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# A simple and sensitive analytical tool for determination of ampyrone and its application in real sample analysis using carbon paste electrode

**Arunkumar T. Buddanavar, and Sharanappa T. Nandibewoor***

*P.G. Department of Studies in Chemistry, Karnatak University, Dharwad, India*

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**ABSTRACT**

*Electrochemical methods have been widely used for the determination of electro active compounds due to their simplicity, sensitivity, stability, and low cost. A carbon paste electrode was used for the electro analytical determination of ampyrone in real sample by cyclic, linear sweep and differential pulse voltammetric techniques. The oxidation of ampyrone was irreversible and exhibited a diffusion controlled process. The oxidation mechanism was proposed. The dependence of the current on pH, the concentration and scan rate was investigated to optimize the experimental conditions for the determination of ampyrone. It was found that the optimum pH for the determination of ampyrone was 3.0. The current measured by differential pulse voltammetry presents a good linear property as a function of the concentration of ampyrone in the range of $1.0x10^{-8}$ to $1.0x10^{-6}$ with a limit of detection $0.102 \times 10^{-9}$ M. In addition, the reproducibility, precision and accuracy of the method were checked as well. Electro analytical determination of ampyrone in human urine and plasma samples was carried out using differential pulse voltammetry. The method finds its applications in quality control laboratories.*

***Keywords**:* Electro-oxidation, Ampyrone, Voltammetry, Real samples

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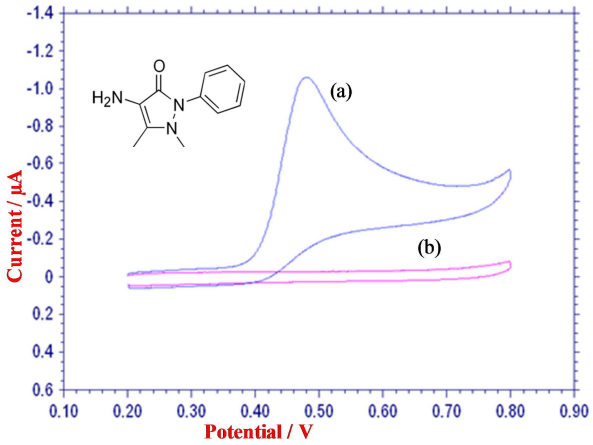
## INTRODUCTION

Ampyrone (4-Amino-2, 3-dimethyl-1-phenyl-3-pyrazol-5-one, structure as given in Scheme 1 AMP) is an analgesic, anti-inflammatory and antipyretic drug. Ampyrone residues in the environment pose a potential threat to human health. Ampyrone stimulates liver microsomes and is also used to measure extracellular water. In view of health hazards due to the presence of ampyrone, its determination becomes important tool for drug quality control. The attention of most of the electrochemists towards electrochemical methods, electrode material based on carbonaceous compounds has been widely applied in electrochemistry. This is due to their special properties including low resistivity, chemical inertness, and unique surface chemistry, large sensitivity, excellent stability, low cost, which make them a proper choice to determine a wide range of substances in electro catalytic area and has been widely used to study the redox behavior of electro active compounds. Several analytical methods have been described in the literature for determination of ampyrone, including liquid and gas chromatography/ spectrophotometry [1-3], liquid chromatography [4], mass spectrometry [5], solid-phase spectrophotometry [6], and different high-performance liquidchromatographic methods [7-9]. The main problems encountered in using these methods are time consuming, extraction and separation procedures. Determination of ampyrone by voltametric techniques, we have undertaken the present investigation.




**Scheme 1. Chemical Structure of ampyrone**

The electroanalytical method for the determination of ampyrone using carbon paste electrode has not been reported yet. The aim of this study is to establish the suitable experimental conditions and to investigate the voltammetric behavior and oxidation mechanism of ampyrone at carbon paste electrode by cyclic, linear sweep and differential pulse voltammetric methods for the direct determination of ampyrone in real samples.

