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Square Wave Voltammetric Determination of Ampyrone in Human Biological fluids using Pencil Graphite Sensor

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ABSTRACT

Electrochemical behavior of Ampyrone (AMP) was investigated at Pencil Graphite Sensor in 0.2 M Phosphate buffer solution at pH 3.0 by using cyclic (CV), differential pulse (DPV) and square wave voltametric (SWV) techniques. AMP showed one oxidation peak 0.5348 V at scan rate of 100 mV s^{-1}. The effect of scan rate and concentration was found to be diffusion-controlled electrode process. The electrocatalytic effect of AMP was dependent on pH. The developed method was found to be precise, selective and rapid for the simultaneous determination of AMP. The proposed method has been applied for the determination of AMP in real sample.

Highlights:

1. Developed a new voltammteric method which is cost effective in technology against conventional methods.
2. This method is helpful in performing identification and quantification of *Ampyrone* drug in real samples.
3. The electro-redox mechanism involves two electron-one proton transfers.

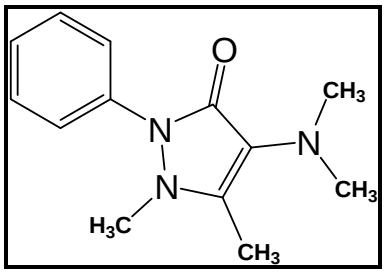
Keywords: Ampyrone, Square Wave Voltammetry, Pencil Graphite Sensor, Analytical applications.

INTRODUCTION

Ampyrone (4-Amino-2, 3-dimethyl-1-phenyl-3-pyrazol-5-one) (AMP) (Scheme 1) is an analgesic, anti-inflammatory and antipyretic drug. AMP residues in the environment pose a potential threat to human health. AMP stimulates liver microsomes and is also used to measure extracellular water. In view of health hazards due to the presence of AMP, 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 [**1**] have been described in the literature for determination of AMP, including liquid and gas chromatography/spectrophotometry [**2, 3**], liquid chromatography [**4**], mass spectrometry [**5**].

The advance in experimental technique in the field of analysis of drugs is due to their simplicity, low cost and relatively short analysis time when compared with the other techniques. Electrochemical methods have proved to be very sensitive for the determination of organic molecules, including drugs and related molecules in pharmaceutical dosage forms and biological fluids. Carbon electrodes, especially pencil graphite electrode (PGE), are widely used in the electrochemical investigations because of their low cost and suitability for detection of various organic and biological compounds. The objective of the present work is to develop a convenient and sensitive method for the determination of AMP. Hence, we report the electrochemical behavior of AMP and its determination at PGE using square wave (SWV), differential pulse (DPV) and cyclic voltammetry (CV) techniques. It was further successfully applied for the sensitive and selective determination of AMP in human biological samples.



Scheme 1. Structure of AMP.

MATERIALS AND METHODS

Instrumentation: Electrochemical measurements were carried out on a CHI 630D electrochemical analyzer.(CH Instruments Inc., Austin, TX). The voltammetric measurements were carried out in a 10ml single compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode and a pencil graphite electrode (PGE) as the working electrode. All the potentials are given against the Ag/AgCl (3.0 M KCl). The pH measurements were performed with Elico LI 120 pH meter (Elico Ltd., India). All experiments were carried out at an ambient temperature of 25°C ± 0.1°C.

Reagents and Chemicals: AMP is purchased from Sigma-Aldrich India. A stock solution of AMP (1.0mM) was prepared in millipore water. Phosphate buffers from pH 3.0-10.2 were prepared according to method of Christian and Purdy [**6**] other reagents used were of analytical or chemical grade. All solutions are prepared with Millipore water.

Area of 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 [**7**].

$$I_{pa} = (2.69x10^5)n^{3/2}AD_o^{1/2}C_o\,\upsilon^{1/2} \qquad (1)$$

where I_{pa} refers to the anodic peak current, n is the number of electrons transferred, A is the surface area of the electrode, D_0 is diffusion coefficient, υ is the scan rate and C_0 is the concentration of $K_3Fe(CN)_6$. For 1.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, n = 1. D_0= 7.6 × 10^{-6} cm^2s^{-1}, then from

the slope of the plot of I_{pa} versus $\upsilon^{1/2}$ relation, the surface area of electrode was calculated. In our experiment, the surface area of PGE was calculated to be 0.011 cm^2.

