and the results are depicted in Fig. 3. The plot of peak current Vs pH shows that the peak current increased gradually with increasing pH then it reaches a maximum value at pH 6.0, which was adopted as optimum pH value for subsequent investigations. The influence of pH factor on the cyclic voltammetric peaks were illustrated in Fig. 2.

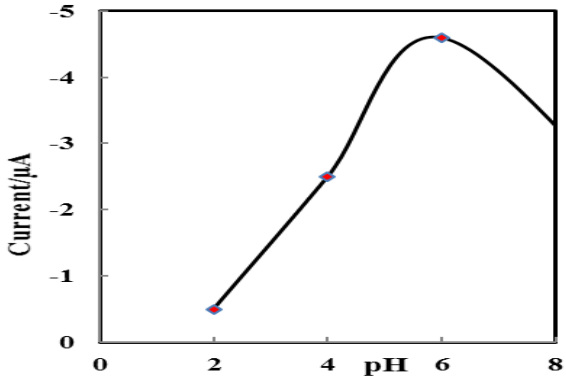

Figure 3: The plot the of current Vs pH of amfepramone in PB solution, Concentration: $2.25x10^{-7}$ M at glassy carbon electrode in different pH values (pH 2.0-8.0).

Effect of scan rate

The effect of scan rate on the electroreduction of 1.0 × 10^{-3} mol L^{-1} AFPN at GCE in phosphate buffer solution of pH 6.0 at different potential sweep rates was examined by cyclic voltammetry. Fig. 2 exhibits the cyclic voltammograms of AFPN at GCE with different scan rates, in the range of 50 mV/s –150 mV/s. For AFPN no anodic peak is observed on the reverse scan in various potential sweep rates shown in Fig.4. The cathodic peak current varied linearly with the scan rates I (µA) = 0.0878 *v* (mV s^{-1}) + 1.1538 (R^2 = 0.998) which shows that the reduction of AFPN on GCE was a typical absorption-controlled process.

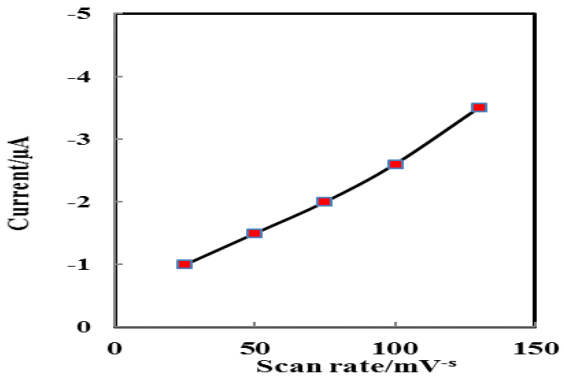

Figure 4: Effect of scan rate on AFPN at pH 6.0 in PB solution at Concentration: 2.25x10^{-7}M at different scan rates (50mV/s-150 mV/s).

Differential pulse voltammetric studies

The formulations were analyzed by the proposed DPV methods using supporting electrolytes. It is important to highlight that none of the procedures were interfered by the matrices. The DPV curves in Fig. 5 shows the determination of amfepramone in pharmaceutical formulations and serum samples employing the electrolyte systems studied in this work. Three replicate determinations in three different formulations gave average results, which are shown in Table. 1. As can be seen, good accuracy was obtained comparing the obtained results with the content declared by the pharmaceutical industries on the drug packaging. Recoveries in the range 97.10 % to 98.4% were obtained.

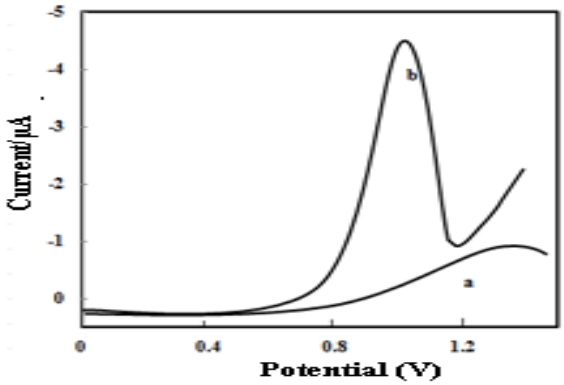

Figure 5: Typical DPV of AFPN in phosphate buffer solution at pH 6.0, Concentration: 2.25x10^{-7} M to 6.42×10^{-4}M at GCE.

