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SIMULTANEOUS VOLTAMMETRIC DETERMINATION OF *p*-AMINOPHENOL AND PARACETAMOL USING ACTIVATED GLASSY CARBON ELECTRODE: CYCLIC VOLTAMMETRY AND DIFFERENTIAL PULSE VOLTAMMETRY STUDY

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Abstract: In this study, we demonstrated simultaneous voltammetric determination of p-aminophenol (PAP) and paracetamol (PAR) at activated glassy carbon electrode (aGCE) using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The electrochemical responses of PAP and PAR were compared at bare GCE (bGCE) and aGCE. The aGCE showed an excellent electrocatalytic effect and decreased over-potential for simultaneous determination of PAP and PAR. It was found that the redox process at aGCE for both PAP and PAR is adsorption-controlled process. Electrochemical reaction of

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PAP and PAR at aGCE was found to be reversible involving the transfer of two electron and two proton processes. The oxidation peak current is linear in the concentration range of PAP from 10 μM to 240 μM at the presence of 80 μM PAR and the oxidation peak current of PAR is directly related to its concentration from 4 μM to 60 μM at constant concentration of PAP (80 μM) at aGCE. The limit of detection (LOD) of PAP and PAR were found to be 0.324 μM and 0.146 μM, respectively and limit of quantification (LOQ) of PAP and PAR were 1.08 μM and 0.487 μM, respectively. The validity of the demonstrated method was checked by using commercial tablet which contain different amount of PAP and PAR and satisfactory percent recoveries were obtained.

***Keywords*:** p-aminophenol, Paracetamol, Activated glassy carbon electrode, Simultaneous electrochemical determination, Differential pulse voltammetry

Introduction

N-acetyl-*p*-aminophenol, (PAR) also commonly called acetaminophen or paracetamol is a prominent pain relieving and antipyretic medicine that is promptly taken after administration. It has few side effect and little poisonous quality when used as a part of prescribed measurement.[1-2] PAR is a synthetic derivative of *p*-aminophenol (PAP) and it undergoes hydrolysis in a conditions, like, elevated temperature and acidic or basic media to PAP.[3-5] PAP and PAR, the hydrolytic degradation product, is the most well-known toxin, highly harmful and mutagenic impact.[6] PAP has two oxidizable gatherings OH group and NH_2 group. These gatherings give more responsive or reactive sites; in this manner aminophenols are fascinating in electrochemical materials.[7]

Several analytical techniques, for example, spectrophotometry,[8-9] spectrofluorometry,[10] voltammetric,[11-13] chromatographic techniques[14-15] are utilized for the determination of PAP and PAR. From the techniques said above, voltammetric techniques are usually used for the detection of PAP

and PAR in pharmaceutical arrangements due to their more specific, less expensive and less time-consuming in comparison to others.[16-17]

The transfer of electrons at electrode-electrolyte interface the important phenomenon in electrochemical reactions. Due to the physico-chemical properties, glassy carbon electrode (GCE) is one of widely used electrode material in electroanalysis. It has some interesting features which makes it suitable electrode such as, higher inertness, very small pore sizes, a small permeability to gas and liquid, excellent biocompatibility and extremely low coefficient of thermal expansion.[18-20] Electrode pretreatment in electrochemical analysis is getting much attention because of its simplicity, sensitivity, efficiency and low cost. Because of the absence of harmful materials and some other tiresome and complex modification steps, pretreatment of GCE is considered as simple and environment-friendly.[21] Surface pretreatment is a method used to improve the electrochemical nature of GCE surface. The electrochemical pretreatment process helps to obtain accurate, definite and reproducible electrochemical signals. The activation of the GCE significantly enhanced the selectivity and the sensitivity of the electrochemical sensors to the analytes of interest by introducing different functional groups on the GCE surface.[22-24] During surface activation of GCE, the electrode surface can be oxidized, thus, more functional groups can be introduced at the surface of electrode that facilitate the redox reaction. So electrochemically activated GCEs are demonstrating fast electron transfer rate and low background current, better sensitivity, reversibility, reproducibility, stability and reduced over potential compared with their precursors.[25-28] Since, PAP is the degradation product of PAR, it is found as an intermediate product in pharmaceutical synthesis of PAR,

which brings teratogenic effect and nephrotoxicity,[29] it is necessary to have an efficient method for their determination.