Analytical procedure: The electrodes were transferred into another 10 mL of phosphate buffer (0.2 M, pH 3.0) containing proper amount of AMP. After accumulating for 20 s at open circuit under stirring and following quiet for 10 s, potential scan was initiated and cyclic voltammograms were recorded between 0.0 V and 1.0 V, with a scan rate of 0.1mV s^{-1}. The parameters for SWV were as follows: initial potential, 0.3 V; final potential, 0.6 V; increase in potential, 0.004 V; amplitude, 0.025 V; frequency, 15 Hz; quiet time, 2s; sensitivity, 1.0×10^{-5} A/V.

RESULTS AND DISCUSSION

Cyclic voltammetric behavior of AMP: The electrochemical behavior of AMP at PGE was studied by cyclic voltammetry in 0.2 M phosphate as the supporting electrolyte at pH 3.0. The cyclic voltammogram obtained for 1.0 mM AMP solution at a scan rate v = 0.1 mV s^{-1} as shown in figure 1. Anodic peak occurs at E_{pa} = 0.5348V. On the reverse scan, no corresponding reduction peak was observed indicating that, the electrode process of AMP is an irreversible one. The blank solution without AMP was shown by curve (a), here in peak (a) lower intensity broader peak is observed and at curve (b) intensity of peak increased due to electrocatalytic behavior of pencil graphite. Due to their inherent electrochemical and economical characteristics PGEs found in recent years a wide spread application in various fields.

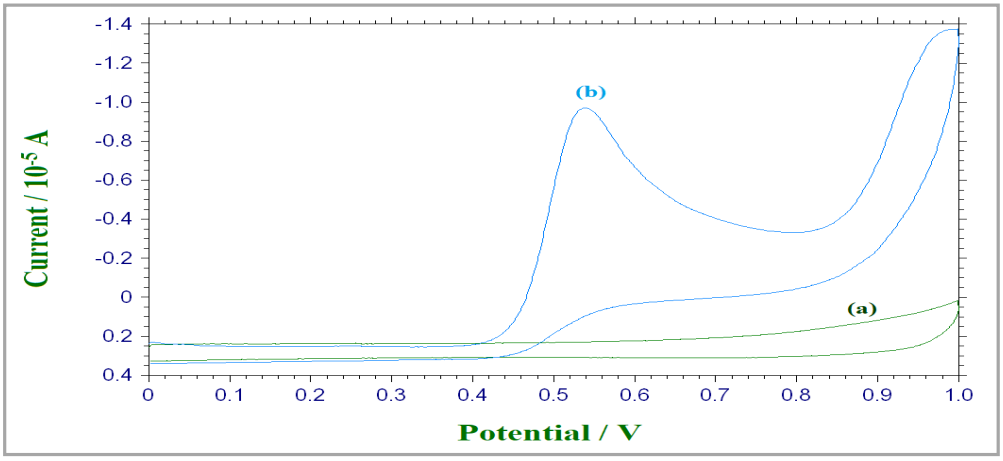


Figure 1. Cyclic voltammogram obtained for 1.0mM AMP on pencil graphite electrode in pH 3.0, 0.2M buffer: (a) blank run without AMP at v=0.1 Vs^{-1} and (b)AMP.

Effect of pH: An electrode reaction might be affected by the pH of the medium. The electro-oxidation of 1.0 mM AMP was studied over the pH range of 3.0−10.2 in phosphate buffer solution by using cyclic voltammetry and the results are shown in figure 2A. The sharp oxidation peak appeared between pH 3.0–10.2 and thereafter sharpness of oxidation peak gradually disappeared. The pH of solution influenced the peak current considerably. With increase in the pH, peak potential linearly shifted to less positive values and the linear relation between E_{pa} and pH (Figure 2B) can be expressed as, E_{pa}(V) = 0.604 - 0.033 pH;(r= 0.935). The slope of the plot E_{pa} versus pH was found to be 0.033 V close to the theoretical value of 30 mV which indicates the involvement of two electrons and a proton transfer in the rate determining step [**8-10**]. From the plot of I_{pa} versus pH (Figure 2C), it is clear that the intensity increased to a high value at pH 3.0, and then the peak intensity decreased. Because the best result with respect to sensitivity accompanied by sharper response was obtained at pH 3.0, this value was selected for further experiments.

