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Simultaneous determination of *N*-acetyl-*p*-aminophenol and *p*-aminophenol with poly(3,4-ethylenedioxythiophene) modified glassy carbon electrode

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ABSTRACT

A sensitive and selective method was developed for the determination of *N*-acetyl-*p*-aminophenol (APAP) and *p*-aminophenol (PAP) using poly(3,4-ethylenedioxythiophene) (PEDOT)-modified glassy carbon electrode (GCE). Cyclic voltammetry and differential pulse voltammetry were used to investigate the electrochemical reaction of APAP and PAP at the modified electrode. Both APAP and PAP showed quasireversible redox reactions with formal potentials of 367 mV and 101 mV (vs. Ag/AgCl), respectively, in phosphate buffer solution of pH 7.0. The significant peak potential difference (266 mV) between APAP and PAP enabled the simultaneous determination both species based on differential pulse voltammetry. The voltammetric responses gave linear ranges of 1.0×10^{-6}–1.0×10^{-4} mol L^{-1} and 4.0×10^{-6}–3.2×10^{-4} mol L^{-1}, with detection limits of 4.0×10^{-7} mol L^{-1} and 1.2×10^{-6} mol L^{-1} for APAP and PAP, respectively. The method was successfully applied for the determination of APAP and PAP in pharmaceutical formulations and biological samples.

1. Introduction

N-acetyl-*p*-aminophenol (APAP), also known as paracetamol or acetaminophen, is one of the most commonly used drugs in the world. It is the preferred alternative to aspirin, to patients who cannot tolerate aspirin [1]. APAP is an acylated aromatic amide that was first introduced in medicine by Von Mering in 1893 and had been in use as an analgesic for home medication for over 50 years [2]. It has been accepted as an effective drug for the relief of pain and fever in adults and children [3]. *p*-Aminophenol (PAP), the primary hydrolytic degradation product of APAP, can exist as a synthetic intermediate in pharmaceutical preparations or as a degradation product of APAP. PAP is considered as an impurity for APAP by European Pharmacopoeia (Ph. Eur. 2000) and numerous specifications of manufacturers [4].

Standard usage of APAP has no detrimental effect on the human body but overusage of the drug could lead to some serious side effects such as kidney damage [5] and liver failure [6], while PAP is a substance of modest toxicity and can cause nephrotoxocity and tetragenic effects [7,8]. Availability of APAP without prescription has increased the use of the compound for self-poisoning [9]. Consequently it is vital to develop a simple, selective and reliable technique for the determination of APAP and its impurities.

The various methods reported for the determination of APAP and PAP in body fluids and pharmaceutical formulations include spectrophotometry [10,11], liquid chromatography [12,13], capillary electrophoresis [14–16], and chemiluminescence [17]. Spectrophotometric and chemiluminescence methods use extraction of the analytes prior to detection, while liquid chromatography and capillary electrophoresis take longer time that hampers the suitability of the methods for routine analysis.

Electrochemical techniques based on chemically modified electrodes have attracted much attention because of their fast response, high sensitivity and selectivity in the determination of trace level of analytes [18]. The use of bare electrode, such as glassy carbon electrode (GCE) for the electrochemical determination of APAP and its impurities is limited because of the sluggish electron transfer and fouling which result in poor sensitivity, selectivity and reproducibility [19]. Owing to these limitations, various types of chemically modified electrodes have been used for the electrochemical studies of APAP, for example, graphene-modified GCE [20], carbon film resistor electrode [21], carbon-coated nickel magnetic nanoparticles modified electrodes [22], C_{60}-modified GCE [23], multi-walled carbon nanotube (MWCNT) modified basal plane pyrolytic graphite electrode [24], boron-doped diamond electrode [25], carbon ionic liquid electrode [26], carbon–ceramic modified electrodes [27], and polyaniline–MWCNT composite modified electrode [28]. Although these modified electrodes demonstrated good sensitivity, selectivity and low detection limit, none of them has been applied for the simultaneous determination of APAP and its impurities. To our knowledge, no work has so far been reported on

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the simultaneous determination of APAP and its main impurity PAP based on conducting conjugated polymer-modified electrodes.

Conducting conjugated polymers (CCPs) have widely been used for surface modification of electrodes. CCPs have π-conjugated structures that are characterized by high electrical conductivity. These offer good electrocatalytic behavior, which explains their use as transducers in the preparation of efficient electrochemical sensors [29,30].

Thin films of CCPs can be easily synthesized on the electrode surface by electrochemical methods. Their physico-chemical properties strongly depend on the electropolymerization conditions such as solvent type, supporting electrolyte, electrode material, polymerization potential, and electropolymerization method [31]. The formation of charge carriers on the conjugated backbone is realized by oxidation (p-doping) or reduction (n-doping) that allows the appearance of a metal-like intrinsic conductivity. In the case of p-doping of polymers such as polypyrrol or polythiophene, the cationic charges carried by the polymer backbone are counter balanced by negative charges carried by anions [31].