Therefore, in this study the aGCE was used for simultaneous electrochemical determination of PAP and PAR. The cyclic voltammetric response of the PAP and PAR was studied before and after the activation of GCE. The aGCE exhibits better electrochemical performance than the traditional electrodes. Therefore, the aGCE has been used for the electrochemical investigations of PAP and PAR.

Results and Discussion

CV of PAP and PAR at bGCE and aGCE

The redox properties of PAR and PAP were studied at bGCE using CV and the result was shown on Figure 1A. The CV of PAP shows reversible redox process at bGCE with oxidation and reduction potentials of about 0.155 V and 0.28 V, respectively at bGCE (Figure 1A, curve *i*). Whereas, PAR shows irreversible redox peak at bGCE and the oxidation potential was observed at about 0.502 V (Figure 1A, curve *ii*). This shows that slow electron transfer behavior of bGCE mainly for elctrocatalysis of PAR.

As shown in Figure 1B, the redox properties of PAP and PAR were also observed at aGCE. In this case the redox properties of both PAP and PAR shows reversible and fast electron transfer rate at aGCE. The oxidation potential and reduction potential of 0.1 mM PAP was observed at 0.083 V and 0.062 V, respectively (Figure 1B, curve *i*). Also, the anodic and cathodic potential of 0.1 mM PAR at aGCE was about 0.364 V and 0.336 V, respectively (Figure 1B, curve *ii*). Moreover, the anodic potential was

shifted to more negative and peak to peak separation was decreased for both PAP and PAR on aGCE as compared to bGCE.

The peak to peak separation of 0.271 V was sufficient for simultaneous electrochemical detection of PAP and PAR at aGCE (Figure 2). Moreover, the redox peak currents were enhanced on aGCE than bGCE. This also shows the good electrocatlytic ability of aGCE toward determination of PAP and PAR.

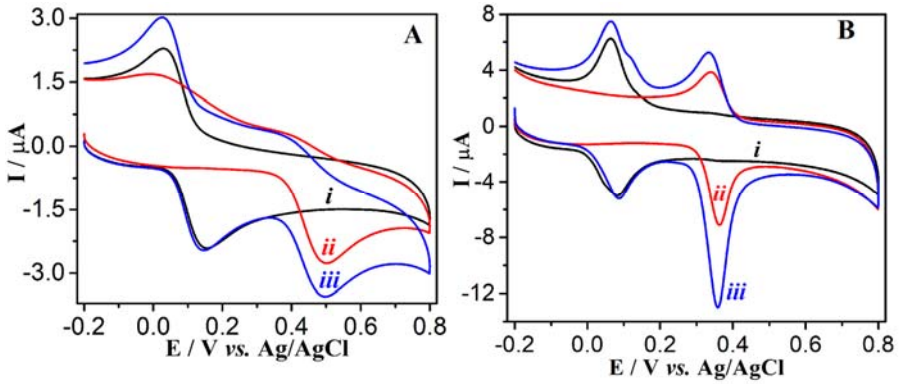

Figure 1. CVs of 0.1 mM of PAP (i) and PAR (ii), and equimolar mixture (iii) of the two at bGCE (A) and aGCE (B).

