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Electro-chemical oxidation and determination of granulocytosis risk inducing drug 4-aminoantipyrine at gold electrode

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A voltammetric technique for electrochemical sensing and quantification of granulocytosis risk inducing metabolite of aminopyrine, 4-Aminoantipyrine (4-AAP) at gold electrode has been developed. The gold electrode shows excellent selectivity and sensitivity towards the oxidation of 4-AAP in phosphate buffer solution (PBS) of pH=3.0. It is observed from the electrochemical studies of scan rate that overall electrode process is diffusion controlled and pH effect suggests involvement of protons in electrochemical oxidation of 4-AAP. A linear current response towards the electrochemical oxidation of 4-AAP in the concentration range of $1.0 \times 10^{-6} – 2.5 \times 10^{-5}$ M is observed with limit of detection of 3.8×10^{-8} M (S/N = 3). The interference studies have shown that the gold electrode exhibited excellent selectivity in presence of large excess of interferents. The method is employed to recovery study of 4-AAP in spiked urine and serum samples.

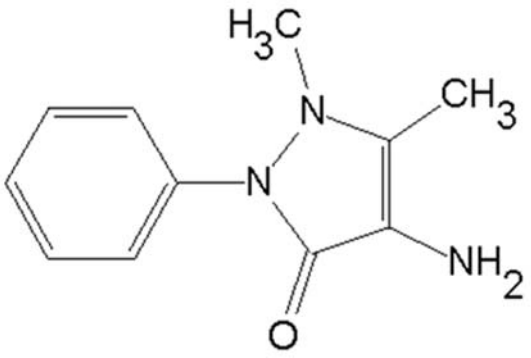
Keywords: 4-Aminoantipyrine, Gold electrode, Electro-oxidation, Diffusion controlled process, Detection limit, Recovery study

The scope of drug analysis includes the analytical investigation of bulk-drug materials, intermediates in their synthesis, products of drug research, drug formulations, impurities and degradation products of drugs, the drugs and their metabolites in the biological samples. The main goal is to obtain data that can contribute to the maximal efficacy and maximal safety of drug therapy and the maximal economy of the production of drugs. Thus, the determination of drugs in pharmaceutical formulations and urine is necessary for quality-control procedures in the industry, in order to ensure the correct dose in patients during treatment and for physiological pharmacokinetics.

4-aminoantipyrine (4-AAP, Ampyrone, chemical structure given in Scheme 1) is an analgesic, anti-inflammatory and antipyretic drug. It shows many of the side effects on human body like the residue of 4-AAP in the environment posses a potential threat to human health. Due to the risk of agranulocytosis, an acute condition involving a severe and dangerous leukopenia, its use as a drug is discouraged. 4-AAP stimulates liver microsomes and is also used to measure extracellular water. In view of health hazards due to the presence of 4-AAP, its determination becomes important.

A review in the literature reveals that several methods have been developed for the determination of 4-AAP: liquid and gas chromatography, spectrophotometric method,[1-3] liquid chromatography/mass spectrometry,[4] capillary electrophoresis,[5] solid phase spectrophotometry,[6] different HPLC methods[7-9] and voltammetric methods.[10,11] However, major drawbacks of some of these methods such as titration, fluorometric methods, and chromatographic methods are time-consuming, some are expensive, some need special training operators, need complicated preconcentration, multisolvent extraction and they suffer from the insufficiency of sensitivity and or selectivity, respectively. Electrochemical methods satisfy many of the requirements for such tasks, particularly owing to their inherent specificity, simplicity, high sensitivity, good stability, low-cost instrumentation, small dimensions, on-site monitoring, and rapid response.[12] Electrochemical methods, especially differential pulse voltammetry (DPV), make it possible to decrease analysis time as compared to the time required by chromatographic methods. The advantages of DPV over other electroanalytical techniques are greater speed of analysis, lower consumption of electro active species, and fewer problems with blocking of the electrode surface.

The aim of this study is to establish the suitable experimental conditions, to investigate the voltammetric behaviour and oxidation mechanism of 4-AAP at gold electrode by cyclic and differential pulse voltammetry



Scheme 1 — Chemical structure of 4-aminoantipyrine

and to develop a method for the direct determination of 4-AAP in real samples. The proposed method has advantages such as no time-consuming sample preparation step prior to drug assay, high sensitivity, rapid response, good reproducibility, and low detection limit.