Validation of the method

Once the optimum chemical conditions and instrumental parameters for the DPV determination of AFPN were established, several analytical characteristics of the proposed methods were evaluated. Under the optimized conditions, a linear correlation between DPV peak intensity and the drug concentration was obtained over the range 2.25×10^{-7}M to 6.42×10^{-4} M shown in Fig. 6. The calibration equation was calculated by least-squares method and it has the form. The limits of detection (LOD) and limits of quantification (LOQ) were determined using the formula: LOD or LOQ = *k* S.D.a/b, where k = 3 for LOD and 10 for LOQ, a is the standard deviation of the intercept, and b is the slope. Also lower limit of detection (LOD) defined as the concentration of AFPN corresponding to the intersection of the extrapolated linear segment of the calibration graph which is 3.2x10^{-8}M. The obtained LOQ value is 6.2x10^{-9}M. This obtained sensitivity was significantly preferable than those reported for other analytical technique used for determination of AFPN.

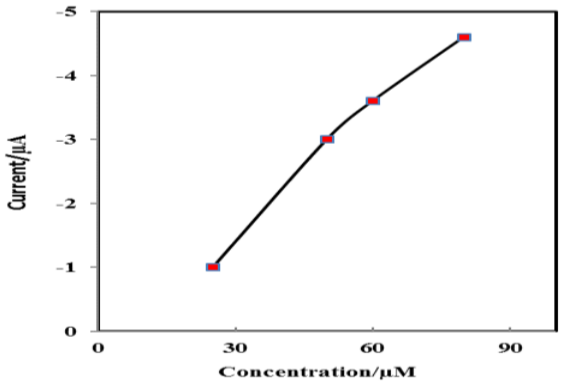

Figure 6: Linear correlation between DPV peak intensity and the drug concentration

CONCLUSION

The described methods provide a sensitive determination of amfepramone in tablets and capsules of pharmaceutical formulations by DPV. The determination of amfepramone in these formulations was possible employing an electrolyte systems and a simple solubilization of the sample in methanol. Recovery experiments for amfepramone in synthetic mixture containing fenproporex, mazindol, sibutramine, fluoxetine, caffeine, diazepam, and metformin as adulterants confirmed the satisfactory accuracy of the methods. The DPV method using a phosphate buffer (pH 6.0) as supporting electrolyte seemed also to be useful for the selective determination of amfepramone in formulations commercialized all over the world as natural medicines, in which different classes of drugs can be present as adulterants. The detection limit obtained for amfepramone in this supporting electrolyte allows its sensitive determination as adulterant in formulations, which may contain the drug at therapeutically doses and even at low doses.

REFERENCES

1. Nappo SA, Carlini EA. Medicamentos, Drogas e Saúde, Hucitec: São Paulo, 1995.
2. Weiser M, Frishman WH, Michaelson MD, Abdeen M. A, J, Clin, Pharmacol, 1997, 37, 453.
3. Mello Jr., EL, Maiora RS, Careyc RJ, Hustonb JP, Tomaza C, Muller CP. Eur, J, Pharmacol,2005, 511, 43.
4. Jasinski DR, Nutt JG, Griffith JD. Clin, Pharmacol, Ther, 1974, 16, 645.
5. Khan SA, Spiegel DA, Jobe P. C, Am, Farm, Phys, 1987, 36, 107.
6. Carney MW, Brit J. Psychiatr, 1988, 152, 146.
7. Brooke D, Kerwin R, Lloyd K, Brit J. Psychiatr, 1988, 152, 572.
8. Marquardt H, Schäfer S, Lehrbuch der Toxikologie, Wissenschaftliche Verlagsgesellschaft: Stuttgart, 2004.
9. Parodi B, Caviglioli G, Bachi V, Cafaggi S, Romussi G, Pharmazie 1993, 48, 678.
10. Almeida AE, Ribera ML. J, Liq, Chromatogr, Rel, Technol, 1999, 22, 1759.
11. Farina AM, Iorio MA, Doldo A. Spectrosc, Lett, 1988, 21, 455.
12. Tan X, Zhang S, Li Q. Huaxue Yanjiu Yu Yingyong 1996, 8, 279.
13. Dos Santos MMC, Famila V, Gonçalves MLS. Anal, Bioanal, Chem, 2002, 374, 1074.

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