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Up Growth Effect of Cetyltrimethyl Ammonium Bromide with Carbon Paste Electrode for the Electrochemical Determination of Allopurinol and Its Biological Activities

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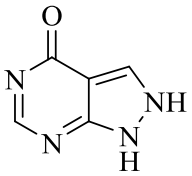
Abstract- A stable sensor for the determination of allopurinol (AP) was upgrade by effect of surfactant cetyltrimethyl ammonium bromide (CTAB) with running phosphate buffer solution (pH=3.0) at a carbon paste electrode (CPE). A sensitive electrochemical method was improved distinctly in the presence of low concentration of CTAB, the oxidation peak current signifying that CTAB exhibits noticeable enhancement effect to the determination of AP as well as under optimal conditions the linear sweep voltammetry response to AP has a linear concentration over the range from 0.6 µM to 60.0 µM, with limit of detection and quantification 97.2 nM and 0.324 µM respectively. This constructed voltammetry sensor was successfully applied to determination of AP in the human serum sample, urine sample and tablet. Finally this was shown to be sensitive and efficient.

Keywords- Allopurinol, Surfactant effect, Electrochemical detection, Drug monitoring, Carbon paste electrode

1. INTRODUCTION

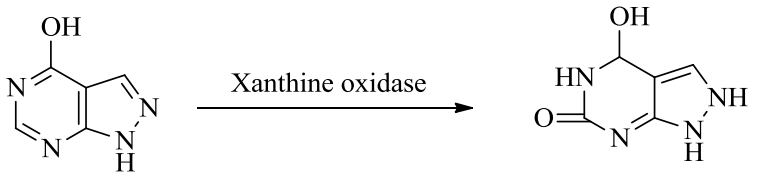
Drug monitoring is one of the important mechanisms and is a branch of electrochemistry. Therefore, the development of rapid response, high sensitive, simple and reliable method for the determination of dynamic drug is of great importance and attention. Allopurinol (4-

hydroxypyrazolo[3,4-d] pyrimidine) (AP) as shown in Scheme 1, is a radical sifting clinical drug and widely used in the treatment of hyperuricemia and chronic gout [1-3] and it can be controlled either intravenously or orally. The bioavailability is about 67 to 90% with a peak plasma concentration arising within one hour; the volume of distribution is approximately 1.62/kg [4]. It is additionally used in the novel therapeutic strategy for the treatment of human heart failure [5]. AP is greatly used to reduce the gout, hyperuricemia, Lesh-Nyan, renal failure, kidney disease, heart disease, high blood pressure and diabetes [6].



Scheme 1. Chemical structure of allopurinol

It is frequently used in patients with severe gout, although, optimization of allopurinol dosage by measuring oxypurinol serum levels might be necessary. Another indication for therapeutic drug monitoring (TDM) is to verify a patient's adherence to the use of allopurinol, which in general is reported to be a point of concern [7,8]. Usually drugs containing allopurinol (ALO) (daily dose 100-300 mg) are used. ALO (structural analogue to HYP and XO inhibitor) is preferentially oxidized to oxypurinol (OXY), which is excreted in the urine together with HYP and XAN as more soluble products compared to UA. From a medical point of view, it is necessary to monitor these substances, because, for instance, at a higher dosage of ALO, there is a danger of xanthine nephropathy caused by accumulation of natural purines [9]. Allopurinol is a structural isomer of hypoxanthine (a naturally occurring purine in the body) and acts to inhibit xanthine oxidase. In the presence of xanthine oxidase, allopurinol will be converted to alloxanthine as shown in Scheme 2, after that the formation of uric acid from xanthine and hypoxanthine will be inhibited [10].



Allopurinol Alloxanthine

Scheme 2. Inhibition of uric acid production

The purine substrates and products of XDH/XO, hypox-anthine, xanthine, and uric acid are related by 2-electron, 2-pro-ton and O-atom transfer reactions so each is electrochemically active. The approximate electrochemically redox potentials determined at