Among different CCP modifications, the PEDOT-modified electrode has gained interest in a wide range of areas such as the determination of pesticides [32] and phenolic compounds [33]. Recently, our group reported the voltammetric determination of paracetamol with PEDOT-modified GCE [34]. The modified electrode has been found to show much better results compared to bare GCE and has a lot of potential applications for electroanalytical investigations.

The present study reports the preparation of PEDOT-modified GCE and its application for the simultaneous determination of APAP and PAP. The method offers well-resolved voltammetric responses for APAP and PAP. The response for APAP in the presence of other interferents, such as ascorbic acid, *p*-nitrophenol and uric acid has also been investigated. The PEDOT-modified electrode was applied for the determination of APAP in tablets and biological samples.

2. Experiments

2.1. Reagents and apparatus

APAP (Sigma, Germany), PAP (Sigma–Aldrich, UK), tetrabutylammonium tetrafluoroborate (Bu_4NBF_4) (Sigma–Aldrich, Germany), acetonitrile (Scharlau Chemie, Spain), disodium hydrogen phosphate (Techno Pharmchem, India), sodium dihydrogen phosphate (BDH, England), hydrochloric acid (Riedel-deHaen, Germany), and sodium hydroxide (BDH, England) were used without further purification. 3,4-Ethyleneoxythiophene (EDOT) was distilled repeatedly under vacuum until a colorless liquid was obtained and was kept in the dark. Stock solutions of APAP and PAP in phosphate buffer solutions (0.1 mol L^{-1} NaH_2PO_4 and 0.1 mol L^{-1} Na_2HPO_4) were prepared by using deionized water. The pH of the phosphate buffer solution was adjusted by adding drops of concentrated hydrochloric acid and sodium hydroxide.

The voltammetric experiments were carried out using BASi Epsilon-EC_USB, controlled by a Dell computer with a conventional three-electrode configuration. The PEDOT-modified GCE was used as the working electrode, a platinum wire electrode served as the counter electrode with Ag/AgCl/saturated KCl as the reference electrode. The pH of the buffer solutions was measured with a Jenway model 3510 pH meter.

2.2. Preparation of the modified electrode

Prior to modification, a GC electrode was polished with 0.05 μm alumina on the polishing cloth and cleaned with deionized water followed by immersing the polished electrode into a 0.1 M EDOT monomer. Then, the monomer was electropolymerized on the GCE by running cyclic voltammetry from −400 mV to 1300 mV for 8 cycles. The stabilization of the modified electrode has already been described in our previous paper [34].

2.3. Sample preparation

Five tablets (500 mg APAP per tablet) of pharmaceutical formulations were accurately weighed and finely powdered in a porcelain mortar. An adequate amount of the powder was weighed and transferred to a 100 mL flask containing 30 mL of 0.1 mol L^{-1} phosphate buffer (pH 7.0). The flask was thoroughly shaken until most of the sample dissolved and the mixture was centrifuged. Finally, the clear solution was filtered through a Whatman 41 filter paper and the pH of the supernatant was adjusted to 7.0. The recovery of the tablet sample solutions was obtained by using standard addition method.

2.4. Urine preparation

A urine sample was collected from a healthy person 4 h after intake of two tablets of 500 mg APAP per tablet. The extraction procedure of urine sample was carried out according to the literature recently reported [9]. A mixture of 0.2 mol L^{-1} NaOH and urine sample was manually swirled for 2 min followed by ethyl acetate addition. After centrifugation at 4500 rpm for 5 min, the organic phase was separated from the aqueous. The ethyl acetate was removed using a Rotavapor under low pressure and reduced temperature (50 °C). The dried samples were reconstituted with phosphate buffer solution (pH 7.0).

2.5. Electrochemical measurements

The electrochemical experiments of APAP and PAP at the PEDOT-modified GCE were done by using cyclic voltammetry and differential pulse voltammetry (DPV). A potential window between +0 mV and +500 mV together with the optimized pulse amplitude (25 mV) and pulse width (75 ms) was used to obtain the differential pulse voltammograms. Prior to each experiment, the modified electrode was regenerated by running cyclic voltammetry between −100 mV and 700 mV in the phosphate buffer solution until the peak for APAP and PAP disappeared.

3. Results and discussion

3.1. Electropolymerization of EDOT at GCE

The electropolymerization of the EDOT monomer at a GCE was made by running successive cycles between −400 mV and 1300 mV vs. Ag/AgCl/saturated Cl^- at a scan rate of 50 mV s^{-1} as shown in Fig. 1. The polymerization was carried out from a non-aqueous solution containing 0.1 mol L^{-1} Bu_4NBF_4 in acetonitrile. The cyclic voltammograms show the redox peaks that are characteristic for polymer formation [35] and the current increases with each successive cycling, which indicates an enhancement in the film thickness of PEDOT polymer on GC with each cycle. The inset in Fig. 1 demonstrates the cyclic voltammogram of the PEDOT modified electrode in the phosphate buffer solution without APAP and PAP.