## MATERIALS AND METHODS

### 2.1. Reagents and chemicals

Ampyrone was purchased from Sigma - Aldrich and used. A 0.01 M (M = mol/dm$^3$) stock solution was prepared in double distilled water. The phosphate buffer solutions from pH 3.0–8.0 (ionic strength 0.2 M), were prepared according to the method of Christian and Purdy [10]. All other chemicals used were of analytical or chemical grade. All solutions were prepared with double distilled water.

### 2.2. Instrumentation

Electrochemical measurements were carried out on a CHI 630D electrochemical analyzer (CH Instruments Inc., USA). The voltammetric measurements were carried out in a 10 ml single compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode and a self-made carbon paste electrode (CPE) as a working electrode. All the potentials in this paper are given against the Ag/AgCl (3 M KCl). The pH of solution was measured with an Elico LI120 pH meter (Elico Ltd., India).

### 2.3. Preparation of carbon paste electrode

The carbon paste was prepared by thoroughly mixing 5 g of graphite powder with 1.8 ml of paraffin oil homogenously by hand for 30 minutes in a mortar with pestle [11]. The homogenized paste was allowed to rest for a minimum period of 24hrs, and then the paste was packed into the tip of the plastic syringe. The surface of the electrode was smoothened on weighing paper until it had a shiny appearance. The electrode body was constructed by pressing a small rod of stainless steel (diameter 2 mm) inside a micropipette tip (1 ml volume capacity) leaving a depression at the surface tip approximately 1 mm for housing the carbon paste, and a thin wire was inserted through the opposite end to establish electrical contact. The carbon paste electrode was immersed in the supporting electrolyte placed in the cell and several sweeps were applied to obtain a low background current. The paste was carefully removed prior to pressing a new portion into the electrode after every measurement.

### 2.4. Area of the electrode

The area of the electrode was obtained by the cyclic voltammetric method using 1.0 mM $K_3Fe(CN)_6$ as a probe at different scan rates. For a reversible process, the following Randles - Sevcik formula was used [12].

$$I_{pa} = 0.4463 (F^3/RT)^{1/2} n^{3/2} A_0 D_o^{1/2} C_0 v^{1/2} \quad (1)$$

where $I_{pa}$ refers to the anodic peak current, n is the number of electrons transferred, $A_0$ is the surface area of the electrode, $D_0$ is diffusion coefficient, υ is the scan rate, and $C_0$ is the concentration, respectively. For 1.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, T =298K, R = 8.314 J $K^{-1}$ $mol^{-1}$, F = 96480 C $mol^{-1}$, n =1, $D_0 = 7.6 \times 10^{-6}$ $cm^2$ $s^{-1}$, then from the slope of the plot of $I_{pa}$ vs $v^{1/2}$ the electroactive area was calculated. In our experiment the slope was 9.75 x $10^{-5}$µA $(V\ s^{-1})^{1/2}$ and the area of electrode was calculated to be 0.0177 $cm^2$.

**2.5. Experimental procedure**

The carbon paste electrode was first activated in phosphate buffer (0.2 M, pH=3.0) by cyclic voltammetric sweeps between 0.0 and +1.0 V until a stable cyclic voltammogram was obtained. Then electrodes were transferred into another 10 ml of phosphate buffer (0.2 M, pH=3.0) containing proper amount of ampyrone (AMP). After open-circuit accumulation for 180 s with stirring and following quiet for 10s, potential scan was initiated and cyclic voltammograms were recorded between 0.0 and + 0.8 V, with a scan rate of 50 mV $s^{-1}$. All measurements were carried out at room temperature of 25±0.1 °C.