pH 8 of these purines (from various studies) in addition to related half reactions of the clinically important xanthine oxidase inhibitor allopurinol (an isomer of hypoxanthine). In each case, the oxidation of each molecule is electrochemically irreversible or quasi-reversible and requires very high over potentials relative to the true thermo-dynamic redox potentials. Of most significance to this study, the product of XDH/XO activity, uric acid, is itself able to be oxidized to a transiently stable imine that is electrochemically detectable. There have been many reports of uric acid electrochemistry using a variety of working electrodes. Uric acid imine undergoes a slower decomposition ultimately to allantoin at neutral pH. In this article, we reconcile previously unexplained electrochemical behavior of XDH by showing that the uric acid imine, on the voltammetric time scale, is capable of acting as an NAD^+ mimic in accepting electrons from XDH following turn-over in an unusual enzyme electro-autocatalytic system [11]. An assessment of the literature revealed that, there are only rare analytical methods for the determination of AP in pharmaceutical preparations and biological conditions relying on the use of capillary electrophoresis (CE/UV) [12], capillary electrophoresis with end-column amperometric detection (CE-EC) [13], high-performance liquid chromatography with UV detection (HPLC-UV) [14], flow injection electrochemiluminescent (FI-ECL) in presence of $Ru(bpy)_3^{2+}$ [15], nanolayer molecularly imprinted polymer with multiwall carbon nanotubes (MIP-MWCNT) [16], sensitive voltammetric an edge plane-oriented pyrolytic graphite electrode and glassy carbon electrode (g-PGEe/g-GCE) [17], flow injection analysis by enzymeless at glassy carbon electrodes [18] and high-performance liquid chromatography with UV detection (HPLC-UV) [19] were several methods are described for determination of AP. Unfortunately, the problems come across in using such methods are either the essential for time-consuming extraction procedures or lack of information. However, developing a voltammetry sensors fulfill many of the requirements for such tasks predominantly owing their integral selectivity, rapid resolution, fast and sensitivity.

As far as we know, there has been yet no report on enhanced surfactant electrodes for the determination of AP. At low concentrations, surfactant molecules adsorbed on the electrode surface and make a film of surfactant on electrode surface. Surfactants, containing hydrophobic and hydrophilic clusters can modification the properties of the electrode surface and finally stimulus the electrochemical processes of additional substances [20]. Interrelated work has been done our research group for enhancing agents in electro analytical chemistry to propagate the detection limits of some biomolecules [21-25]. In this existing work, experimental results indicated that cationic surfactant-CTAB had a noticeably group up effect on the electrochemical determination of AP at carbon paste electrode. Accordingly, the intention of the present investigation was to optimize under electrochemical conditions for the monitoring AP at carbon paste electrode. In a several electrodes mainly carbon paste electrodes plays an important role in electrochemical investigations because of their low

background current, low cost, renewability with many electron mediators, have been widely used in these studies [26].

The present study is concerned with low concentration CTAB effect on electrode activities and electrochemical reaction of AP. Compared with that in the absence of CTAB, the oxidation peak current of AP significantly enhance in the presence of CTAB. This process has some obvious advantages together with high sensitivity, informal repair, no time-consumed sample preparation, low cost, reproducibility and good detection limit. Finally, the method informed in this article was found to be straight forward, modest, efficient and sensitive in addition to constituting an effective method for exploration by linear sweep voltammetry (LSV) of the AP.

2. EXPERIMENTAL

2.1. Instrumentation

Electrochemical measurements were carried out using a CHI602E electrochemical work station (CH Instrument Ltd. Co., USA). A standard single compartment three electrode cells was used Ag/AgCl as a reference electrode, Pt wire as a counter electrode and a self-made carbon paste electrode as a working electrode. All the potential were given against Ag/AgCl (3 M KCl). The pH measurements were made with Elico pH meter model LI120 (Elico Ltd., India) with glass electrodes.

2.2. Reagents and chemicals

All chemicals were of analytical grade and used without additional purification. AP was obtained from sigma Ltd., India. A stock solution of AP (1.0 mM) was prepared in by dissolving doubly distilled water. The phosphate buffers (KH_2PO_4/K_2HPO_4) from pH 3.0–10.4 were prepared according to the method of Christian and Purdy [27]. The AP containing tablets i.e. Zyloric (GlaxoSmithKline: GSK) were purchased from a local pharmacy. Cetyltrimethylammonium bromide (CTAB) surfactants obtained from Hi-Media Pvt. Ltd., were dissolved in doubly distilled water to form 10.0 µM solutions. Graphite powder (particle size < 50micron) and mineral oil (IR grade) were obtained from S.D. Fine Chem., India. All other reagents used were of analytical or chemical grade. All solutions were prepared with doubly distilled water.

2.3. Preparation of electrode

The carbon paste electrode was prepared by mixing 1.0 gram graphite powder and 0.5 ml mineral oil in an agate mortar, and the mixture was then homogenized. After that, the paste was packed into the void of the electrode body and the surface was smoothened on a

weighing paper. Unless otherwise stated, the paste was carefully removed prior to pressing a new portion into the electrode after each measurement.