Effect of pH on electrochemical behavior of PAP and PAR

The effect of solution pH on redox behavior of PAP and PAR was carefully investigated on aGCE using CV and the results were shown in Figure 3A. The pH value of the buffer has a substantial effect on both peak current and peak potential of oxidation and reduction of the PAP and PAR. The studies were carried out in the pH region from 2 to 8. As shown in Figure 3B and C, pH increases with peak current of redox reaction up to pH 6 and then start decreasing for pH greater than 6. Therefore, pH 6 was chosen as the optimum pH for the rest study. Moreover, both the oxidation peak potential (E_{pa}) and reduction peak potential (E_{pc}) of PAP and PAR shifted towards negative potential with the pH increasing from 2 to 8,

demonstrating that protons are involved in the electrode reaction. The relationships between the peak potentials and pH could be expressed by the equations: E_{pa} = 0.669 - 0.052pH (R^2 = 0.9934) for PAP (Figure 3B) and E_{pa} = 0.40 – 0.050pH (R^2 = 0.994) for PAR (Figure 3C), respectively. The values of the slopes were close to the theoretical value of 0.059 V/pH, suggesting equal numbers of proton and electron are involved in the redox reaction.[30]

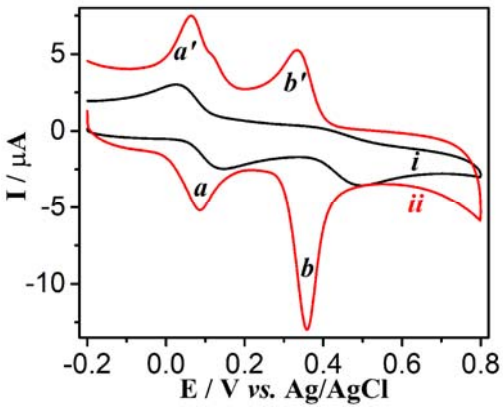

Figure 2. Comparison of the CVs of PAP (a and a') and PAR (b and b') at bGCE (i) and aGCE (ii).

Effect of varying scan rate on electrochemical behavior of PAR and PAP

The effect of scan rate (ν) on the redox responses of 0.1 mM PAP and PAR at aGCE was studied using CV in 0.1 M PBS over 20 to 300 mV/s and the results were shown in Figure 4.

Both the oxidative and reductive peak currents of PAP and PAR increased linearly with scan rate of 20 - 300 mV/s (Figure 4 Inset (A) PAP and (B) PAR). This indicated that the redox reaction of PAP and PAR at aGCE was adsorption-controlled process.

Differential pulse voltammetric investigation of PAP and PAR

DPV was selected to evaluate the analytical performance of aGCE for simultaneous determination of PAP and PAR. As seen in Figure 5A and B, two distinct peaks were observed at 0.12 V for PAP and 0.38 V for PAR with sufficient peak to peak potential separation, about 0.26 V, which is enough for simultaneous detection of PAP and PAR.