Materials and Methods

Reagents and chemicals

All of the solutions were freshly prepared using double-distilled water. 4-AAP with analytical grades were used as received. The buffer solutions (pH 3.0 to 10.4) were prepared from orthophosphoric acid and its salts.[13] Other reagents used were of analytical or chemical grade.

Instrumentation

CHI 630D electrochemical analyzer (CH Instruments Inc., USA) was employed for all the voltammetric measurement. A conventional three-electrode system was used, including a Ag/AgCl as reference electrode, a platinum wire as auxiliary electrode and a 2 mm diameter gold electrode as working electrode. All the pH values were measured with a Elico LI120 pH meter (Elico Ltd., India) and experiments were carried out at an ambient temperature of 25 ± 1 °C.

The area of the electrode was obtained by the cyclic voltammetric (CV) 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.[14]

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

where I_p refers to the peak current, n is the number of electrons transferred, A_0 is the surface area of the electrode, υ is the scan rate, D_0 and C_0 are diffusion coefficient and concentration of $K_3Fe(CN)_6$, respectively. For 1.0 mM $K_3Fe(CN)_6$ in 0.1 M KCl electrolyte, T = 298 K, 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_p vs $v^{1/2}$, relation, the electro-active area was calculated. In our experiment the slope was 2.2×10^{-5} μA $(V\ s^{-1})^{1/2}$ and the area of electrode was calculated to be 0.29656 cm^2.

Analytical procedure

The polishing was done on micro cloths (Buehler) glued to flat mirrors. A different micro cloth was used for each size of alumina. The particle sizes used were 0.3, 0.1, and 0.05 μm, and the final particle size was 0.05 μm. After initial cleaning of the electrode, it was necessary to polish only with the 0.05 μm particle size during the experiments. Before transferring the electrode to the solution, it was washed with double-distilled water. The electro-oxidation of 4-AAP was measured at a scan rate of 50 mVs^{-1} by cyclic voltammetry between 0.1 V to 0.8 V and differential pulse voltammetry (DPV) between 0.3 V to 0.8 V with a sensitivity of 1×10^{-6} A/V.

Results and Discussion

Electrochemical behaviour of 4-AAP

The electrochemical behaviour of 4-AAP at gold electrode was investigated using CV technique at pH = 3.0. The cyclic voltammograms obtained for 1.0 mM 4-AAP solution at a scan rate of 50 mV s^{-1} exhibits a well-defined irreversible anodic peak at about 5.62 V at gold electrode. The results are shown in Fig. 1. Usually gold electrode shows an oxidation and a reduction peak at around 1.2 V and 0.7 V, respectively.[15] But we did not observed any oxidation or reduction peaks of gold electrode in our experiment since the potential range selected is between 0.1 to 0.8 V. In this range there is no possibility of gold undergoing oxidation and hence no reduction of gold is expected.

Influence of pH

The effect of pH on peak potential and peak current of 1.0 mM 4-AAP was investigated in phosphate buffer solution over the pH range of 3.0–10.4 using cyclic voltammetry. A well-defined sharp oxidation peak appeared at pH 3.0 (Fig. 2a), and thereafter the intensity of oxidation peak observed was extremely lower. This may be due to deprotonation of 4-AAP. In solution, pK_a of 4-AAP is 4.07, hence deprotonationation is more favourable at acidic pH values ie. for 3.0, and deprotonation capacity of

4-AAP decreases as the pH value goes on increases. With the increase in solution pH, the peak potential linearly shifts to less positive values and the linear relation between E_p and pH (Fig. 2b) can be expressed as:

$$E_p(V) = -0.037\,pH + 0.648; (R^2 = 0.938) \quad \ldots(2)$$

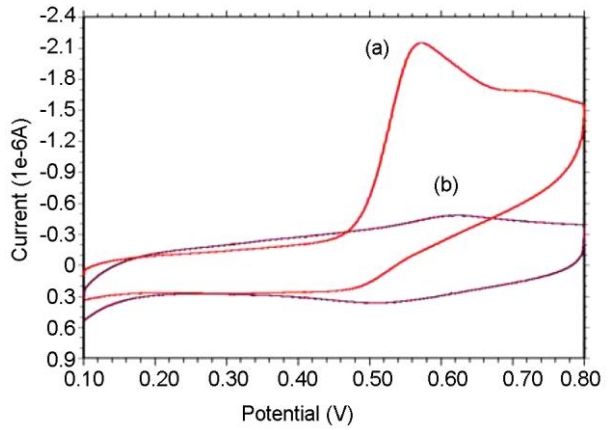


Fig. 1 — Cyclic voltammogram obtained for 1 mM 4-AAP on GE in 0.2 M phosphate buffer (pH = 3.0) : (a) 4-Aminoantipyrine and (b) blank at v = 50 mV s^{-1}