The area of the electrode was obtained by the cyclic voltammetric method using 1.0 mM $K_4Fe(CN)_6$ as a review of different scan rates. For a reversible process, the fallowing Randles-Savcik equation can be used [28].

$$I_p = 0.4463\left(\frac{F^3}{RT}\right)^{1/2} n^{3/2} A_0 D_0^{1/2} C_0 v^{1/2} \quad (1)$$

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, v is the scan rate and C_0 is the concentration respectively, of $K_4Fe(CN)_6$. For 1.0 mM $K_4Fe(CN)_6$ in 0.1 M KCl electrolyte, T=298 K, R=8.314 J K^{-1} mol^{-1}, F=96,480 C mol^{-1}, n=1, $D_0=7.6\times10^{-6}$ cm^2 s^{-1}, then from the slope of the plot of I_{pa} *vs.* $v^{1/2}$, relation, the electroactive area was calculated. In our experiment the slope was 2.7×10^{-4} μA $(V\ s^{-1})^{1/2}$ and the area of electrode was calculated to be 0.369 cm^2.

2.4. Analytical procedure

Unless otherwise stated, the CPE was first activated in phosphate buffer (pH 3.0, Ionic strength=0.2 M) containing 10 μM CTAB was used as the supporting electrolyte for determination of AP by cyclic and linear sweep voltammetric sweeps between 0.0 and 2.0 V until a stable cyclic voltammograms was obtained. Then electrodes were transferred into another 10 ml of phosphate buffer (0.2 M, pH 3.0) containing proper amount of AP and CTAB. The accumulation step was carried out under open-circuit with 3 min stirring solution, then cyclic and linear sweep voltammograms from 0.4 to 1.6V were recorded after 20s quite time, with a scan rate of 100 mV s^{-1}. All measurements were carried out at room temperature of 25±0.1 ˚C.

2.5. Sample preparation

A quantity of 5 tablets were weighed and ground to a homogeneous fine powder in a mortar. A portion equivalent to a stock solution of concentration of about 1.0 mM was accurately weighed and dissolved in doubly distilled water. The contents were sonicated for 10 min to affect widespread dissolution. The excipient was separated by filtration and the residue was washed three times with doubly distilled water. The solution was transferred into a 100 ml calibrated flask and diluted to a final volume with same solvent. Appropriate solutions were prepared by taking suitable aliquots from this stock solution and diluting them with the phosphate buffer solutions. Each solution was transferred to the voltammetric cell and analyzed by standard addition method. The linear sweep voltammograms were recorded between 0.4 and 1.6 V after open-circuit accumulation for 20 s with stirring. The oxidation

peak current of AP was measured. The parameters for linear sweep voltammetry (LSV) were, pulse width of 0.06 s, pulse increment of 4 mV, pulse period of 0.2 s, pulse amplitude of 50 mV and scan rate of 100 mVs^{-1}. To study the accuracy of the proposed method and to check the interferences from excipient used in the dosage form, recovery experiments were carried out. The concentration of AP was calculated using standard addition method.

3. RESULTS AND DISCUSSIONS

3.1. Cyclic voltammetric behavior of AP

The electrochemical behavior of AP at CPE and in the presence of CTAB was investigated using cyclic voltammetry (CV). The results are shown in Fig. 1. No apparent cyclic voltammetric signals were detected in the phosphate buffer (pH 3.0) solution in the presence (curve c) and absence (curve d) of CTAB, which point out that CTAB is an electrochemically inactive material in the working potential range. The AP shows an anodic peak at about 1.39 V (curve b) at CPE in the absence of CTAB. After the addition of 10 µM CTAB, the oxidation peak current of AP increases significantly (curve a). This indicates that CTAB can make the electron transfer of AP more easily and show obvious enhancement effect to the oxidation of AP. The peak current enhancement was undoubtedly attributed to the interaction of CTAB with AP and CPE. It is well known that surfactants can be adsorbed on a hydrophobic surface to form surfactant film, which may alter the over voltage of the electrode and influence the rate of electron transfer.