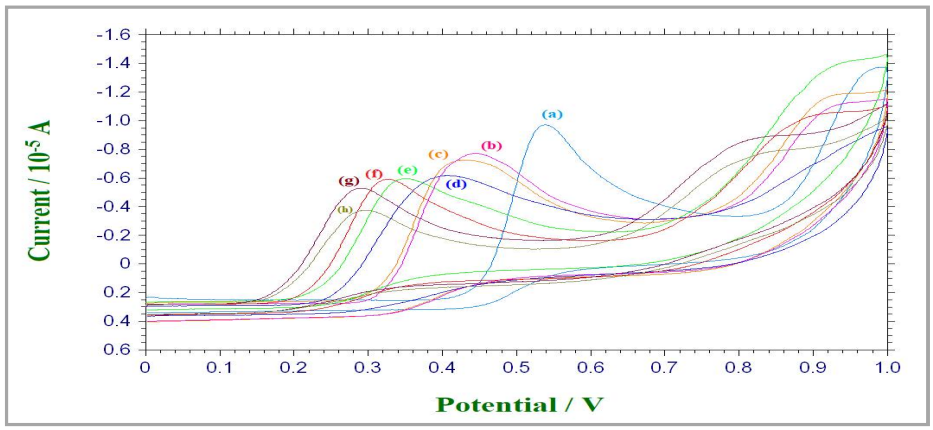


Figure 2A. Influence of pH on the shape of the peaks in phosphate buffer solution at (a) pH 3.0, (b) pH 4.0, (c) pH 5.0, (d) pH 60, (e) pH 7.0, (f) pH 8.0, (g) pH 9.0, (h) pH 10.2 with potential scan rate 0.1 Vs^{-1}.Other conditions are in figure 1.

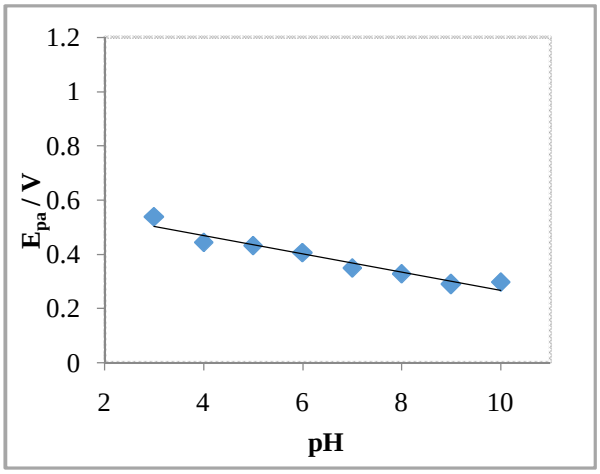


Figure 2B. Influence of pH on the peak potential of AMP.

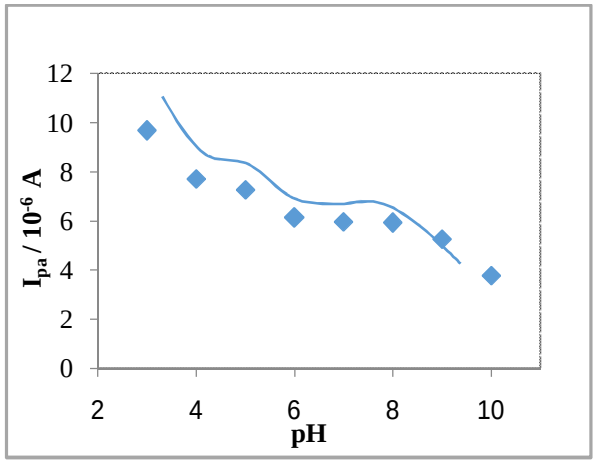


Figure 2C. Variation of oxidation peak currents with pH.