The slope (37.0 mV/pH) is close to the theoretical value of 30 mV/pH that involves two electron and a proton transfer in the rate determining step.[16-18] From the plot of I_p vs. pH (Fig. 2c), it is clear that the intensity increases to a high value at pH = 3.0, then the peak intensity decreases. Because the best result with respect to sensitivity accompanied with sharper response was obtained with pH = 3.0, it was selected for further experiments.

Influence of scan rate

Useful information involving an electrochemical mechanism generally can be acquired from the relationship between peak current and scan rate. Therefore, the voltammetric behaviour of 4-AAP at different scan rates was also studied using cyclic voltammetry (Fig. 3a). Scan rate studies were carried out to assess whether the electrochemical processes on gold electrode were under diffusion or adsorption controlled. The influence of the square root of scan rate on the peak current showed a linear relationship in the range of 25 to 400 mV s^{-1} for CV (Fig. 3b), which is typical of a diffusion-controlled process[19, 20] and the equations can be expressed as:

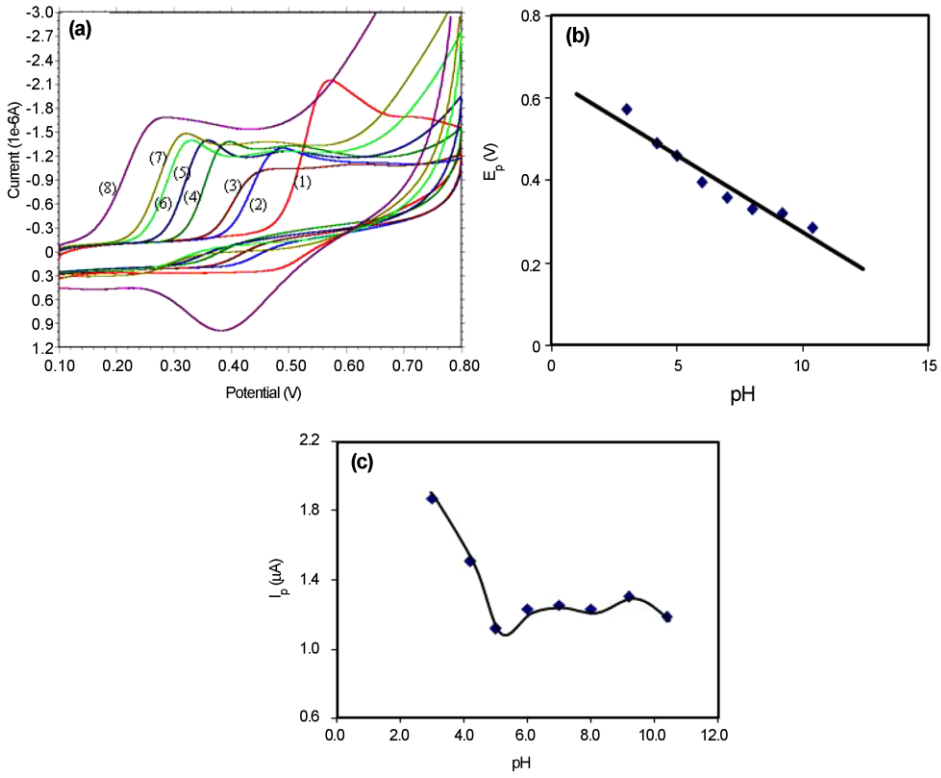


Fig. 2 — (a) Cyclic voltammograms of 1 mM 4-AAP at different pH: (1) 3.0, (2) 4.2, (3) 5.0. (4) 6.0, (5) 7.0, (6) 8.0, (7) 9.2 and (8) 10.4, (b) Influence of pH on the potential of 1 mM 4-AAP on GE at scan rate of 50 mV s^{-1} in phosphate buffer and (c) Variation of current with pH of 1 mM 4-AAP on GE at scan rate of 50 mV s^{-1} in phosphate buffer

