



Electroanalytical determination of acetaminophen using nano-TiO₂/polymer coated electrode in the presence of dopamine

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ABSTRACT

We report a new method for selective determination of acetaminophen (AP) in physiological condition. A new hybrid film modified electrode was fabricated using inorganic semiconducting nano-TiO₂ particles and redox active polymer. Redox polymer, poly(acid yellow 9) (PAY) was electrochemically deposited onto nano-TiO₂ coated glassy carbon (GC) electrode. Surface characterizations of modified electrode were investigated by using atomic force microscope and scanning electron microscope. The PAY/nano-TiO₂/GC hybrid electrode shows stable redox response in the pH range 1–12 and exhibited excellent electrocatalytic activities towards AP in 0.1 M phosphate buffer solution (pH 7.0). Consequently, a simple and sensitive electroanalytical method was developed for the determination of AP. The oxidation peak current was proportional to the concentration of acetaminophen from 1.2×10^{-5} to 1.20×10^{-4} M and the detection limit was found to be 2.0×10^{-6} M (S/N = 3). Possible interferences were tested and evaluated that it could be possible to selective detection of AP in the presences of dopamine, nicotinamide adenine dinucleotide (NADH), ascorbic acid and uric acid. The proposed method was used to detect acetaminophen in commercial drugs and the obtained results are satisfactory.

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1. Introduction

Nanomaterials have received much interest by virtue of their excellent properties suited for applications in various fields such as electronic, pharmaceutical, biomedical, cosmetic, energy, and catalysis [1–6]. The nano-TiO₂ films and membranes had good structural and catalytic properties, including homogeneity without cracks and pinholes, high surface area and porosity, narrow pore size distribution, small crystallite size, high crystallinity, and active anatase phase [1]. Devices containing functional molecules anchored to nanocrystalline TiO₂ electrodes have attracted a significant amount of attention [2–6]. Nanocrystalline TiO₂ electrodes coated with light-absorbing dyes are fundamental components of dye-sensitized solar cells [7] and a number of display devices utilizing TiO₂ electrodes with adsorbed electrochromic compounds have been reported [8]. In each case, the redox chemistry of the bound molecular species is intimately involved with the device performance. Nano-TiO₂, known as their biocompatible and environmentally benign properties, have been proposed as a potential interface for the immobilization of biomolecules and

applied in photochemistry [9,10] and electrochemistry [11–13] tentatively.

A wide range of polymer and inorganic mesoporous metal oxide have been combined to form nanocomposite materials with unique mechanical, electrical, magnetic and adhesive properties [1,14]. Inorganic nanoparticles of different nature and size can be combined with the conducting polymers, giving rise to a host of nanocomposites with interesting physical properties and important applications [14,15]. This is related to some distinctive properties of nanoparticles and anomalous cooperative properties of systems and it has several advantages than its individuals.

Dopamine (3,4-dihydroxyphenylethylamine) is an important neurotransmitter of the catecholamine group that exists in the mammalian central nervous system and is well characterized by its electrochemical activity [16]. In recent years, there has been considerable interest in developing new methods to measure this neurotransmitter in biological samples [17]. Identification and determination of DA with electrochemical techniques are more promising approach. However, DA oxidation required high overpotential at bare electrodes and its oxidation products were strongly adsorbed onto bare electrode surface results electrode fouling and unstable analytical signal [16,18]. Ascorbic acid (AA), uric acid (UA) and reduced form of nicotinamide adenine dinucleotide (NADH) are the important biomolecules often coexist with DA. Electrochemical oxidation of AA, UA and NADH at bare electrode results highly

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overlapped peak which lead poor selectivity and reproducibility of the electrode. To determine these biomolecules selectively in the presence of each other has been a major goal of electroanalytical research [19] and several methods mainly based on the chemical modification of traditional electrode materials, have been developed to resolve the problem [20–38].