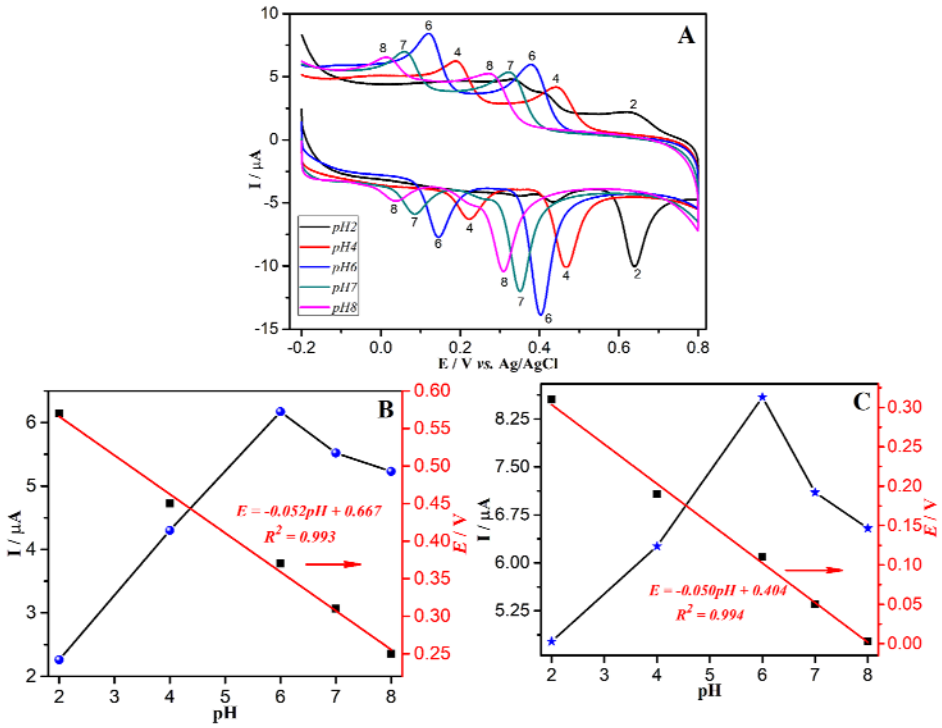

Figure 3. A) CVs at the aGCE for PAP (0.1 mM) and PAR (0.1 mM) in pHs of 2, 4, 6, 7 and 8; Oxidation peak current vs. pH and E vs. pH of 0.1 mM of B) PAP and C) PAR.

Under optimal experimental conditions, aGCE was used to detect PAP and PAR in the mixed solution. Figure 5A and B shows, the DPV responses for the PAP and PAR mixture prepared by changing the concentration of one of the analytes while the other kept constant. The peak current value of PAP is directly proportional to its concentration from 10 µM to 0.24 mM in the presence of 80 µM PAR (Figure 5A), and there is no

substantial variation in the peak current of PAR. Likewise, the peak current value of PAR is also linearly related to its concentration from 4 μM to 0.6 mM at constant concentration of PAP 80 μM (Figure 5B), and the presence of 80 μM PAP does not affect its determination. The linear regression equations of PAP and PAR are I (μA) = -0.268C_{PAP} (μM) - 3.37 (R^2 = 0.994) and I (μA) = -0.487C_{PAR} (μM) - 2.30 (R^2 = 0.998), respectively. The corresponding LOD for PAP is 0.043 μM and for PAR is 0.039 μM (3σ/*m*). The calculated LOD for PAP is 0.14 μM and PAR is 0.32 μM. Limit of quantification of the PAP and PAR and PAP were 1.08 μM and 0.487 μM, respectively.

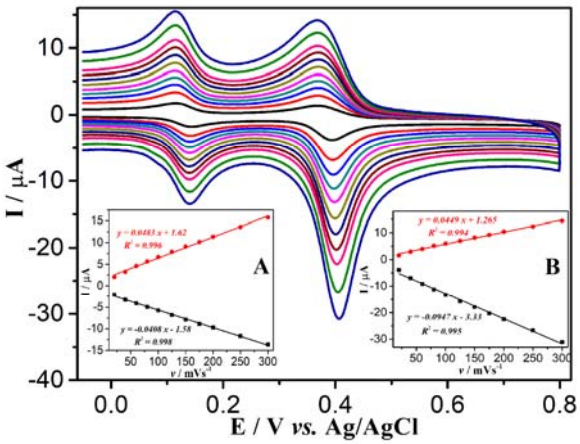

Figure 4. CVs of the aGCE for PAP (0.1 mM) and PAR (0.1 mM) in PBS (0.1 M pH 6.0) at scan rates from 20 to 300 mV/s. Insets: The plots of anodic and cathodic peak currents of (A) PAP and (B) PAR *vs.* scan rates.

Real sample analysis

The aGCE was applied to detect PAP and PAR in paracetamol tablet products. The standard addition method was used to evaluate the percent recoveries and the results were shown in Table 1. The percentage recoveries were calculated to be 98.08% and 107.5% for PAP and PAR, respectively. The results revealed the possibility of the proposed methods for the quantitative detection of PAP and PAR in tablet.