The redox behavior of $[Fe(CN)_6]^{3-/4-}$ was taken as a molecular probe to obtain the optimum film thickness of PEDOT/GC electrodes. The anodic peak currents for 1.0 mmol L^{-1} of $Fe(CN)_6^{3-}$ as a function of the square root of the scan rate is shown in Fig. 2. Linear responses of the anodic peak currents as function of the square-root of scan rate for both the bare and PEDOT-modified GCEs indicate that the reaction is diffusion-controlled. Hence, the active electrode

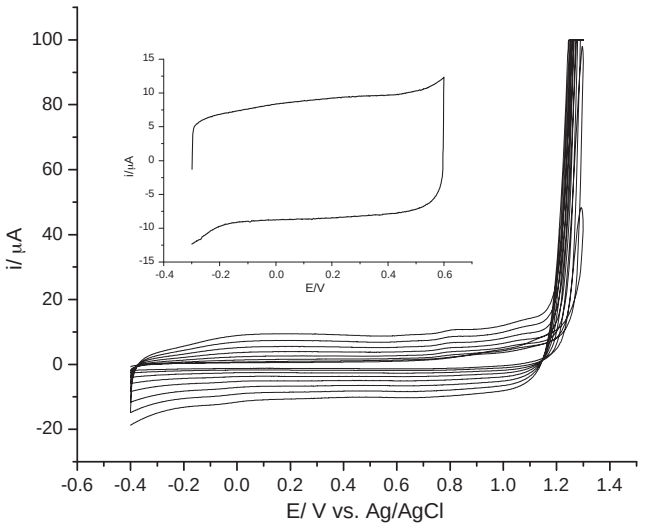

Fig. 1. Electropolymerization of EDOT at glassy carbon electrode at scan rate 50 mV s^{-1}.

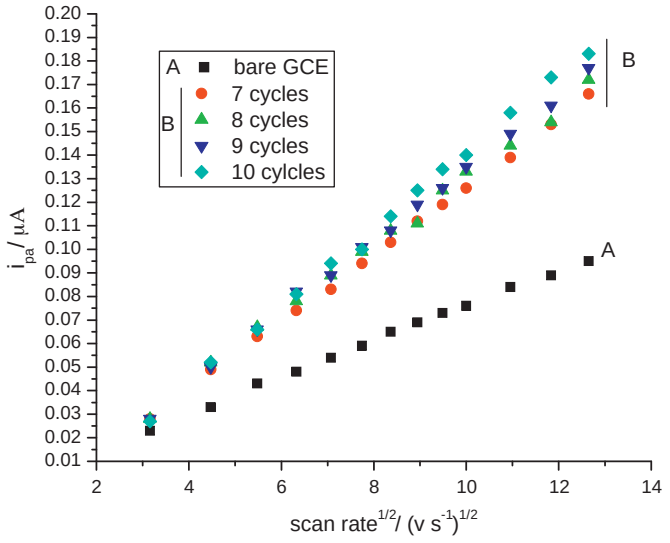

Fig. 2. Plots of anodic peak current of $Fe(CN)_6^{3-/4-}$ (1 mmol L^{-1}) vs. square root of scan rates for: (A) bare GCE; (B) PEDOT-modified electrode with 7, 8, 9, 10 cycle numbers of CV during electropolymerization.

area was determined based on the expression given in Eq. (1) for diffusion controlled reactions [36]:

$$i_{pa} = 0.446nFcA\left(\frac{DnF}{RT}\right)^{1/2}\nu^{1/2} \quad (1)$$

where n is the number of electron(s) involved in the redox reaction, D is the diffusion coefficient, c is the molar concentration, A is the active electrode area, ν is the scan rate and F, R, and T have their usual meanings. The active electrode areas of the PEDOT-modified electrode from Eq. (1) were found to be 0.111, 0.114, 0.116 and 0.119 cm^2 for 7, 8, 9, and 10 cycles, respectively. The modified active electrode areas are much larger than that of the bare GCE (0.068 cm^2). In addition, the active areas do not show significant changes for the cycles between 7 and 10. However, memory effects were observed at higher cycles and the peak currents were low at lower cycles. Hence, to suppress memory effects at higher cycles and to obtain reasonably high peak currents, all modified electrodes were prepared by running eight cycles during electropolymerization.