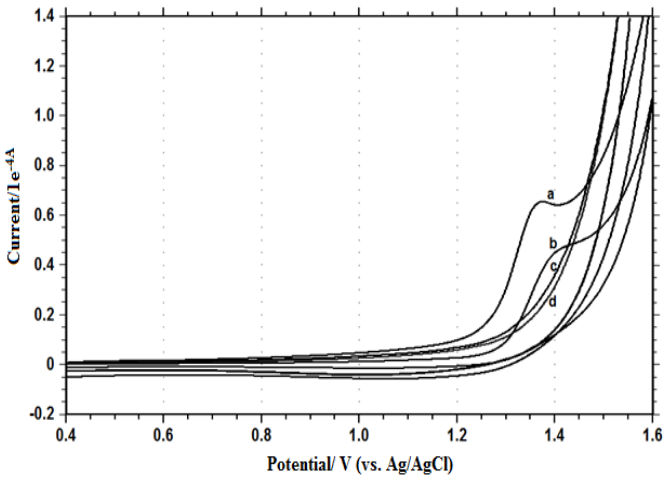


Fig.1. Cyclic voltammograms at the carbon paste electrode in 0.2 M phosphate buffer solution (pH 3.0): (a) in the presence of AP and CTAB; (b) in the presence of AP; (c) in the presence of CTAB; (d) in the absence of CTAB; Scan rate: 100 mVs^{-1}; *t*(acc): 50 s (at open circuit), AP: 1.0×10^{-3} M, CTAB: 10 µM

In the presence of CTAB, the electrode surface may form a hydrophilic film with positive charge. This hydrophilic layer increases the concentration of AP at the electrode surface. The working potential range of AP was taken as 0.4-1.6 V and it is well known fact that each substrate has its own capability to undergo oxidation at a particular potential where it's current will increase, likewise AP underwent oxidation at higher potential of 1.39 V showing higher peak current at pH 3.0. On the reverse scan, no corresponding reduction peak was observed, specifying that the electrode process of AP is an irreversible one. It was found that the oxidation peak current of AP showed a remarkable decrease during the successive cyclic voltammetric sweeps. A decrease in the oxidation peak current occurs with the number of successive sweeps. This phenomenon may be due to the fact that the adsorption of AP or its oxidative product occurs at the electrode surface. As a result, the voltammograms corresponding to the first cycle was generally recorded [29-31].

3.2. Influences of accumulation potential and time

The influence of accumulation potential and accumulation time was studied using cyclic voltammetric method. Open circuit accumulation is widely used in electroanalytical chemistry to accumulate analyzed and develop the sensitivity. The influence of accumulation was studied time ranging from 10 to 90 s on the oxidation of AP at CPE.

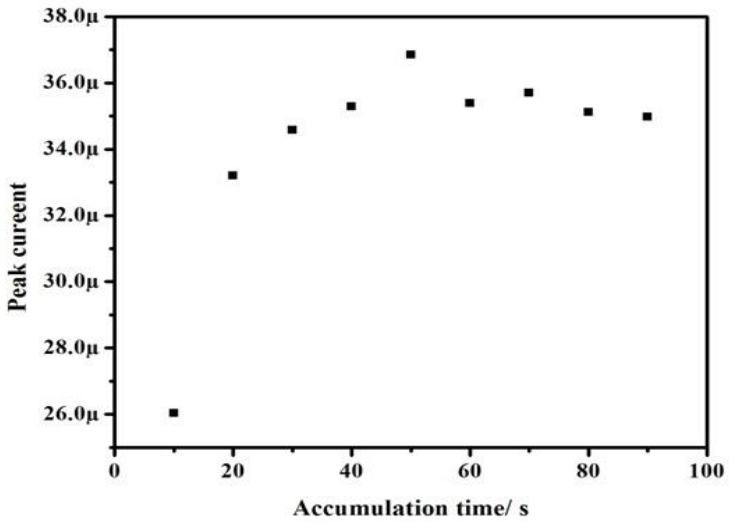


Fig. 2. Variation of the anodic peak current with accumulation time

The current increased gradually as accumulation time increased from 10 to 50 s. However, with further increasing, accumulation time beyond 50 s, the peak current decreases (Fig. 2). Therefore, optimal accumulation time of 50 s was employed in further experiments. With the change of accumulation potential, the peak current of AP varied only. Thus, the accumulation potential has practically no effect on the peak current of AP, which in turn

indicates that adsorption of CTAB is independent of the charge on the electrode surface. It is reliable with the fact that CTAB is adsorbed on the electrode surface through hydrophobic interaction with mineral oil. Consequently the accumulation was carried out at open-circuit conditions.

3.3. Influence of concentration of CTAB

Amongst different surfactants used, such as sodium dodecyl sulfate (SDS), sodium dodecylbenzene sulfonate (SDBS) and cetyltrimethyl ammonium bromide (CTAB). From Fig. 1, it is clear that CTAB could only enhance the oxidation peak current of AP at carbon paste electrode effectively. In presence of CTAB, the monomer surfactant molecules due to the cationic charge of themselves, get arranged on the anionic surface of the electrode and give the electrode opposite chare (positive), so that, AP get adsorbed on the electrode surface.