**2.6. Plasma Sample Preparation**

Human blood samples were collected in dry and evacuated tubes (which contained saline and sodium citrate solution) from a healthy volunteer. The samples were handled at room temperature and were centrifuged for 10 min at 1500 rpm for the separation of plasma within 1 h of collection. The samples were then transferred to polypropylene tubes and stored at 20 $^0$C until analysis. The plasma samples, 0.2 mL, were deprotonised with 2 mL of methanol. After vortexing for 15 min, the mixture was then centrifuged for 15 min at 6000 rpm, and supernatants were collected. The supernatants were spiked with known amounts of ampyrone. Appropriate volumes of this solution were added to phosphate buffer pH = 3.0 as supporting electrolyte and the voltammograms were then recorded.

## RESULTS AND DISCUSSION

**3.1. Cyclic voltammetry of ampyrone**

The electrochemical behavior of ampyrone at carbon paste electrode was investigated using cyclic and linear sweep voltammetry. Ampyrone was oxidized on carbon paste electrode between pH 3.0 and 8.0. The cyclic voltammograms of ampyrone at pH 3.0 in phosphapate buffer was as shown in Fig. 1.

The blank solution was shown by curve (b) and anodic peak corresponding to ampyrone oxidation appeared at about + 0.482 V as shown in curve (a), which corresponds to the oxidation of the amine group. On scanning in the negative direction, no reduction peak was observed, showing that the oxidation of ampyrone is an irreversible process.

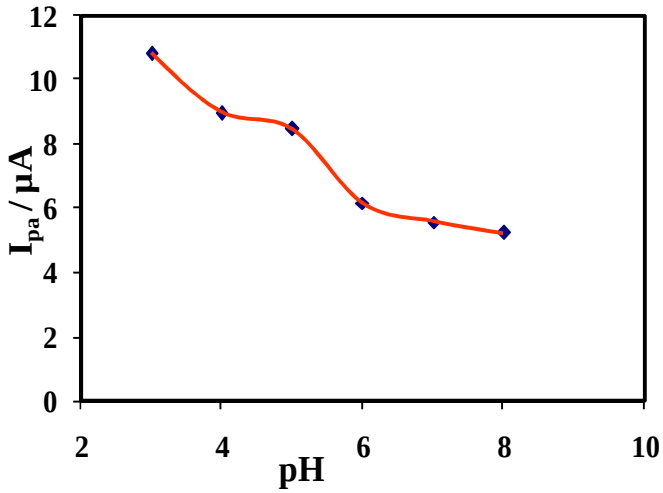
**3.2. Influence of pH**

The effect of pH of the buffer solution on the electrochemical behavior of ampyrone was studied in the pH range of 3.0 to 8.0 in phosphate buffer at a scan rate of 0.05 $Vs^{-1}$. With the increase in pH of the solution, the peak potential linearly shifted to less positive value (Fig. 2A).The relation between $E_p$ and pH according to the equation (1) (Fig. 2B).

$E_p$ (V) = 0.586 - 0.036 pH; ($r$ = 0.975) (1)