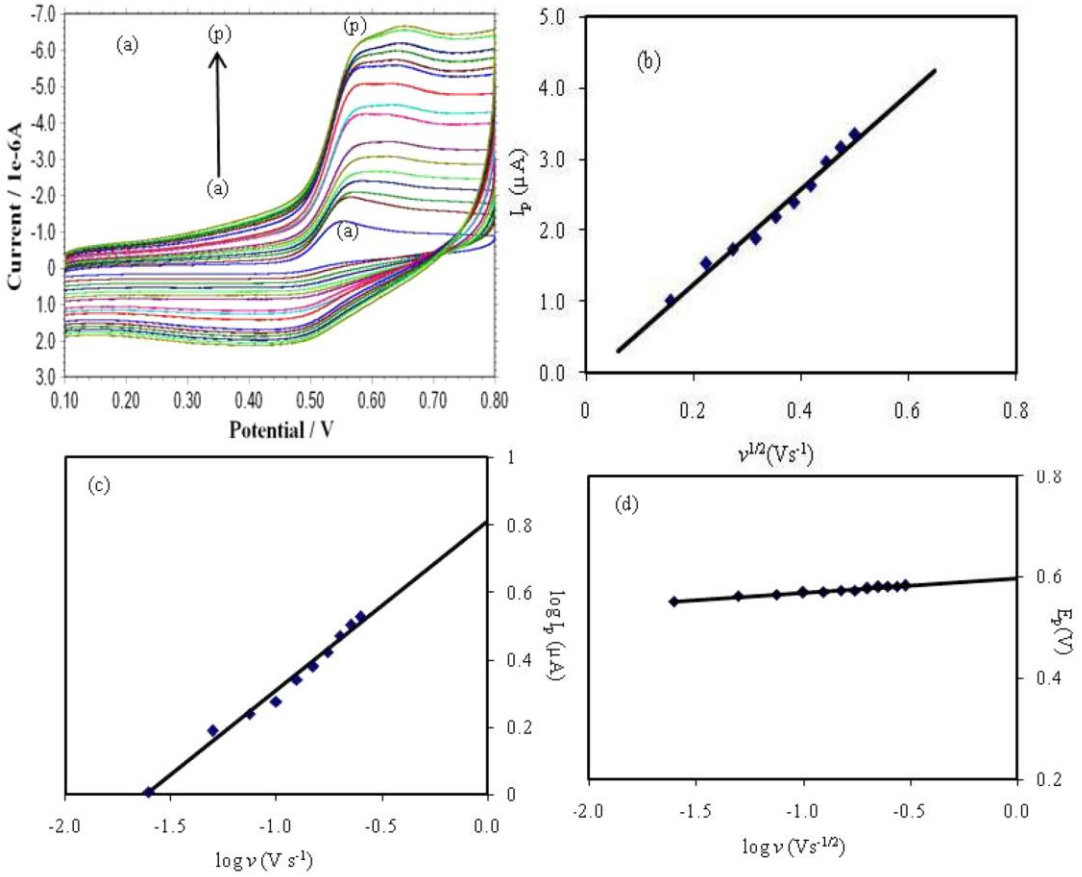


Fig. 3 — (a) Cyclic voltammograms of 1 mM 4-AAP at different scan rate (a–p; 25, 50,75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375 and 400 mV s^{-1}) in 0.2 M phosphate buffer (pH 3.0), (b) Dependence of peak current on the square root of scan rate, (c) Dependence of the logarithm of peak current on logarithm of scan rate and (d) Relationship between peak potential and logarithm of scan rate

$$I_p = 6.807\upsilon^{1/2}(V^{1/2}s^{-1/2}) + 0.121; (R^2 = 0.988) \quad \ldots(3)$$

A plot of logarithm of anodic peak current versus logarithm of scan rate produced a straight line with a slope of 0.51 (Fig. 3c), which is close to the theoretical value of 0.5 for a purely diffusion-controlled process,[19] which in turn further confirms that the process is diffusion controlled where the electroactive species 4-AAP diffuses from the bulk solution to a planar electrode surface; the equations can be expressed as:

$$\log I_p(\mu A) = 0.510 \log \upsilon(Vs^{-1/2}) + 0.82; (R^2 = 0.988) \quad \ldots(4)$$

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 the scan rate (Fig. 3d), which can be expressed by the following equations:

$$E_p(V) = 0.028 \log \upsilon(Vs^{-1/2}) + 0.597; (R^2 = 0.987) \quad \ldots(5)$$