Therefore in presence of CTAB, AP potential shifts toward the lower potential and also, the peak current increases significantly. The optimum concentration of CTAB was determined using different concentration of CTAB. It is clear that, the critical micelle concentration (CMC) of CTAB can be affected by the experimental conditions as well as the presence of various materials or additives that are added to the measurement cell. It will be better, if we use critical aggregation concentration (CAC) in these systems instead of CMC because, in presence of other substances the micellization phenomena will not occur only by the surfactant molecules, but co-micellization of additive and surfactant molecules occur. It is clearly implicit that CAC for the mentioned condition has been reached at 1.0×10^{-5} M of CTAB. On the other hand, with further increase in the CTAB concentration, i.e., beyond 1.0×10^{-5} M, peak cur-rent decreased which indicates that, CTAB layer formed on the electrode surface blocks the electron transfer between AP and the electrode.

3.4. Influence of pH 1.0×10^{-5}

The electrode reaction might be affected by the pH of the medium. The electrooxidation of AP at 1.0×10^{-3} concentration was studied over the pH range of 3.0–10.4 in phosphate buffer solution using cyclic voltammetry. The oxidation peak appeared between pH 3.0–9.2 and after that sharp oxidation peak gradually extinct (Fig. 3A). With increase in the solution pH, peak potential linearly shifted to less positive values and the linear relation between E_p and pH (Fig. 3B) was obtained. The solution pH influenced the peak current considerably. The peak current decreased linearly with the increase in pH of solution. So, the buffer solution with pH 3.0 was selected for further experiments.

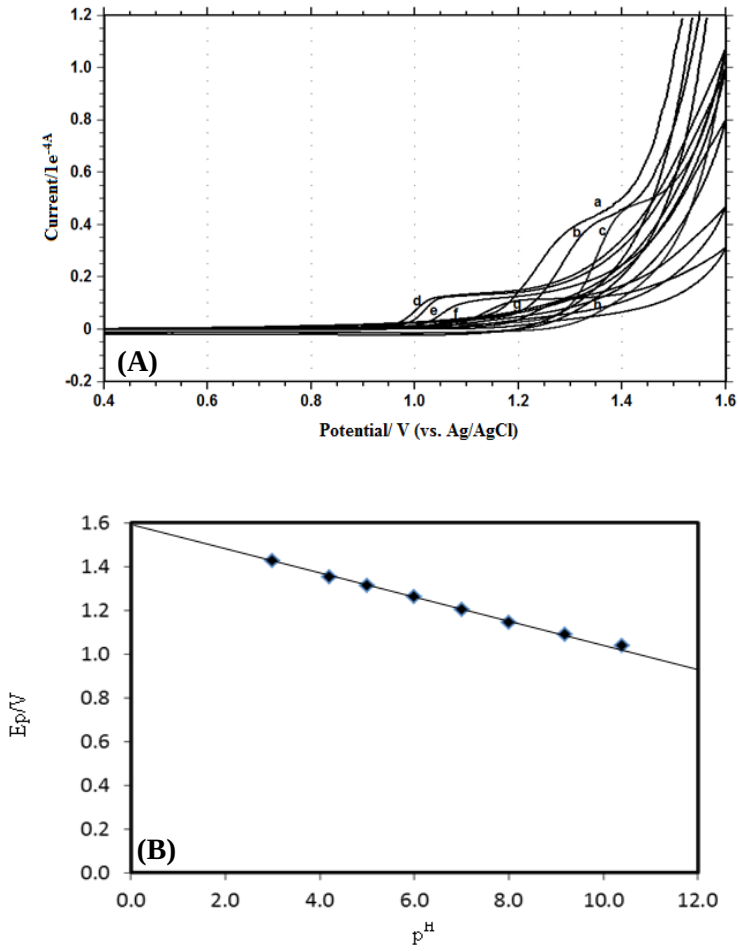


Fig. 3. (A) Influence of pH on the shape of anodic peak. pH: 5.0 (a), 4.2 (b), 3.0 (c), 10.4 (d), 9.2 (e), 8.0 (f), 7.0 (g), 6.0 (h); (B) Influence of pH on the peak potential E_p/V of AP. Other conditions are as in Fig. 1

3.5. Influence of scan rate

Useful information involving electrochemical mechanism generally can be acquired from the relationship between peak current and scan rate. Therefore, the electrochemical behavior of AP at different scan rates from 25 to 300 mVs^{-1} (Fig. 4) was also studied. The influence of scan rate on anodic peak current showed a good linear relationship and the equation can be expressed as follows (Eq. 2):

$$I_p = 0.4463\left(\frac{F^3}{RT}\right)^{1/2} n^{3/2} A_0 {D_0}^{1/2} C_0 v^{1/2} \quad (2)$$