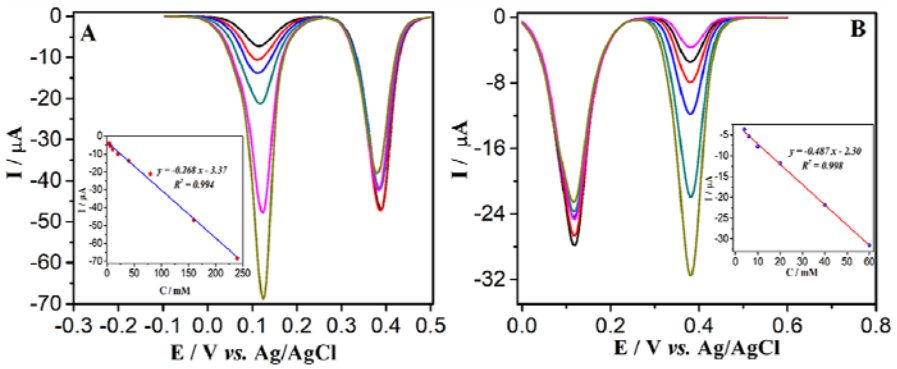

Figure 5. DPV at aGCE for different concentrations of PAP (from 10 µM to 0.24 mM) in the presence of 80 µM PAR (B) PAR (from 4 µM to 0.6 mM) in the presence of 80 µM PAP, (Inset: the corresponding calibration curves).

Table 1. Determination and recovery of PAR and PAP in commercial tablets using aGCE.

Sample	Analyte	Detected (µM)	Added (µM)	Found (µM)	Recovery (%)
Tablet	PAR	40	15	53.5	98.1
			20	57.4	95.6
			25	66.3	102
	PAP	-	15	14.6	97.3
			20	19.4	97
			25	26.9	107.5

Comparison with results of other studies

Comparison was made between the linear range and the LOD of PAP and PAR at aGCE and other electrochemical analysis methods reported for the simultaneous electrochemical detection of PAP and PAR and shown in Table 2. The experimental results show that aGCE has comparable linear range and LOD as compared with other electrochemical analysis methods for detecting PAP and PAR.

Interference study

There are several possible interferents which affect the selectivity of aGCE for the simultaneous electrochemical detection of PAP and PAR. Different interfering substances were added as an interferent into a solution containing PAR and PAP. Equimolar amounts of ascorbic acid (AA), glucose (GL), K^+, Na^+, Cl^-, and NO_3^- have no interfering effect with the detection of PAP and PAR.

But equimolar uric acid (UA) was moderately affect the oxidation peak current of both PAR and PAP, and with slight shift of peak potential of PAR toward negative side (Figure 6). The signal change caused by addition of UA was greater than 5% which indicate the positive interference effect.

Table 2. Comparison of linear range and LOD for PAP and PAR with other reported electrochemical methods.

Method	Method	Linear range (μM)		LOD (μM)		Ref.
		PAP	PAR	PAP	PAR	
MoS_2@N-dopped hallow carbon nanospheres/GCE	DPV	0.5-20	0.5-20	0.011	0.015	[4]
Poly(2,2'-(1,4-phenylenedivinylene) bis-8-hydroxyquinaldine) /GCE	SWV	3-150	0.5-200	0.45	0.075	[11]
Poly(3,4-ethylenedioxythiophene) /GCE	DPV	4-320	1-100	1.2	0.40	[12]
Au NPs/Pd/reduced graphene oxide/GCE	DPV	1-300	1-250	0.12	0.30	[31]
Au NPs/tetraaminophenyl porphyrin/MWCNT/GCE	DPV	0.08-60	4.5-500	0.025	0.44	[32]
Bis-Schiff base cobalt complexes/GCE	DPV	5-30	5-30	2.08	1.86	[33]
aGCE	DPV	4-60	10-240	0.14	0.32	This work