For an irreversible electrode process, according to Laviron, E_p is defined by the following equation.[21]

$$E_p = E^0 + \left(\frac{2.303RT}{\alpha nF}\right)\log\left(\frac{RTk^0}{\alpha nF}\right) + \left(\frac{2.303RT}{\alpha nF}\right)\log\upsilon \quad \ldots(6)$$

where α is the transfer coefficient, k^0 the standard heterogeneous rate constant of the reaction, n the number of electron transferred, *v* the scan rate and $E^{0'}$ is the formal redox potential. Other symbols have their usual meanings. This relationship allows αn to be readily calculated from the slope of the E_p vs. log *v*. In this system, the slope is 0.028, taking T = 298 K, R = 8.314 J K^{-1} mol^{-1}, and F = 96480 C mol^{-1}, the αn

was calculated to be 2.11. According to Bard and Faulkner,[22] α can be given as

$$\alpha = \frac{47.7}{E_p - E_{1/2}} mV \quad \ldots(7)$$

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 to be 0.954. Further, the number of electrons (n) transferred in the electro-oxidation of 4-AAP was calculated to be 2.21 ~ 2. The value of k^0 can be determined from the intercept of the previous plot if the value of $E^{0'}$ is known. The value of $E^{0'}$ in Eqn. (6) can be obtained from the intercept of E_p vs. *v* curve by extrapolating to the vertical axis at $v = 0$.[23] In our system the intercept for E_p vs. log *v* plot was 0.597 and $E^{0'}$ was obtained to be 0.554 and the k^0 was calculated to be 2.8×10^3 s^{-1}.

Calibration curve and detection limit

Differential pulse voltammetric (DPV) method was used for quantification of 4-AAP since DPV has a much higher current sensitivity and better resolution than cyclic voltammetry. It was used to estimate the lower detection limit from the voltammograms in which the peaks are sharper and better-defined than those obtained by cyclic voltammetry. According to the obtained results, it was possible to apply this technique to the quantitative determination of 4-AAP. The influence of the differential pulse parameters such as amplitude and frequency on the peak current was investigated. The peak current (I_p) increased with the increase in differential pulse amplitude from 5 to 75 mV or differential pulse frequency in the range of 5 to 55 Hz, but the peak potential shifted to less negative values and the peak changed unshapely. So, 50 mV was chosen as the optimum amplitude and 15 Hz was chosen as the optimum frequency. Fig. 4a represents the DPV voltammograms of 4-AAP at the gold electrode. The electrode showed linear behaviour in the concentration range of 1.0×10^{-6} to 2.5×10^{-5} M as represented in Fig. 4b. For higher concentrations deviation from linearity was observed. This may be due to the adsorption 4-AAP or its oxidation product on the electrode surface. The calibration plot was fitted with the following equation

$$I_p(\mu A) = 0.079[4-AAP](\mu M) + 1.043; R^2 = 0.959) \quad \ldots(8)$$

Related statistical data of the calibration curves were obtained from the five different determinations. The limit of detection (LOD) and quantification (LOQ) were 3.8×10^{-8} M and 1.61×10^{-7} M, respectively (Table 1). The LOD and LOQ were calculated using the following equations

Table 1 — Characteristics of 4- AAP calibration plot using differential pulse voltammetry at gold electrode

Linearity range(M)	1.0×10^{-6} - 2.5×10^{-5}
Slope of the calibration plot	0.079×10^{-6}
Intercept	1.043
Correlation coefficient (r)	0.959
RSD of slope (%)	1.282
RSD of intercept (%)	5.161
Number of data points	13
LOD (M)	3.8×10^{-8}
LOQ (M)	1.61×10^{-7}
Repeatability of peak potential (RSD %)	0.1879
Repeatability of peak current (RSD %)	0.9364
Reproducibility of peak potential (RSD %)	0.6551
Reproducibility of peak current (RSD %)	1.214