This indicates that the electrode process was controlled by adsorption rather than diffusion. A plot of logarithm of anodic peak current *vs*. logarithm of scan rate gave a straight line with a slope of 0.8261(Fig. 4 (Inset)) close to the theoretical value of 1.0, which is the expected value for an ideal reaction of surface species [32]. So, it confirms that the process

appears to have an important adsorptive component and the equation can be expressed as Eq. 3:

$$\text{Log}\frac{I_p}{1e^{-4A}} = 0.8261 \log v/\text{Vs}^{-1} + 1.5323,\ r = 0.9842 \quad (3)$$

The E_p of the oxidation peak was also dependent on scan rate. The peak potential shifted to more positive values on increasing the scan rate, which confirms the irreversibility of the oxidation process and a linear relationship between peak potential and logarithm of scan rate can be expressed by the Eq. (4):

$$\frac{E_p}{V} = 1.4648 + 0.0543 \log v/\text{Vs}^{-1};\ r = 0.9719 \quad (4)$$

For an adsorption-controlled and irreversible electrode process, according to Laviron [33], E_p is defined by the Eq. (5):

$$E_P = E^{ó} + \frac{2.303RT}{anF}\log\frac{RTk^0}{anF} + \frac{2.303RT}{anF}\log v \quad (5)$$

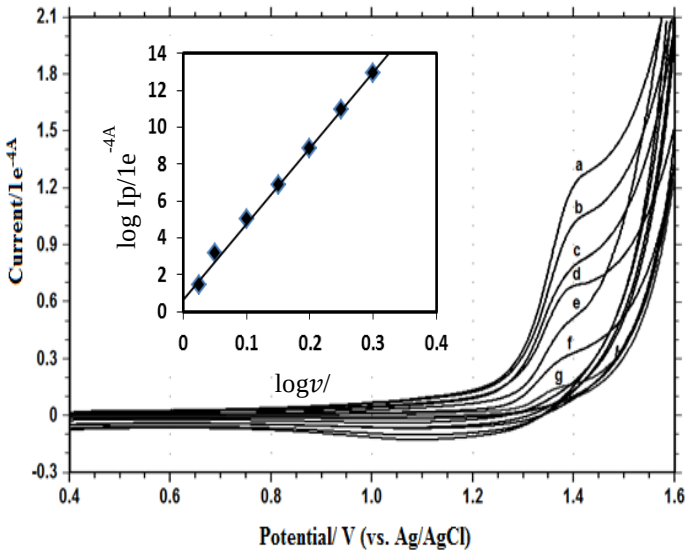


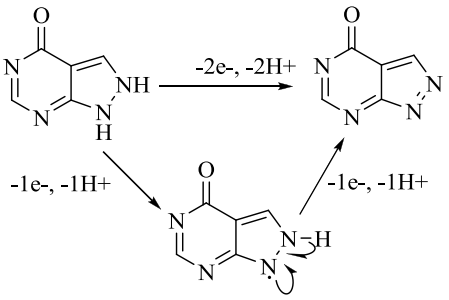
Fig. 4. Cyclic voltammograms of 1.0×10^{-3} M AP at CPE in the presence of CTAB with different scan rates. (a)-(g) were 25, 50, 100, 150, 200, 250 and 300 mVs^{-1}, respectively. Other conditions are given as in Fig. 1. Inset: Dependence of the logarithm of peak current log $I_p/1e^{-4A}$ on logarithm of scan rate log v/Vs^{-1}

Where α (alpha) is the transfer coefficient, k^0 is the standard heterogeneous rate constant of the reaction, n is the number of electrons transferred; v (nu) is the scan rate and $E^{ó}$ 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 0.0543, taking T=298 K, and substituting the values of R and F, αn was calculated to be 1.085.

Generally α is assumed to be 0.5 in total irreversible electrode process [22]. Further, the number of electron (n) transferred in the electrooxidation of AP was calculated to be 2.17≃2. The value of k^0 can be determined from the intercept of the above plot if the value of $E^{ó}$ is known. The value of $E^{ó}$ in Eq. (2) can be obtained from the intercept of E_p *vs.* v curve by extrapolating to the vertical axis at v=0 [34]. In our system the intercept for E_p *vs.* log$v$ plot was 1.4648 and $E^{ó}$ was found to be 1.383; k^0 was calculated to be 1.3×10^3 cms^{-1}.

3.6. Mechanism

Taking into account that AP contains heterocyclic amines in its molecular structure, it presents as basic center with the availability of nonbonding electron as donor. So, we may assume that the oxidation step of AP is located on the heterocyclic amines. AP loses an electron from the heterocyclic amines to form cation radical, further oxidation, which on losing a proton and electron in subsequent steps to form another cation radial. Thus resulted 4H-pyrazolo [3,4-d] pyrimidin-4-one compound was formed. The mechanism is shown in Scheme 3.