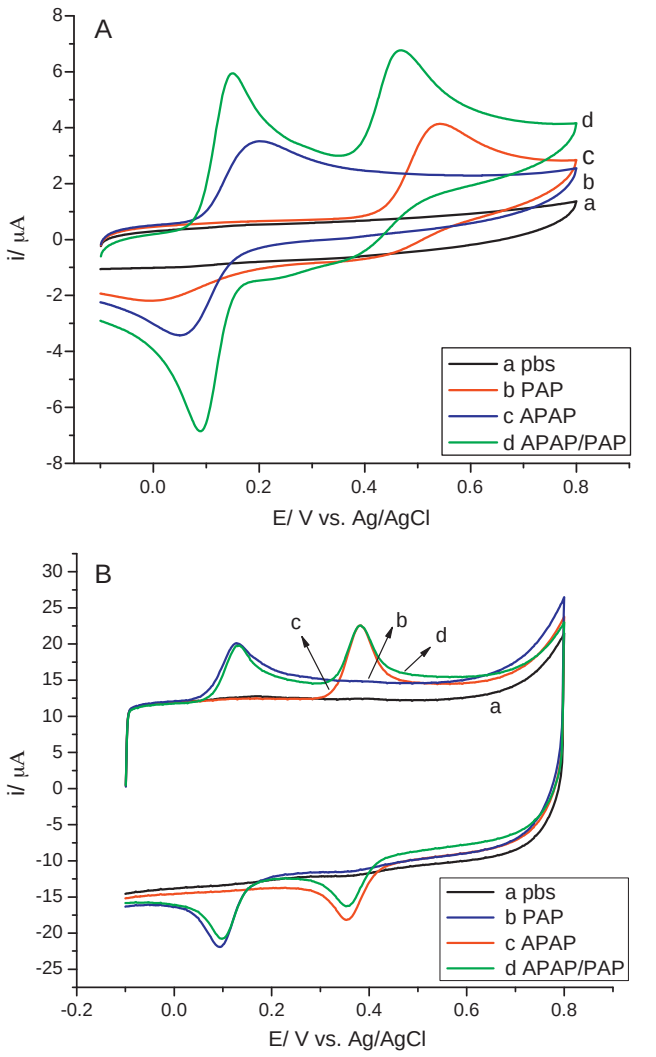

Fig. 3. CV of (A) bare GC and (B) PEDOT-modified GCE in a solution containing APAP (0.2 mmol L^{-1}) and PAP (0.2 mmol L^{-1}), at pH 7.0, phosphate buffer solution with a scan rate of 50 mV s^{-1}. In both CVs: (a) phosphate buffer solution (pbs), (b) PAP, (c) APAP, and (d) mixture of APAP and PAP.

3.2. Electrochemical behaviors of APAP and PAP on PEDOT/GCE

In order to investigate the electrocatalytic activity of the PEDOT-modified GCE, cyclic voltammetry (CV) experiments for APAP and PAP were performed in phosphate buffer pH 7.0. Fig. 3(A) and (B) depicts the cyclic voltammograms of APAP and PAP at bare GCE and PEDOT-modified GCE. The modified electrode has shown a large capacitive current which could be caused by a higher active electrode area of the PEDOT polymer film on the GCE [20]. At the bare GCE, 0.2 mmol L^{-1} APAP gives an irreversible behavior with an anodic peak at 540 mV and a small cathodic peak at −10 mV vs. Ag/AgCl (saturated KCl) while the PEDOT-modified electrode shows a quasireversible reaction. The substantial reduction of overpotential (about 200 mV) and the considerable enhancement in the peak currents (4–5 times) of the modified electrode suggest that the PEDOT-modified electrode exhibits an excellent electrocatalytic activity (Fig. 3 and Table 1).

On the other hand, 0.2 mM PAP demonstrates irreversible behavior at the bare GC electrode and shows almost reversible peaks at the PEDOT-modified GCE. The oxidation and reduction peak potentials of PAP at the modified electrode appeared at 129 and 102 mV, respectively, at a scan rate of 50 mV s^{-1}. The peak-

Table 1
The cyclic voltammetric results for APAP and PAP at the bare GC and PEDOT-modified GCE.

Parameters	APAP		PAP		Mixture			
	GC	PEDOT/GC	GC	PEDOT/GC	APAP		PAP	
					GC	PEDOT/GC	GC	PEDOT/GC
i_{pa} (μA)	4.14	22.54	3.51	19.93	6.76	22.54	5.94	20.17
i_{pc} (μA)	−2.19	−18.12	−3.41	20.05	–	−16.31	−6.82	−21.95
E_{pa} (mV)	543	382	198	129	468	382	151	130
E_{pc} (mV)	−10	354	49	102	–	354	90	93
ΔE_p (mV)	553	28	149	27	–	28	61	37
$E^{o\prime}$ (mV)	267	368	123	116	–	368	121	112

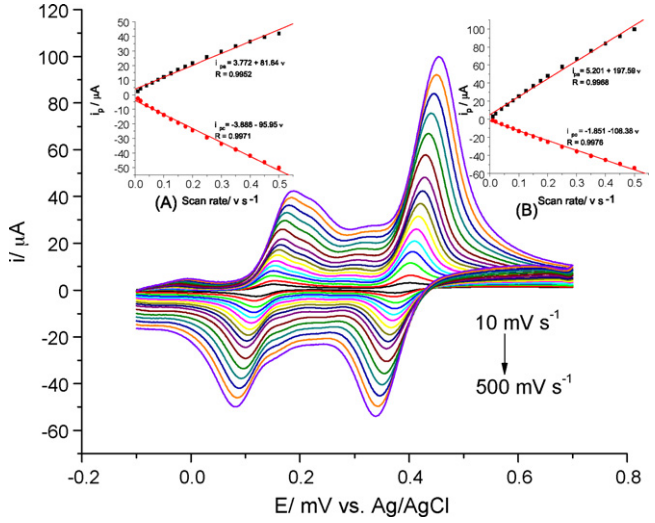

Fig. 4. CVs of the PEDOT modified GCE for APAP (0.2 mmol L^{-1}) and PAP (0.2 mmol L^{-1}) in phosphate buffer solution (0.1 mol L^{-1} pH 7.0) at scan rates from 10 to 500 mV s^{-1}. Insets are the plots of the peak currents of (A) PAP and (B) APAP vs. scan rates (background subtracted).

to-peak potential separation is lowered to 27 mV for the modified electrode compared to 149 mV for the bare GC electrode; see Fig. 3 and Table 1.