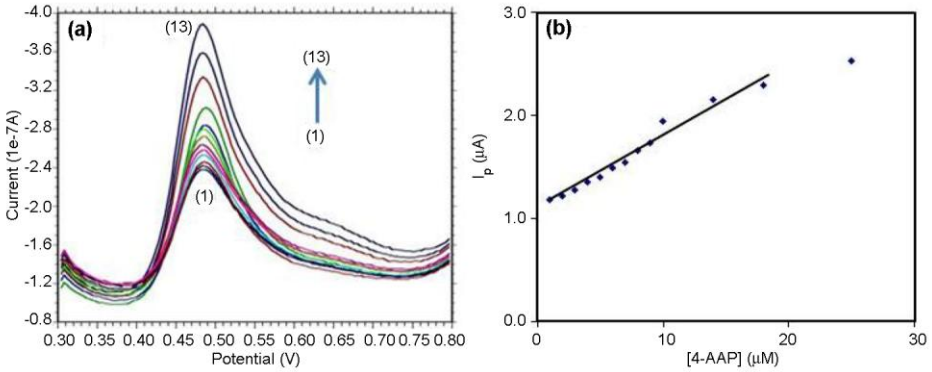


Fig. 4 — (a) Differential pulse voltammograms with increasing concentration of 4-AAP in pH 3.0 PBS on graphite pencil electrode with 4-AAP concentrations: (1) 1.0, (2) 2.0, (3) 3.0, (4) 4.0, (5) 5.0, (6) 6.0, (7) 7.0, (8) 8.0, (9) 9.0, (10) 10.0, (11) 14.0, (12) 18.0 and (13) 25.0 μM and (b) Plot of peak current against the concentration of 4-AAP

Table 2 — Comparison of detection limits for 4- AAP related dipyrone derivative drugs at different methods

Dipyrones	Methods	LOD	Ref.
1) Dipyrone(DP)(1-Phenyl-2,3-Dimethyl-5-Pyrazolone-4-Methylaminomethanesulfonate Sodium)	1. Flow injection amperometric determination	2.78×10^{-4} M	3
	2. Diffusion layer titration at dual-band electrochemical cell	3.6 μM	25
	3. Nano-Riboflavin-Modified Glassy Carbon Electrode(voltammetry)	0 .0502 μM	26
	4.Titanium Phosphate/Nickel Hexacyanoferrate mod graphite electrode(voltammetry)	3.75×10^{-4} M	27
2) 4-aminophenazone (4-Aminoantipyrine)	Graphite pencil electrode(voltammetry)	0.45×10^{-7} M	10
3) 4-Aminoantipyrine	Gold electrode	3.8×10^{-8} M	Present work

$$LOD = \frac{3s}{m} \quad ...(9)$$

$$LOQ = \frac{10s}{m} \quad ...(10)$$

where, s is the standard deviation and m is the slope (sensitivity) obtained from the calibration curves.[24] The detection limits reported at different analytical methods for 4-AAP related dipyrone derivative drugs[25-27] are tabulated in Table 2. The proposed method was better as compared to other reported electrochemical methods.

For validation of the proposed method, various parameters, such as repeatability, reproducibility, precision and accuracy of analysis, were obtained by performing five replicate measurements for 0.5×10^{-6} M standard 4-AAP over a single day (intraday assay, n=5) and for 3 days over a period of 1 week (interday assay). Satisfactory mean percentage relative standard deviations (% RSD) were obtained and are reported in Table 1.

Effect of excipients

The influences of various foreign species on the determination of 4-AAP was investigated. The tolerance limit was taken as the maximum concentration of the foreign substances which caused an approximately ±5% relative error in the determination. The effects of these excipients on the voltammetric response was determined by analyzing sample solutions containing a fixed amount of 4-AAP (1.0×10^{-5} M) spiked with various excess amount of each excipient under the same experimental conditions. The experimental results (Table 3) showed that 100-fold excess of citric acid, dextrose, glucose, gum acacia, lactose, starch, and sucrose did not interfere with the voltammetric signal of 4-AAP. Thus, the procedures were able to assay 4-AAP in the presence of excipients, and hence they can be considered specific.

Table 3 — Influence of potential excipients on the voltammetric response of 1.0×10^{-5} M 4-AAP

Excipients(1.0 mM) + Drug (1.0×10^{-5} M)	Potential observed (V)	Signal change (%)
Only 4-Aminoantipyrine	0.465	0
Tartaric acid + 4-AAP	0.472	+1.5
Citric acid + 4-AAP	0.442	-4.94
Glucose + 4-AAP	0.445	-4.3
Gum acacia + 4-AAP	0.434	-6.66
Lactose + 4-AAP	0.449	-3.44
Dextrose + 4-AAP	0.466	+0.215
Sucrose + 4-AAP	0.472	+1.5
Starch + 4-AAP	0.482	+3.65

Detection of 4-AAP in urine and human serum samples

For further evaluation of the validity of the proposed method, recovery tests for 4-AAP in urine and human serum samples were carried out. A quantitative analysis can be carried out by adding the standard solution of 4-AAP into the detect system of urine samples. The calibration graph was used for the determination of spiked 4- AAP in urine samples. The detection results of four urine samples obtained with recovery percentage and RSD are listed in Table 4a.