Scheme 3. Detailed plausible mechanism; of electrooxidation of AP at carbon paste electrode in the presence of CTAB

3.7. Calibration curve and detection limit

In order to develop a voltammetric method for determining the drug, we selected the linear sweep voltammetric mode, because the peaks were sharper and better defined at lower concentration of AP than those achieved by cyclic voltammetry, with a lower background current, resulting in upgraded resolution. According to the obtained results, it was possible to put on this technique to the quantitative determination of AP. The phosphate buffer solution of pH 3.0 was selected as the supporting electrolyte for the quantification of AP as it gave a maximum peak current at pH 3.0. Linear sweep voltammograms obtained with increasing amounts of AP showed that the peak current increased linearly with increasing concentration, as shown in Fig. 5. Using the optimum conditions described above, linear calibration curves were obtained for AP in the range of 0.6–60.0 µM. The linear equation was $I_p/1e^{-4A}$=6.666 [AP]/µM+2.203; r=0.9909. Deviation from linearity was observed for more concentrated

solutions, due to the adsorption of AP or its oxidation product on the electrode surface. Related statistical data of the calibration curves were obtained from five different determinations. The limit of detection (LOD) and quantification (LOQ) were 97.2 nM and 0.324 µM, respectively [35-37]. The LOD and LOQ were calculated using the following equations:

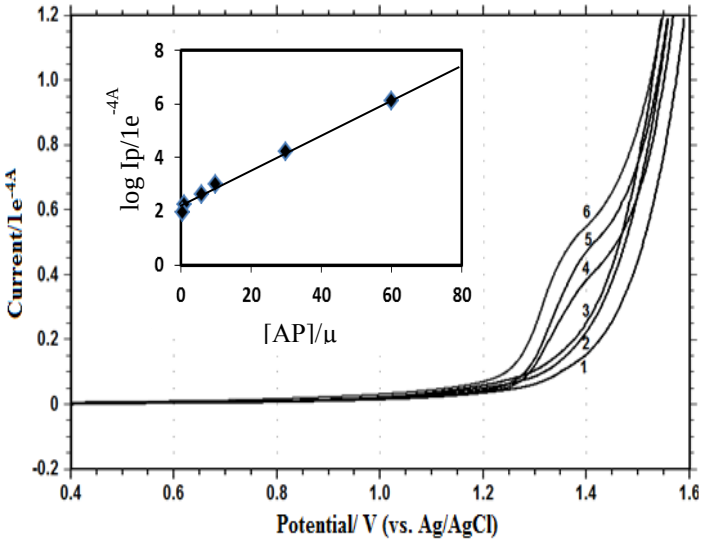


Fig. 5. Linear sweep voltammograms of CTAB modified CPE in AP solution at different concentration: 0.6 (1), 1.0 (2), 6.0 (3), 10.0 (4), 30.0 (5) and 60.0 (6)×µM. Inset: Plot of peak current $I_p/1e^{-4A}$ against concentration of AP/µM

LOD=3 s/m; LOQ=10 s/m. Where, s is the standard deviation of the peak currents of the blank (five runs), and m is the slope of the calibration curve. The detection limits reported for different classical methods and electrodes are tabulated in Table 1. This method was better compared to other reported classical/electrochemical methods [13-18]. Precision of the method was investigated by intra- and interday determination of AP at two different concentrations (*n*=6) within the linear range. In order to ascertain the repeatability of the analysis, 6 measurements of 1.0×10^{-3}AP solution were carried out using carbon paste electrode in the presence of CTAB at intervals of 30 min. The RSD value of peak current was found to be 2.83%, which indicated that carbon paste electrode in the presence of CTAB has good repeatability. As to the reproducibility between days, it was similar to that of within a day repeatability if, the temperature was kept almost unchanged. Owing to the adsorption of AP 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 modified again, by taking new carbon paste for next readings.

Table 1. Comparison of linear range and detection limits for AP with different classical methods and electrodes

Classical method/type of electrode	**Linear range (µM)**	**Detection limits (µM)**	**Detection Potential (V)**	**Ref.**
CE-EC	0.2-100.0	0.001 (1×10^{-8})	1.20	[13]
HPLC-UV	0.4-20	0.0006 (0.6 ng)	-	[14]
FI-ECL- $Ru(bpy)_3^{2+}$	0.01-0.5	0.005 (5 nM)	1.5	[15]
NMIP-MWCNT	0.01-1.0	0.0068 (6.88 nM)	0.53	[16]
g-PGEe/g-GCE	-	0.05 (50 nM)	-0.1 to +1.85	[17]
FIA	150	0.5	-	[18]
CPE with CTAB	**0.6-60.0**	**0.0972 (97.2 nM)**	**1.439**	**Present work**

CE-EC: Capillary electrophoresis with end-column
FI-ECL: Flow injection electrochemiluminescent
NMIP-MWCNT: Nanolayer molecular imprinting multiwall carbon nanotube
g-PGEe/g-GCE: Roughened pyrolytic graphite and glassy carbon electrode
FIA: Flow injection analysis

3.8. Detection of AP in urine samples

The developed linear sweep voltammetric method for the AP determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known amounts of AP.