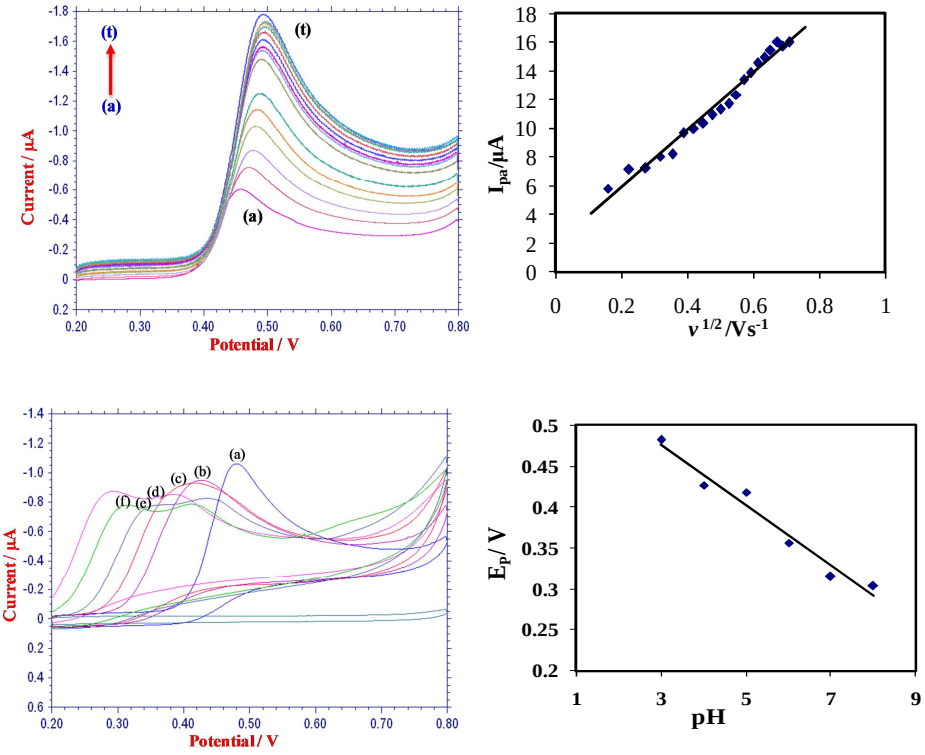
The slope of the plot $E_p$ versus pH was found to be 36.0 m V which is close to the theoretical value of 30 mV which indicates the involvement of two electrons and a proton transfer in the rate determining step [13-15].

The plot of $I_{pa}$ v/s pH (Fig. 2C) clearly shows the peak current is affected by the pH value. Ampyrone showed the sharp and highest peak intensity at pH = 3.0, then as the pH concentration increases peak intensity (current/$I_{pa}$) decreased continuously. Best result with respect to sensitivity accompanied with higher peak resolution obtained with pH=3.0, so it was selected for further experiments.




### 3.3. Influence of scan rate

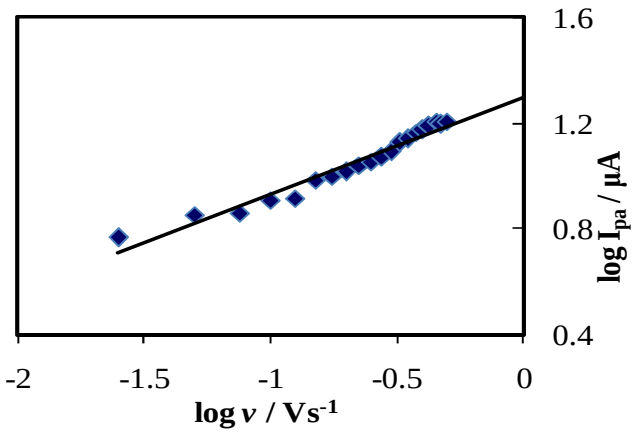
The useful information involving electrochemical mechanism generally can be acquired from the relationship between peak current and scan rate. The voltammetric behavior of ampyrone was examined at different scan rates from 0.025 $mVs^{-1}$ to 0.50 $mVs^{-1}$ using linear sweep voltammetry (LSV) (Fig. 3A). Scan rate studies were carried to assess whether the process on carbon paste electrode was diffusion controlled or adsorption controlled. The influence of square root of scan rate on the peak current (Fig. 3B) showed a linear relationship in the range of 0.025 to 0.50 mV $s^{-1}$for LSV which is of a typical diffusion controlled process [16].