The applicability of the DPV to the determination of 4-AAP in spiked human plasma sample was also investigated. The recoveries from human plasma were measured by spiking drug free plasma with known amounts of 4-AAP. A quantitative analysis can be carried out by adding the standard solution of 4-AAP in the detect system of plasma sample. The calibration graph was used for the determination of spiked 4-AAP in plasma samples. The detection results obtained for four plasma samples with recovery percentage and RSD are listed in Table 4b.

Pharmacokinetics study

Pharmacokinetics is the study of the time line of drug absorption, distribution, metabolism, and excretion. Clinical pharmacokinetics is the application

Table 4 — Application of DPV to the determination of 4-AAP in spiked human urine and blood plasma sample

Sample	Added ($\times 10^5$ M)	Found[a] ($\times 10^5$ M)	Recovery (%)	R.S.D (%)	Bias (%)
		(a) Human Urine			
1	1.0	0.985	98.54	0.0079	-1.46
2	3.0	3.156	105.12	0.0108	5.12
3	5.0	4.866	97.25	0.0036	-2.74
4	8.0	7.96	99.54	0.0035	-0.44
		(b) Blood plasma			
1	1.5	1.502	100.16	0.217	0.25
2	3.0	2.985	99.52	1.47	-0.90
3	4.5	4.671	103.80	5.068	3.80
4	6.0	6.069	101.15	0.841	1.15

[a]Average of five determinations

Table 5 — Response of peak current of 8×10^{-5} M 4-AAP in urine sample at different time interval

Time (min)	Peak current (μA)	Concentration ($\times 10^{-5}$M)
0	1.658	8.0
10	1.544	7.44
20	1.491	7.19
30	1.442	6.95
40	1.387	6.69
50	1.3142	6.34
60	1.2631	6.09
70	1.2012	5.79
80	1.110	5.35
Elimination rate constant	0.3224 h^{-1}	
Half life of drug	2.14 h	

of pharmacokinetic principles to the safe and effective therapeutic management of drugs in an individual patient. Primary goals of clinical pharmacokinetics include enhancing efficacy and decreasing toxicity of a patient's drug therapy. The development of strong correlations between drug concentrations and their pharmacologic responses has enabled clinicians to apply pharmacokinetic principles to actual patient situations. A drug's effect is often related to its concentration at the site of action, so it would be useful to monitor this concentration. Receptor sites of drugs are generally inaccessible to our observations or are widely distributed in the body, and therefore direct measurement of drug concentrations at these sites is not practicable. We cannot directly sample drug concentration in this tissue. However, drug concentration in the blood or plasma, urine, saliva, and other easily sampled fluids can be measured.

Response of peak current at different time interval for 8×10^{-5} M concentration of 4-AAP in urine sample is as shown in Table 5. From the plot of urine drug concentration versus time (Supplementary Data, Fig. S1), the pharmacokinetics data can be calculated. Some of the pharmacokinetics data calculated is listed in Table 5.

Conclusions

The voltammetric oxidation of 4-AAP at gold electrode in phosphate buffer solution (pH = 3.0) has been investigated. 4-AAP undergoes two electron-one proton changes and is a diffusion controlled process. The peak current was linear to 4-AAP concentrations over a certain range, under the selected conditions. This helps in voltammetric determination of the selected analyte in amounts as low as 3.8×10^{-8} M and can be used successfully to assay the drug in spiked urine and plasma samples. High percentage recovery and study of excipients showed that the method is free from the interference of commonly used excipients and additives in the formulations of drug. In addition, the results obtained in the analysis of 4-AAP in spiked real samples demonstrated the applicability of the method in real sample clinical analysis. Some of the pharmaco-kinetics data were calculated. The proposed methods are suitable for quality control laboratories where economy and time are essential.

Supplementary Data

Supplementary Data associated with this article are available in the electronic form at http://nopr.niscair.res.in/jinfo/ijca/IJCA_60A(02)228-235_SupplData.pdf.

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