Effect of scan rate: The effect of scan rate on the electro-oxidation of AMP was examined by cyclic voltammetry. The oxidation peak current linearly increased with the increasing the scan rate between 0.1 and 0.35 Vs^{-1} as shown in figure 3A. These results confirmed that, the scan rate played major role and also the oxidation of AMP at PGE was a typical diffusion-controlled process [11]. The influence

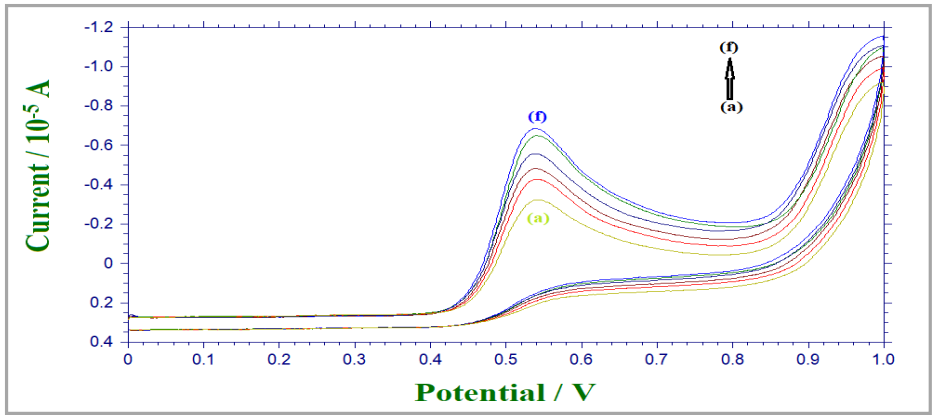


Figure 3A. Cyclic voltammograms obtained for 1.0×10^{-6} M AMP in buffer solution of pH 3.0 at scan rate of (a) 0.1, (b) 0.15, (c) 0.2, (d) 0.25, (e) 0.3, (f) 0.35 Vs^{-1}.

of the square root of the scan rate on the peak current showed a linear relationship (Figure 3B). This behavior is typical of diffusion-controlled process and can be expressed as $I_{pa}(\mu A) = 9.65\ v^{1/2}$ ($V^{1/2}s^{-1/2}$) + 1.203; (r = 0.990). A linear relationship was observed between log I_{pa} and log v (Figure 3C), corresponding to the equation log $I_{pa}(\mu A)$ = 0.391 log v (Vs^{-1}) + 1.016; (r = 0.992).

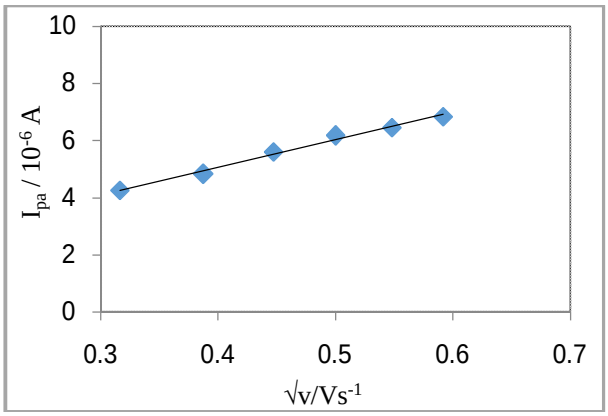


Figure 3B. Dependence of the oxidation peak current on scan rate.

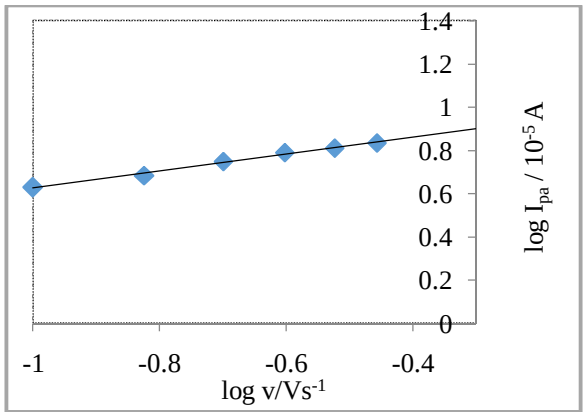


Figure 3C. Dependence of logarithm of peak current on logarithm of scan rate.