The PEDOT-modified GCE showed two well-defined redox peaks at the formal potentials ($E^{o\prime}$), calculated as the midpoint of anodic and cathodic peak potentials, of 112 mV and 368 mV corresponding to PAP and APAP, respectively. Since the reduction peak potential of APAP was shifted to a more positive potential, distinct redox peaks were obtained for APAP. The difference between the formal potentials of APAP and PAP was found to be 256 mV. Hence, the simultaneous determination of APAP and PAP in phosphate buffer becomes possible with the PEDOT-modified GCE. The effective electrocatalytic resolution of the peaks for APAP and PAP at the PEDOT modified electrode may be due to the excellent behavior of the PEDOT polymer such as high conductivity, strong adsorptive capability, significant increment in active electrode area [28,33].

The effect of scan rate on the electrochemical responses for APAP and PAP was assessed by cyclic voltammetry as shown in Fig. 4. The redox peak currents at the modified GCE for APAP and PAP increased linearly with the scan rate in the range 10–500 mV s^{-1} (inset, Fig. 4; linear regression equations: (A) $i_{pa} = 5.201 + 197.59\nu$, $R = 0.9968$; $i_{pc} = -1.851 - 108.38\nu$, $R = 0.9976$ for APAP and (B) $i_{pa} = 3.772 + 81.64\nu$, $R = 0.9952$; $i_{pc} = -3.888 - 95.95\nu$, $R = 0.9971$ for PAP). The results suggest that the reactions at the modified electrode for both APAP and PAP be a surface-confined process.

The plots of the anodic and cathodic peak potentials vs. the logarithm of the scan rates are used to calculate the electron-transfer coefficient (α) based on the slopes of the lines $RT/(1-\alpha)nF$ and $-(RT/\alpha nF)$, respectively, where n is the number of electrons involved in the redox reactions, F, R and T have their usual meanings. The linear regression equations of the plots were found to be $E_{pa} = 0.1498 + 0.074 \ln \nu$, $R = 0.9918$; $E_{pc} = 0.1147 - 0.066 \ln \nu$, $R = 0.9916$ for PAP and $E_{pa} = 0.1923 + 0.016 \ln \nu$, $R = 0.9908$; $E_{pc} = 0.077 - 0.014 \ln \nu$, $R = 0.9892$ for APAP. The value for the electron-transfer coefficient was calculated to be 0.53 for both PAP and APAP.

3.3. Effect of pH

The redox responses for APAP and PAP at the PEDOT-modified GCE were examined in different buffer solutions (0.1 mol L^{-1} of acetate pH 4.53, phosphate pH 7.01, sodium tetraborate pH 9.50) using cyclic voltammetry (Fig. 5A). CV of sodium tetraborate is not shown due to the instability of PAP in the buffer. Well-defined anodic and cathodic peaks for APAP and PAP were obtained in phosphate buffer with higher current responses at lower negative potentials compared to the acetate buffer. Thus, phosphate buffer was chosen for further experiments and the simultaneous determination of APAP and PAP.

Fig. 5B shows the cyclic voltammograms of APAP and PAP in pHs ranging 5.0–9.0 using the PEDOT-modified electrode. The voltammograms illustrate the highest response was obtained at pH 7. As can be seen in the inset of Fig. 5B, the formal potentials ($E^{o\prime}$) shifted towards negative potentials for both APAP and PAP as the pH increased, indicating the redox reactions are accompanied by proton transfer [20,27]. The $E^{o\prime}$ changed linearly with pH according to the equations of $E^{o\prime} = 0.7695 - 0.056$pH, $R = 0.9926$ for APAP and $E^{o\prime} = 0.5317 - 0.058$pH, $R = 0.9937$ for PAP. The slopes of the linear regression equations are close to the ideal 59 mV for each unit pH change suggesting the same numbers of electrons and protons are involved in the redox reactions for both APAP and PAP. The oxidation of APAP involves the formation of *N*-acetyl-*p*-quinone-imine and a loss of two electrons and two protons as reported previously [37,38]. Similarly, the oxidation reaction of PAP proceeds with the formation of *p*-quinone-imine and releasing two electrons and two protons [37,39].

3.4. Analytical performances of the modified electrode

In order to suppress the influence of background current, DPV was selected to examine the analytical performance of the PEDOT-modified GCE. Two well-defined peaks appeared at 367 mV (for APAP) and 101 mV (for PAP) in the voltammograms; see Fig. 6(A) and (B). The peak-to-peak potential separation is 266 mV, which is sufficient to determine the two species simultaneously.