The relation can be expressed as equation (2)

$I_{pa}(\mu A) = 19.98\ v^{1/2}\ (V\ s^{-1}) + 1.883$; (r = 0.978) (2)

The peak potential shifted to more positive values on increasing the scan rate. The linear relationship between peak potential and logarithm of scan rate for LSV (Fig. 3C),




which confirms the irreversibility of the oxidation process can be expressed as equation (3),

$E_p\ (V) = 0.03 \log v\ (Vs^{-1}) + 0.506$ ; (r= 0.993) (3)

For an irreversible electrode process, according to Laviron [17], $E_p$ is defined by the following equation (4),

$$E_p = E^{0'} + \left(\frac{2.303RT}{\alpha nF}\right) \log \left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right) \log v \quad (4)$$

where 'α' is the transfer coefficient, '$k^0$' the standard heterogeneous rate constant of the reaction, 'n' the number of electrons transferred, the scan rate and '$E^{0'}$' is the formal redox potential. Other symbols have their usual meanings. Thus the value of αn can be easily calculated from the slope of $E_p$vs.log *v*. In this system, the slope was 0.030 for LSV, taking T = 298 K, R= 8.314 J $K^{-1}mol^{-1}$, and F=96480 $Cmol^{-1}$.

According to Bard and Faulkner [18], 'α' can be given as, (5)

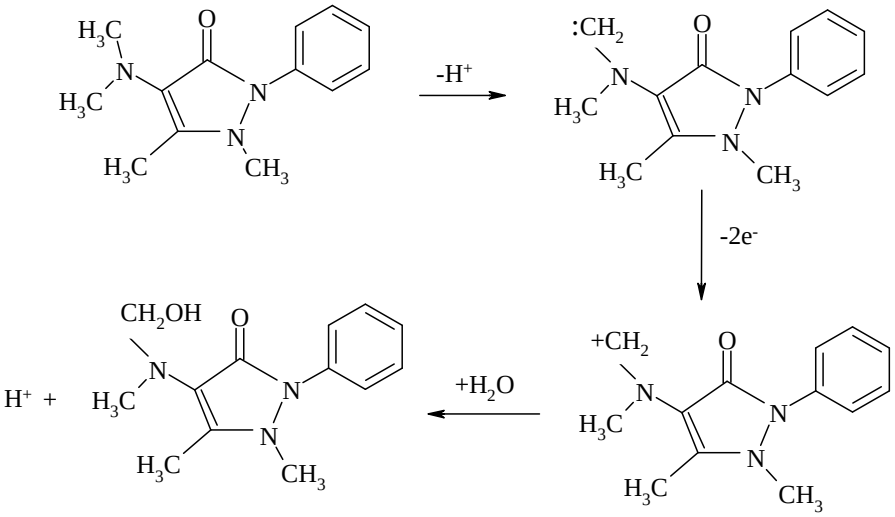
$$\alpha = \frac{47.7}{E_p - E_{p/2}}\ mV \quad (5)$$

where $E_{p/2}$ is the potential where the current is at half the peak value. So, from this we obtained the value of 'α'. The number of electron (n) transferred in the electro oxidation of ampyrone was calculated using linear sweep voltammetry. The value of $k^0$ can be determined from the intercept of the above plot if the value of $E^{0'}$ is known. The value of $E^{0'}$ in Eq. (4) can be obtained from the intercept of $E_p$ vs. *v* curve by extrapolating to the vertical axis at *v* = 0 [19]. In our system the intercept for $E_p$ vs. log *v* plot was 0.506 for LSV method. The values of 'αn' 'n' 'α' '$E^{0'}$' and '$k^0$' obtained from linear sweep voltammetry are tabulated in Table 1.

| Parameters | Values using LSV |
|---|---|
| 'αn' | 1.9707 |
| 'n' | 1.818 |
| 'α' | 1.084 |
| '$E^{0'}$' | 0.467 |
| '$k^0$' | $1.421x10^3$ |