The slope value of 0.391 was comparable with the theoretically expected value of 0.5 for a purely diffusion-controlled current, which, in turn, confirms that the electro-oxidation of AMP was diffusion-controlled in our experiments. With an increase in scan rate, the peak potential shifted to a positive value, and a linear relationship was observed in the range of 0.1-0.35 Vs^{-1}, as shown in figure 3D. The relationship can be expressed as E_{pa} (V)= 0.206log v (Vs-1) +0.554; (r= 0.901).

For an diffusion-controlled and irreversible electrode process, according to Laviron [**12**], E_{pa} is defined by the following equation

$$E_p = E^0 + \left(\frac{2.303RT}{nF}\right)\log\left(\frac{RTk^0}{nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right)\log \quad (2)$$

Where α is the transfer coefficient, k^0 is the standard heterogeneous rate constant of the reaction, n is the number of electrons transferred, v is the scan rate, and $E^{0\prime}$is the formal standard redox potential. Other symbols have their usual meanings. Thus, the value of αn can be easily calculated from the slope of a plot of E_{pa} versus log v. In this system, the slope was 0.206; taking T = 298 K, R = 8.314 J K^{-1} mol^{-1}, and F =96480 C mol^{-1}, the α n value was calculated to be 2.85. According to Bard and Faulkner [**13**], α can be expressed as

$$\alpha = \frac{47.7}{E_p - E_{p/2}}\ mV \quad (3)$$

where $E_{pa}/2$ is the potential when the current is at one-half the peak value. From this expression, the value of α was calculated to be 1.21 Further, the number of electrons (n) transferred in the electro-oxidation of AMP was calculated to be 2.35. The value of $E^{0\prime}$ in Eq. 2 can be obtained from the intercept of the E_{pa} versus v curve by extrapolating to the vertical axis at v = 0 [**14**]. In our system, the intercept for E_{pa} versus log v plot was 0.554, so $E^{0\prime}$ was obtained as 0.530, and k^0 was calculated to be 1.21×10^{-3} s^{-1}.

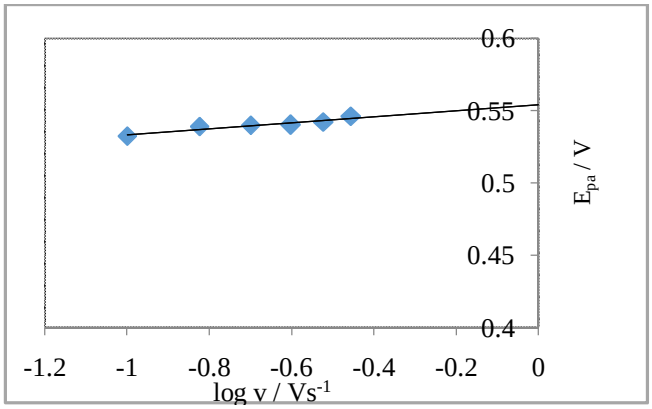
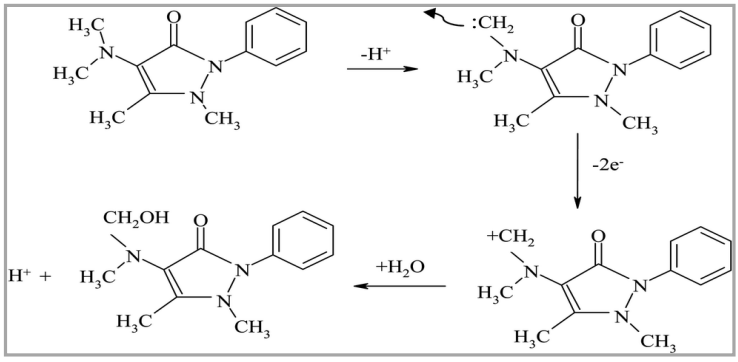


Figure 3D. Relationship between peak potential and logarithm of scan rate.