The performance of the PEDOT-modified GCE for APAP and PAP was studied under the optimized conditions. Fig. 6(A) and (B) shows the DPV responses for the APAP and PAP mixture prepared by varying the concentration of either of the analyte while keeping the other constant. The anodic current is linearly related to the APAP concentration (1–100 μmol L^{-1}) in the presence of 40 μmol L^{-1} PAP (inset Fig. 6A). The linear regression

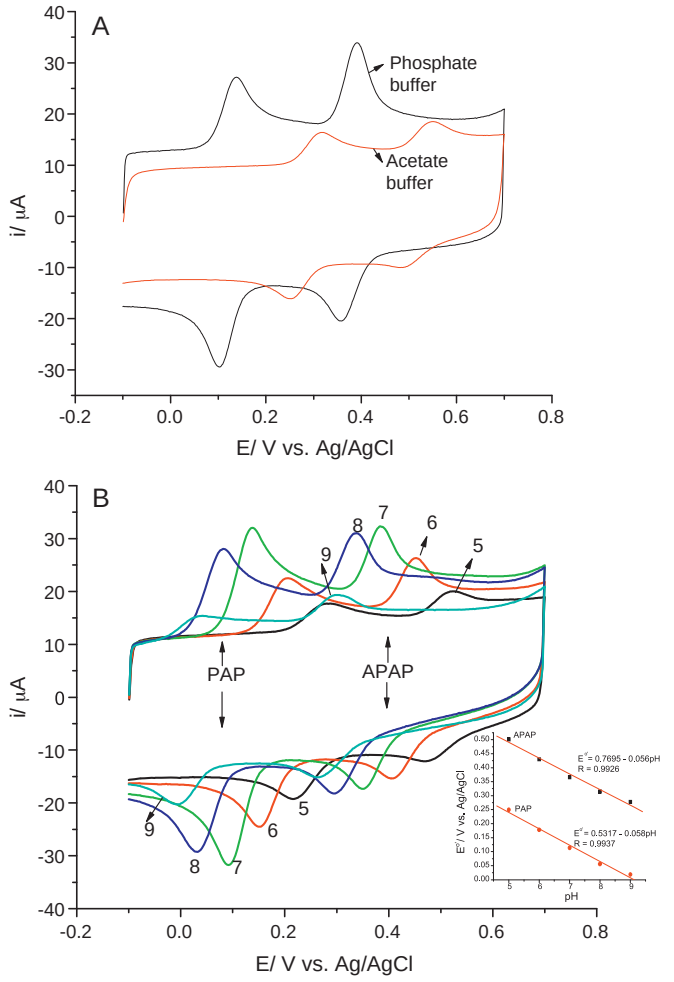

Fig. 5. (A) CVs at the PEDOT-modified electrode for PAP (0.5 mmol L^{-1}) and APAP (0.5 mmol L^{-1}) in (a) acetate buffer (0.1 mol L^{-1}), (b) phosphate buffer (0.1 mol L^{-1}); (B) phosphate buffer of pHs (0.1 mol L^{-1}) of 5, 6, 7, 8 and 9. Inset shows the plot of $E^{o\prime}$s vs. pH. Scan rate of 50 mV s^{-1}.