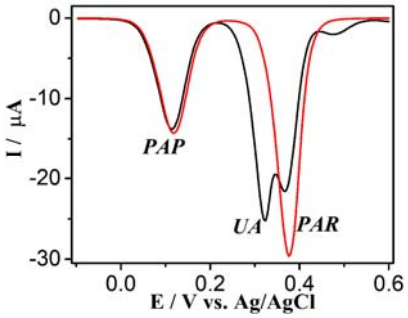

Figure 6. DPVs of 0.1 mM PAP, PAR in the presence of equimolar UA.

Experimental

Chemical and reagents

PAR (APF, Ethiopia), PAP (Godrej industries, India), anhydrous dipotassium hydrogen orthophosphate (BDH, England), potassium dihydrogen phosphate (Sigma-Aldrich Switzerland), phosphoric acid (Riedel-deHaen, Germany), uric acid (Godrej industries, India) were used as received. The stock solution of PAR and PAP were prepared by dissolving an accurate mass of the drugs in appropriate volume of the solvent and stored in a refrigerator until used. 0.1 M phosphate buffer solutions (PBS) (pH 7.0) were prepared by mixing appropriate amount of 0.2 M KH_2PO_4 and K_2HPO_4 each and were used as supporting electrolyte throughout the experiments.

Instrumentations

CV-CHI760E electrochemical workstation [Bioanalytical Systems (BAS), USA] connected to a desktop computer was used for the CV and DPV experiments with three electrode configurations. Besides using aGCE (3.0 mm in diameter) as the working electrode and Ag/AgCl (KCl (sat.)) electrode and platinum wire were used as reference and auxiliary electrode, respectively.

Preparation of the aGCE

Before activation, the surface of GCE (3.0 mm diameter) was polished to a mirror with 0.5 μm alumina slurry with in a polishing cloth and then thoroughly rinsed with distilled water. The GCE was activated by applying a potential of 1750 mV for 200 s in time-based technique in PBS. CV of the activated electrode was run between -200 to 800 mVs until a stable votammogram was obtained.[34]

Preparation of stock and standard solutions

For analysis of standard PAR and PAP, 0.1 M PBS of $K_2HPO_4.2H_2O$ was prepared and pH was adjusted using 0.1M HCl or NaOH. Different concentration of PAR at constant concentration of PAP and different PAP concentration at constant concentration of PAR were prepared using PBS.

Preparations of calibration curve

0.1 M PBS pH 6.0 was used to prepare the stock and working solutions of PAP and PAR. Keeping the optimum experimental parameters constant, a calibration curve using DPV was obtained for PAP in the concentration range from 10 μM to 0.24 mM at constant concentration of PAP and for PAR in the concentration range from 4 μM to 0.1 mM at constant PAP concentration.

Sample preparation

Five tablets of the sample were accurately weighed and powdered finely using a mortar and pestle. The average weight of each tablets powder corresponding to the stock solution concentration 1 mM was added to phosphate buffer solution in 1000 mL volumetric flask and was shaked till it was dissolved. The solution was centrifuged to ensure complete dissolution then filtered by Whatman filter paper. The prepared solution was filled with

PBS (pH 7.0) and further diluted to volume with the same solvent. The diluted solution was containing specified amount of PAR and PAP. From calibration curve concentrations of PAR and PAP were extrapolated and recovery was calculated by dividing the obtained concentration to the spiked.

Conclusions

As reported in this study, aGCE was used as an electrode material for simultaneous determination of PAP and PAR. aGCE electrode gave good electrocatlytic effect toward both analyte than bGCE. The notable peak separation of PAP and PAR allows for simultaneous determination of both analytes. Moreover, the method shows comparable analytical performance, and better selectivity for the detection of PAP and PAR. The practical applicability was demonstrated by detecting PAP and PAR in tablets accurately with satisfactory recovery.

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