Mechanism: In the proposed method, the electro-oxidation of AMP 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 [**15, 16**]. The probable mechanism is as shown in scheme 2.



Scheme 2. Probable electrode oxidation mechanism of AMP.

Calibration curve: In order to develop a voltammetric method for determining the drug, we selected the DPV (Figure 4A) and SWV (Figure 4C) modes, because the peaks are sharper and better defined at lower concentration of AMP, than those obtained by cyclic voltammetry, with low background current, resulting in improved resolution. According to the obtained results, it was possible to apply these techniques to the quantitative analysis of AMP. DPV (Figure 4B) and SWV (Figure 4D) obtained with increasing amount of AMP showed that the peak current increased linearly with increasing concentration. Using the optimum conditions described above, linear calibration curves were obtained for AMP in the range of 0.1 to 1.1 µM for DPV and 0.1 to 1.5 µM for SWV. The linear equation was I_{pa} (µA) = 0.973C (µM) + 3.173 (r^2 = 0.974) and I_{pa} (µA) = 2.318C (µM) + 5.508 (r^2 = 0.971) for DPV and SWV respectively. The SWV presents a good linear response as compared to DPV. Deviation from linearity was observed for more concentrated solutions, due to the adsorption of oxidation product on the electrode surface. Related statistical data of the calibration curves were obtained from five different calibration curves. The limit of detection (LOD) and quantification (LOQ) were 3.25×10^{-9} M and 1.08×10^{-8} M for SWV and 9.19 $\times10^{-8}$and 3.06 $\times10^{-7}$ for DPV respectively. The LOD and LOQ were calculated using the following equations:

$$LOD = 3s/m, \quad LOQ = 10s/m \quad (4)$$

Where, s is the standard deviation of the peak currents of the blank (five runs) and m is the slope of the calibration curve.

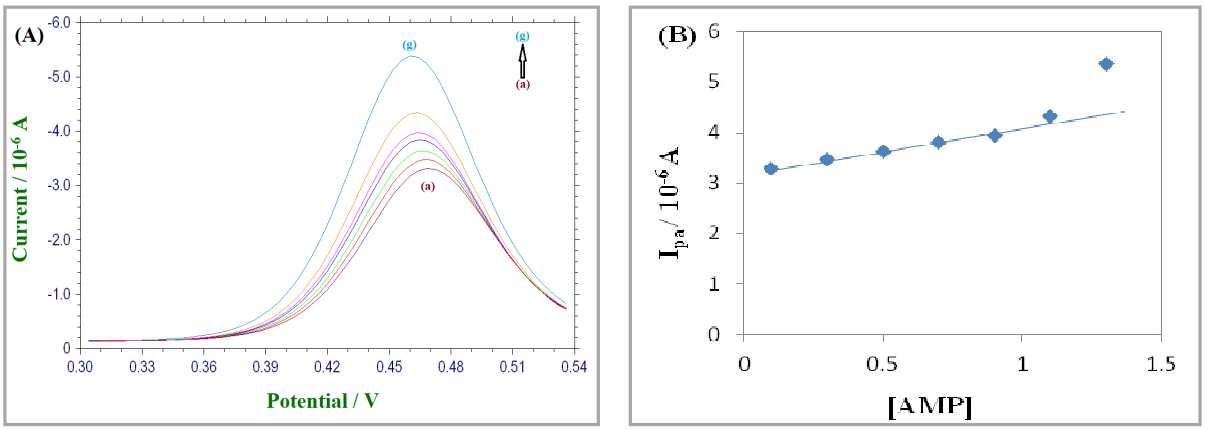


Figure 4. (A) DPVof pencil graphite electrode in AMP solution at different concentrations at (a) 0.1, (b) 0.3, (c) 0.5, (d) 0.7, (e) 0.9, (f) 1.1, (g) 1.3mM; **(B)** plot of peak of pencil graphite electrode in AMP for **DPV**