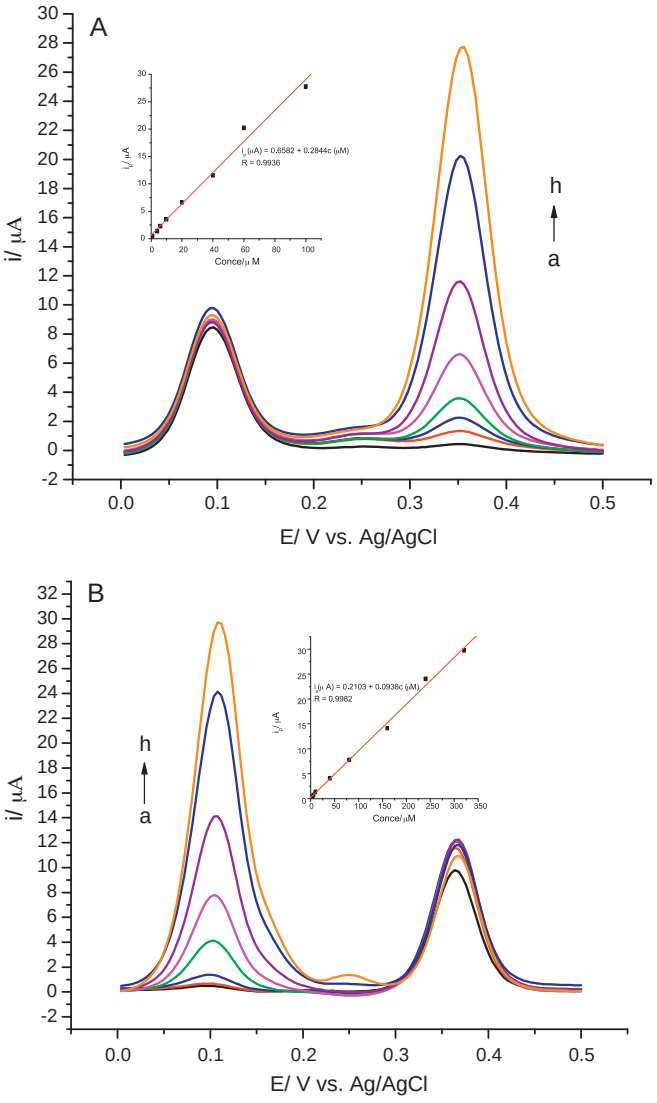

Fig. 6. (A) DPV at the PEDOT-modified GCE in the presence of PAP (40 μmol L^{-1}) for different concentrations of APAP (from a to h): 1.0, 4.0, 6.0, 10.0, 20.0, 40.0, 60.0, and 100.0 μmol L^{-1}. (B) DPV of the modified electrode in the presence of APAP (40 μmol L^{-1}) for different concentrations of PAP (from a to h): 4.0, 6.0 10.0, 40.0, 80.0, 160, 240, and 320 μmol L^{-1}. Scan rate of 20 mV s^{-1} (background subtracted).

equation was i_{pa} (μA) = 0.6582 + 0.284c (μmol L^{-1}), with a correlation coefficient of 0.9936. The detection limit was found to be 4.0×10^{-7} mol L^{-1} based on signal to noise ratio of 3, which is lower than that of C_{60}-modified GCE (5.0×10^{-5} mol L^{-1}) [23] and is comparable to those obtained with GCE modified with carbon coated nickel magnetic nanoparticles (6.0×10^{-7} mol L^{-1}) [22], MWCNTs:graphite (1.6×10^{-7} mol L^{-1}) [24], polyaniline–MWCNT composite (2.5×10^{-7} mol L^{-1}) [28] but higher than graphene-modified GCE (3.2×10^{-8} mol L^{-1}) [20].

Similarly, the i_{pa} increases linearly with increasing concentration of PAP (4.0–320 μmol L^{-1}) in the presence of 40 μmol L^{-1} APAP (inset of Fig. 6B). The linear regression equation was i_{pa} (μA) = 0.2103 + 0.094c (μmol L^{-1}), with a correlation coefficient of 0.9982. The detection limit was 1.2×10^{-6} mol L^{-1} based on signal to noise ratio of 3. The calibration plots of the modified electrode for PAP with and without APAP show similar sensitivity, indicating the two species do not interfere in the electrochemical response of each other.

The reproducibility of four individual PEDOT-modified GCEs for the responses of 40 μmol L^{-1} APAP and 40 μmol L^{-1} PAP was evaluated and the relative standard deviations (RSD) obtained were 4.8% and 4.3%, respectively. The RSD for five successive determinations of 40 μmol L^{-1} APAP and 40 μmol L^{-1} PAP with a modified electrode were 2.1% and 1.9%, respectively, which demonstrate a very good repeatability of the performance of the modified electrode.

The inter-day stability of the PEDOT-modified electrode was tested by preparing three modified electrodes and keeping them at 4 °C when not in use. The response current for 40 μmol L^{-1} APAP was recorded for four consecutive days. The average response was found to be 87 ± 6.4% of the original one at the end of the investigation period.

3.5. Interference study

The selectivity of the PEDOT-modified GCE was studied in the presence of different interfering species. Possible interfering substances such as acetylsalicylic acid, saccharine, ascorbic acid, citric acid, sodium carbonate that can exist in pharmaceutical formulations did not interfere in APAP determination as reported in our earlier work [34].

The interferences of uric acid and *p*-nitrophenol in the determination of APAP and PAP with the modified electrode were investigated by running cyclic voltammetry. *p*-Nitrophenol did not exhibit any peak in the working potential window while uric acid (UA) showed interference as depicted in Fig. 7. The

Table 2
Determination of APAP and PAP in commercial tablets using the PEDOT-modified GCE.

Matrix	Added (μM)		Found[a] (μM)		Recovery (%)	
	APAP	PAP	APAP	PAP	APAP	PAP
Tablet 1 (EPHARM)	–	–	5.94 (±0.07)	–	–	–
	25.0	24.0	30.1 (±0.21)	25.4 (±0.17)	96.6	105.8
	42.0	57.0	51.5 (±0.28)	54.5 (±0.24)	108.5	95.6
Tablet 2 (PANDOL)	–	–	5.79 (±0.11)	–	–	–
	34.0	54.0	41.1 (±0.27)	54.9 (±0.22)	103.9	101.7
	49.0	66.0	58.6 (±0.23)	63.6 (±0.26)	107.8	96.4

[a] Mean value ± standard deviation ($n = 3$).