### 3.4. Mechanism

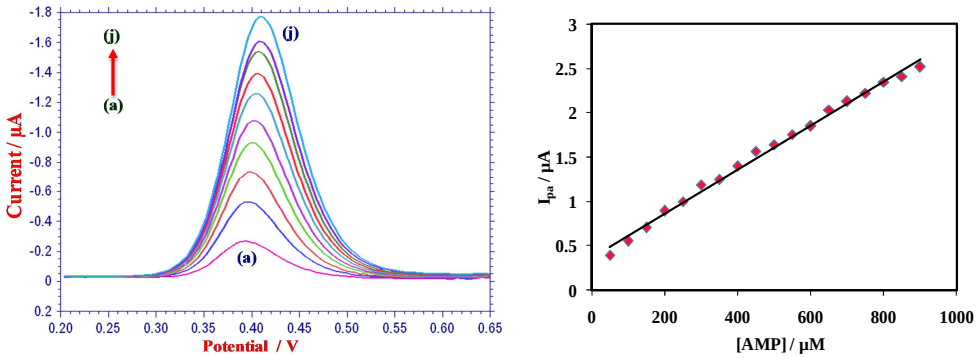
In the proposed method, the electro-oxidation of ampyrone involves two electron and one proton transfer process. The electro oxidation takes place at the amino group of the dipyrone ring as reported in the earlier work of dipyrone derivatives [20, 21]. The probable mechanism is as shown in Scheme 2.




**Scheme 2. Probable electrode oxidation mechanism of ampyrone**

### 3.5. Calibration curve and detection limit

In order to develop a rapid and sensitive voltammetric method for the determination of ampyrone, the differential pulse voltammetric method (DPV) was adopted as the peaks obtained are better defined at lower concentration of ampyrone than those obtained by cyclic voltammetry, with a lower background current, resulting in improved resolution. According to the obtained results, it was possible to apply this technique to the quantitative determination of ampyrone. The phosphate buffer solution of pH = 3.0 was selected as the supporting electrolyte for the quantification of ampyrone as it gave a maximum peak current. Differential pulse voltammograms obtained with increasing amounts of ampyrone showed that the peak current increased linearly with increasing concentration, as shown in Fig. 4A. Using the optimum conditions described above, linear calibration curve was obtained for ampyrone in the range of $1x10^{-8}$to $1x10^{-6}$ M as shown in Fig. 4B.



The linear equation (6) was,

$I_{pa}(\mu A) = 1.238$ [AMP] $\mu M + 0.371$; ( r =0.993 ) (6)

Deviation from linearity was observed for more concentrated solutions, due to the adsorption of oxidation product of ampyrone on the electrode surface. Related statistical data of the calibration curve were obtained from the six different determinations. The limit of detection (LOD) and quantification (LOQ) were $0.102 \times 10^{-9}$ M and $0.341 \times 10^{-9}$ M, respectively. The LOD and LOQ were calculated using the following equations:

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

where, 's' is the standard deviation of the peak currents of the blank, and 'm' is the slope of the calibration curve [22].The comparison of detection limits for ampyrone at different other methods related to derivative of ampyrone are tabulated in Table 2. The proposed method was better as compared to other reported earlier methods [3, 20, 21, 23].

| Methods | Detection limit | Reference |
|---|---|---|
| Dipyrone(DP),(1-Phenyl-2, 3-dimethyl-5-pyrazolone-4-methyl aminomethanesulfonate Sodium) | | |
| 1.Flow injection amperometric Determination | $2.78 \times 10^{-4}$ M | 03 |
| 2.Diffusion layer titration at dual band electrochemical cell | | |
| 3.Nano-riboflavin-modified glassy carbon electrode (voltammetry) | $3.6 \times 10^{-6}$ M | 20 |
| 4.Ttitanium phosphate/nickel Hexacyanoferrate modified graphite electrode (voltammetry) | $0.502 \times 10^{-6}$ M | 21 |
| **Ampyrone** | | 23 |
| 6. A simple and sensitive analytical tool for determination of ampyrone and its application in real sample analysis using carbon paste electrode | $3.75 \times 10^{-4}$M | |
| | **$0.102 \times 10^{-9}$ M** | **Present work** |

### 3.6. Repeatability and reproducibility of the carbon paste electrode

To study the reproducibility of the electrode preparation procedure, a 0.1mM ampyrone solution was measured with the same CPE (renewed every time) for several hours within the day, and the RSD of the peak current was 2.1% (number of measurements = 5). As to the reproducibility between days (RSD of peak current = 1.9%), it was similar to that within a day if the temperature was kept almost unchanged. Owing to the adsorption of ampyrone or its oxidative products on to the electrode surface, the current response of the electrode would decrease after successive use. In this case, the electrode should be prepared again.