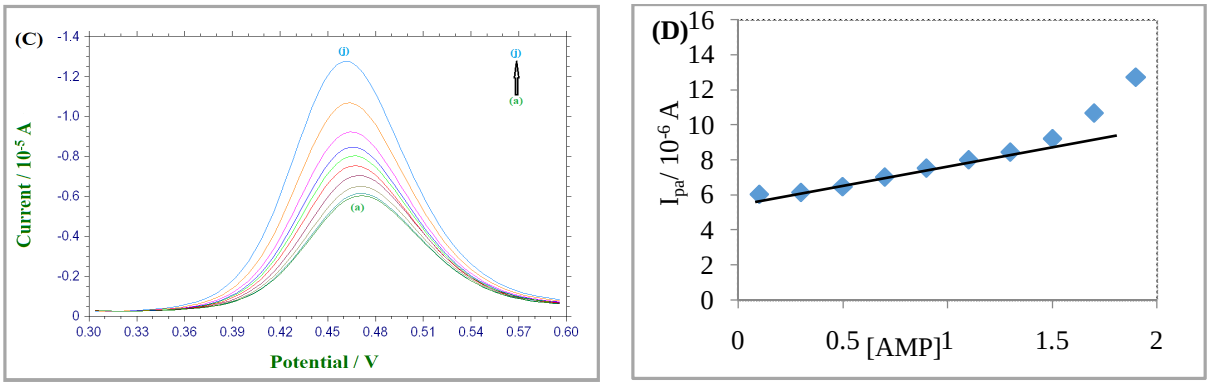


Figure 4. (C) SWV of pencil graphite electrode in AMP solution at different concentrations at (a) 0.1, (b) 0.3, (c) 0.5, (d) 0.7, (e) 0.9, (f) 1.1, (g)1.3, (h)1.5, (i) 1.7, (j) 1.9mM; **(D)**plot of peak current against concentration of AMP for **SWV.**

Effect of interferants: The potential interference for the determination of AMP was also studied. Under the optimized conditions, the oxidation peak of 1.0 µM AMP was individually measured in the presence of different concentrations of the common interferents and then the change of peak potential was checked. It was found that citric acid, glucose, gum acacia, lactose, oxalic acid, sucrose and starch almost had no influence on the detection of AMP. Since the peak potential change was below 5% (Table 1). This revealed that these sensors have good selectivity for AMP determination.

Table 1. Influence of potential excipients on the voltammetric response of 1.0 mM AMP

Excipients (1.0 mM)	Potential observed (E_{pa})	Signal Change (%)
Sucrose	0.5131	2.6560
Glucose	0.5329	-1.1003
Citric acid	0.5212	1.1193
Gum acacia	0.5480	-3.9650
Starch	0.5399	-2.4283
Lactose	0.5236	0.6640
Oxalic acid	0.5317	-0.8727

(a)Mean average of five determinations.

Detection of AMP in urine sample: The applicability of the SWV to the determination of AMP in spiked urine as a real sample was investigated. The recoveries from urine were measured by spiking drug free urine with known amounts of AMP. A quantitative determination can be carried out by adding the standard solution of AMP into the detect system of urine sample. The calibration graph

was used for the determination of spiked AMP urine samples. The recovery determined was in the range from 99.09 to 100.25 %, and the RSDs are listed in table 2. Thus, satisfactory recoveries of theanalyte from the real samples make the developed method applicable in clinical analysis.

Table 2. Determination of AMP in urine samples

Added (µM)	found[(a)] (µM)	Recovery (%)	SD± RSD (%)	Bias (%)
2.5	2.468	99.09	0.0301 ± 0.22	0.914
3.0	3.010	100.25	0.0252 ± 0.26	-0.25
4.5	4.488	99.82	0.0198 ± 0.32	0.184

(a)Mean average of five determinations.

APPLICATION

A systematic procedure is developed to determine the AMP at PGE. Furthermore, the present method could possibly be adopted for pharmacokinetic studies as well as clinical and quality control laboratories.

CONCLUSION

In this work, a PGE has been successfully used for the determination of AMP in phosphate buffer solution (pH=3.0). AMP is irreversibly oxidized at PGE and is diffusion controlled. A suitable oxidation mechanism involving two electrons-one proton was proposed. 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 AMP in spiked urine sample demonstrated the applicability of the method for real sample determination.

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