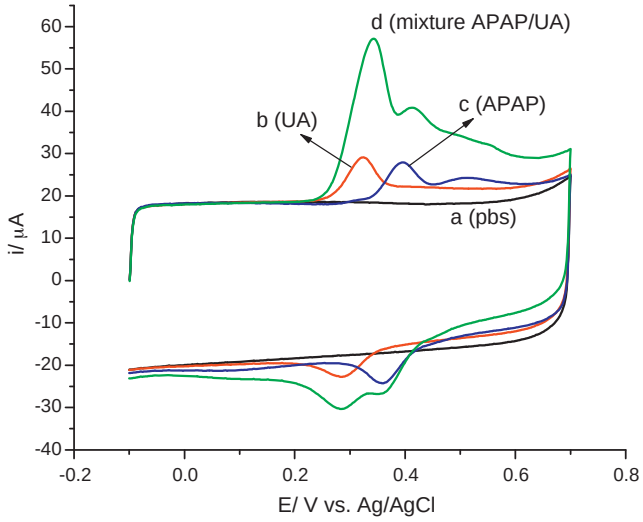

Fig. 7. CVs at the PEDOT-modified GCE for: (a) pbs (pH 7.0), (b) UA (0.5 mmol L^{-1}), (c) APAP (0.5 mmol L^{-1}), (d) mixture of UA (0.5 mmol L^{-1}) and APAP (0.5 mmol L^{-1}), at scan rate 50 mV s^{-1}.

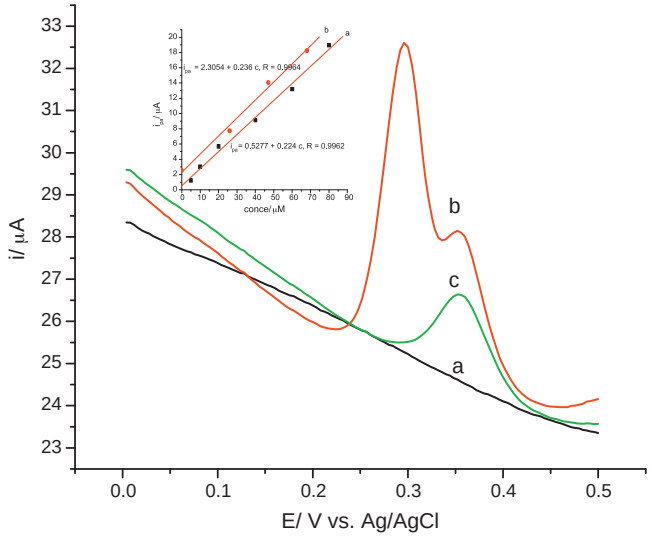

Fig. 8. Differential pulse voltammograms of (a) phosphate buffer solution, (b) unextracted urine sample, and (c) extracted urine sample with a scan rate 20 mV s^{-1}. The inset is the calibration plots for (a) standard APAP and (b) spiked urine sample.

cyclic voltammograms of a mixture of UA (0.5 mmol L^{-1}) and APAP (0.5 mmol L^{-1}) exhibited an enhanced peak with a shoulder (Fig. 7d) by merging the peaks of UA and APAP that appeared at 320 mV (Fig. 7b) and 390 mV (Fig. 7c), respectively. The results indicate that the electrochemical response of the modified electrode for APAP and PAP in biological samples is free of interference from *p*-nitrophenol but suffers from uric acid. However, the interference of uric acid from biological samples can be avoided with ethyl acetate extraction as demonstrated in the urine sample recovery test.

3.6. Analytical application

The method developed was applied for the determination of APAP (500 mg per tablet) and PAP in the tablets to evaluate the validity of the PEDOT-modified GCE. Aliquots obtained by dissolution of APAP tablets were subsequently diluted to get a specified concentration of APAP that lies in the range of the calibration plot and the standard addition method was then employed for recovery tests. The results for APAP and PAP are summarized in Table 2.

Recovery tests are in the ranges 96.6–108.5% for APAP and 95.6–105.8% for PAP. The results show that tablet matrix does not have any interference on the simultaneous determination of the analytes. The amount of APAP in pharmaceutical formulations was found to be 492.0 mg/tablet with 1.6% error, showing a good agreement with the content of APAP given by the manufacturer.

Recovery studies were also carried out for urine sample after intake of APAP. The differential pulse voltammograms of phosphate buffer and urine samples are shown in Fig. 8. The blank urine samples after dilution with the buffer solution reveal double broad peaks (Fig. 8b), due to interference from uric acid. Extraction of urine samples with ethyl acetate removes the interferent from the urine sample (Fig. 8c). A linear calibration plot was obtained for 0–68 μm mol L^{-1} spiked with APAP with a slope of 0.234 μA/μmol L^{-1}, close to the slope obtained for standard APAP solutions (0.224 μA/μmol L^{-1}) as depicted in the inset of Fig. 8. Similar magnitudes in their sensitivity suggest that there is no significant matrix effect in urine sample. The percent recoveries for 26, 47 and 68 μmol L^{-1} spiked with APAP into the extracted urine samples were found to be 88.4%, 109.1% and 102.9%, respectively.

4. Conclusions

A PEDOT-modified GCE was fabricated for the simultaneous determination of APAP and PAP using DPV technique. The results confirm that the presence of PEDOT film on the surface of the electrode significantly affects the kinetics and sensitivity of the electrochemical responses for APAP and PAP. The remarkable peak separation of the two analytes offers the technique a selective determination of APAP in the presence of its main impurity PAP. The proposed method was applied to determine APAP and PAP in tablets with recovery ranging 96.6–108.5% and 95.6–105.8%, respectively. Finally, the PEDOT-modified GCE was successfully used for the determination of APAP in biological samples with a good percent recovery (88.4–109.1%).

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