### 3.7. Effect of excipients

For the possible analytical application of the proposed method, the effect of some common excipients used in pharmaceutical preparation was examined. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than ±5% for determination of ampyrone. The effects of these excipients on the voltammetric response was carried by analyzing sample solutions containing a fixed amount of ampyrone (1.0 µM) spiked with various excess amount of each excipient under the same experimental conditions. The experimental results showed (Table. 3) that 100-fold excess of citric acid, ascorbic acid, dextrose, gum acacia, lactose, oxalic acid, uric acid, starch, sucrose, and tartaric acid did not interfere with the voltammetric signal of ampyrone. Thus, the procedures were able to assay ampyrone in the presence of excipients, and hence it can be considered specific.

| Excepients(1 .0 mM) + Drug (1.0 µM) | Potential (V) | (%) Signal Change |
|---|---|---|
| Only AMP | 0.428 | |
| AMP + Citric acid | 0.424 | 0.934 |
| AMP + Dextrose | 0.424 | 0.934 |
| AMP + Gum Acacia | 0.440 | -2.803 |
| AMP + Lactose | 0.428 | 0.000 |
| AMP + Oxalic acid | 0.428 | 0.000 |
| AMP + Starch | 0.428 | 0.000 |
| AMP + Sucrose | 0.416 | 2.803 |
| AMP + Ascorbic acid | 0.424 | 0.934 |
| AMP + Tartaric acid | 0.424 | 0.934 |
| AMP + Uric acid | 0.0424 | 0.934 |

### 3.8. Detection of ampyrone in urine samples

The applicability of the DPV to the determination of ampyrone in spiked urine as a real sample was investigated. The recoveries from urine were measured by spiking drug free urine with known amounts of ampyrone. A quantitative determination can be carried out by adding the standard solution of ampyrone into the detect system of urine sample. The calibration graph was used for the determination of spiked ampyrone urine samples. The recovery determined was in the range from 97.55 to 99.48 %, and the RSDs are listed in Table 4. Thus, satisfactory recoveries of the analyte from the real samples make the developed method applicable in clinical analysis.

### 3.9. Detection of ampyrone in Spiked Human Plasma Samples

The applicability of the DPV to the determination of *ampyrone* in spiked human plasma sample was investigated. The recoveries from human plasma were measured by spiking drug free plasma with known amount s of ampyrone. The plasma sample was prepared as described in Section 2.6. A quantitative analysis can be carried out by adding the standard solution of ampyrone in the detect system of plasma sample. The calibration graph was used for the determination of spiked ampyrone in plasma samples. The detection results obtained for four plasma samples are listed in Table 4. The recovery determined was in the range from 98 .06% to 100. 14% and the RSD are given in Table4.

| Samples | Added($10^{-7}$M) | Found($10^{-7}$M)[a] | % Recovery | R.S.D(%) |
|---|---|---|---|---|
| urine sample 1 | 2.0 | 1.9896 | 99.48 | 0.1573 |
| urine sample 2 | 4.0 | 3.902 | 97.55 | 2.6208 |
| urine sample 3 | 6.0 | 5.886 | 98.10 | 2.6877 |
| plasma sample 1 | 1.0 | 0.9946 | 99.46 | 0.5177 |
| plasma sample 2 | 3.0 | 2.942 | 98.06 | 2.7038 |
| plasma sample 3 | 5.0 | 5.007 | 100.14 | 0.2896 |