Table 2. Determination of AP in urine samples Scan rate 100 mVs^{-1}, *t*(acc): 50 s (at open circuit), AP: 1.0×10^{-3} M, CTAB: 10 µM by using linear sweep voltammetry

Urine	**Spiked (µM)**	**Detected[a] (µM)**	**Recovery (%)**	**SD±RSD (%)**
Sample 1	7.0	7.105	101.5	0.074±0.053
Sample 2	5.0	4.913	98.2	0.061±0.043
Sample 3	3.0	3.016	100.5	0.011±0.0078
Sample 4	1.0	1.033	103.3	0.023±0.0164
Sample 5	0.5	0.4933	98.66	0.004±0.0033

[a]Average of five determinations

The urine samples were diluted 100 times with the phosphate buffer ((KH_2PO_4/K_2HPO_4)) solution before analysis without further pretreatments. A quantitative determination can be carried out by adding the standard solution of AP into the detect system of urine sample. The calibration graph was used for the determination of spiked AP in urine samples. The

detection outcomes of five urine samples obtained are listed in Table 2. The recovery determined was in the range from 98.2% to 103.3% and the SD and RSD are given in Table 2. Thus, satisfactory recoveries of the analyzed from the real samples and a good agreement between the concentration ranges studied and the real ranges encountered in the urine samples when treated with the drug make the developed method applicable in clinical analysis.

3.9. Detection of AP in real samples

In order to establish the ability of the enhanced effect of surfactant CTAB with CPE, the sensor was applied to determine AP in tablets according to the recommended method. Also, for investigating the capability of the proposed sensor for determination of AP in complex matrix of real clinical samples, and since there are no patient samples available that they would use AP drug, a spike method was chosen. Five measurements were performed for each concentration. As can be seen in Table 3, good recoveries and SD±RSD were found revealing that the recommended method has know-how in determination of AP serum and pharmaceutical samples.

Table 3. Determination of AP in real samples Scan rate 100 mVs^{-1}, *t*(acc): 50 s (at open circuit), AP: 1.0×10^{-3} M, CTAB: 10 µM by using linear sweep voltammetry

Sample	AP added (µM)	Detected[b] (µM)	Recovery (%)	SD±RSD (%)
Serum sample	-	Not detected	-	-
	0.1	0.103	103.0	0.0022±0.0015
	0.3	0.315	105.0	0.0109±0.0077
	0.5	0.493	98.6	0.005±0.0035
	0.7	0.731	104.4	0.0219±0.0156
Tablet (Zyloric)[c]	-	Not detected	-	-
	0.1	0.093	93.0	0.005±0.0035
	0.3	0.301	100.3	0.0007 ±0.0005
	0.5	0.512	102.4	0.0084±0.006
	0.7	0.759	108.42	0.041±0.0292

[b]Average of five determination
[c]Composition: Zyloric: AP, 100 mg; lactose monohydrate, starch, povidone and magnesium stearate

4. CONCLUSION

In the present work, voltammetric oxidation of AP in the presence of cetyltrimethyl ammonium bromide at carbon paste electrode in phosphate buffer solution

(KH_2PO_4/K_2HPO_4) at pH 3.0 has been investigated. The results indicated that, CTAB can adsorb on the surface of CPE via strong hydrophobic interaction and voltammetric responses of AP were facilitated in presence of CTAB than that of bare CPE. AP was irreversibly oxidized at a high potential on CPE in the presence of CTAB and showed an adsorption controlled behavior. The oxidation mechanism involves transfer of two electrons. The peak current was linear to AP concentrations over a certain range, under the selected conditions. The proposed method has distinct advantages over other existing methods regarding sensitivity, accuracy, time saving and minimum detectability. In addition, no sophisticated instrumentation is required. The electrode has been used to determine AP in pharmaceutical samples. In addition, the results obtained in the determination of AP in spiked urine samples demonstrated the applicability of the method for real sample analysis. Furthermore, the present method could possibly be adopted for pharmacokinetic studies as well as clinical and quality control laboratories.

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