[a]*Average of five determination.*

## CONCLUSION

A carbon paste electrode has been successfully used for the oxidation of ampyrone in phosphate buffer solution (pH=3.0). Ampyrone is irreversibly oxidized at a high potential on a CPE. There were two electrons and a proton transfer in electrode reaction and was a diffusion-controlled process. A suitable oxidation mechanism was proposed. The peak current was linear to ampyrone concentrations over a certain range, under the selected conditions. This helps in voltammetric determination of selected analyte as low as 0.102 x $10^{-9}$ M and can be used successfully to assay the drug. The proposed method offered the advantages of accuracy and time saving as well as simplicity of reagents and apparatus. In addition, the satisfactory results obtained in the determination of ampyrone in spiked urine samples demonstrated the applicability of the method for real sample determination. Furthermore, the present method could possibly be adopted for pharmacokinetic studies as well as clinical and quality control laboratories.

## REFERENCES

[1] P. Majlat, *Pharmazie*, **1984**, 39, 325.
[2] E. Emerson, Standard Methods for the Examination of Water and Waste Water, 17th ed.; American Public Health Association: Washington, DC. **1989**, 5.
[3] L. Penney, C. Bergeron, B. Coates, and W. Arosha, *J. AOAC Int*. **2005,** 88, 496.

[4] D. Puig, I. Silgoner, M. Grasserbauer, and D. Barcelo, *Anal. Chem*. **1997**, 69, 2756.
[5] I. Carretero, J. M. Vadillo, and J. J. Laserna, *Analyst*, **1995**, 120, 1729.
[6] N. Isoshi, N. Sachico, W. Kaori, and O. Kunio, *Anal. Sci*. **2000**, 16, 269.
[7] G. Blo, F. Dondi, A. Betti, and C. Bighi, *J. Chromatogr*. A. **1983,** 257, 69.
[8] D. Damm, *Arzneimittelforschung*. **1989**, 39, 1415.
[9] X. Liu, Chin. *J. Pharm. Anal*. **2007,** 27, 1487.
[10] G.D.Christian, W.C.Purdy, *J. Electroanal. Chem.* **1962**, 3, 363.
[11] S. I. Zayed, and H. A. Arida, *Int. J. Electrochem. Sci*. **2013,** 8, 1340.
[12] B. Rezaei, and S. Damiri, *Sens. Actuators B* **2008**, 134, 324.
[13] T. B. Dogan, B. Bozal, B.T. Demircigil, B. Ulsu, and S. A. Ozkan, *Electroanalysis*, **2009**, 21, 2427.
[14] J. Wang, Electroanalytical Techniques in Clinical Chemistry and Laboratory Medicine; VCH: New York **1988**.
[15] P. T. Kissinger, and W. R. Heineman, Laboratory Techniques in Electroanal. Chem. 2nd ed.; Marcel Dekker: New York, **1996**.
[16] D. K. Gosser, Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanisms; VCH: New York, **1993**, 43.
[17] E. Laviron, *J. Electroanal. Chem*. **1979,** 101, 19.
[18] A. J. Bard, and L. R. Faulkner, Electrochemical Methods Fundamentals and Applications, second ed., Wiley, New York, **2006**.
[19] W. Yunhua, J. Xiaobo, and H. Shengshui, *Bioelectrochemistry* **2004**, 64, 91.
[20] R. L. C. Thiago, R. C. Matos, and M. Bertotti, *Talanta* **2003**, 61, 725.
[21] G. Gopalakrishnan, P. Manisankar, B. Muralidharan, and C. Vedhi. *Int. J. Electrochem*. **2011**, 2011, 1.
[22] E. Swartz, and I. S. Krull, Analytical Method Development and Validation; Marcel Dekker: New York, **1997**.
[23] L. R. Cumba, U. O. Bicalho, D. R. Silvestrini, and D. R. Carmo, *Int. J. Chem*. **2012**, 4, 66.