Recently, selective electrochemical determination of DA in the presence of ascorbic acid using sodium dodecyl sulfate micelles as masking agent [39], poly-chromotrope 2B modified GC electrode [40], poly(4-amino-1-1'-azobenzene-3, 4'-disulfonic acid) coated electrode [41], poly(*p*-nitrobenzenazo resorcinol) modified GC electrode [42], nano-Au self-assembly GC electrode [43], Nafion/carbon-coated iron nanoparticles–chitosan composite film modified electrode [44] and PtAu hybrid film modified electrode [45] were reported.

Acetaminophen (*N*-acetyl-*p*-aminophenol or paracetamol) has been used widely all over the world as a pharmaceutical analgesic and antipyretic agent. It is suitable for patients who are sensitive to aspirin and safe up to therapeutic doses [46]. So it is necessary to develop a rapid, precise, simple and reliable method for the determination of acetaminophen. Recently, L-cysteine film modified GC electrode [47], carbon-coated nickel magnetic nanoparticles modified GC electrodes [48], and 4-amino-2-mercaptopyrimidine self-assembled monolayer modified gold electrode [49] has been fabricated and they were applied to the electrochemical determination of acetaminophen.

Catecholamines are small molecules made by nerve tissue (including the brain) and the adrenal gland. The major catecholamine is dopamine. This substance breaks down into other compounds, which leave our body through our urine. A urine test can be done to measure the level of catecholamine in our body. The important drugs such as acetaminophen will interfere with the catecholamine measurements in biological samples [50]. So, it is very important to measure the concentration of acetaminophen in the presence of dopamine. For the first time, in this paper, we report a new method for electrochemical determination of acetaminophen in the presence of dopamine using poly(acid yellow 9)/nano-TiO₂ modified glassy carbon electrode. The electrochemical properties of the poly(AY) coated nano-TiO₂ modified GC electrodes and the valuable applications of this electrode in the fabrication of stable and high sensitive electrochemical sensor for AP was developed. Surface characterization of PAY/nano-TiO₂ and nano-TiO₂ coated electrodes were investigated using scanning electron microscope (SEM) and atomic force microscope (AFM).

2. Experimental

2.1. Reagents and instruments

All chemicals and reagents used in this work were of analytical grade and used as received without further purification. These were 4-amino-1-1'-azobenzene-3,4'-disulfonic acid (AY, dye content about 95%), reduced form of β-NADH, dopamine hydrochloride and uric acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). *p*-Acetaminophen, sulfuric acid (H₂SO₄) and sodium hydroxide (NaOH) were purchased from Wako pure chemicals (Osaka, Japan). Ascorbic acid, sodium acetate (CH₃COONa) and sodium dihydrogen phosphate (NaH₂PO₄) were received from E-Merck (Darmstadt, Germany). Nano-TiO₂ suspension was purchased from Ever-light Chemical Industrial Co., Ltd., Taiwan. Supporting electrolytes were prepared by using doubly distilled deionized water and before each experiment the solutions were deoxygenated by purging with pre-purified nitrogen gas for 10 min.

Electrochemical measurements were performed with CH Instruments (TX, USA) Model-400 potentiostat with a conventional three-electrode cell. A BAS glassy carbon and platinum wire are used as the working electrode and counter electrode, respectively. All the cell potentials were measured with respect to an Ag/AgCl [KCl (sat)] reference electrode. Hitachi scientific instruments (London, UK) Model S-3000H Scanning Electron Microscope was used for surface image measurements. The AFM images were recorded with a Multimode Scanning Probe Microscope System operated in tapping mode using model CSPM4000 Being Nano-Instruments (Beijing, China). All experiments were carried out at room temperature.

2.2. Modification of the electrodes

Prior to electrode modification, the GC electrode was mechanically polished with alumina powder (Al₂O₃, 0.05 μm) up to a mirror finish and ultrasonicated in distilled water for 5 min. Then GC electrode was electrochemically activated by using 20-times cyclic potential sweeps in the range of –0.5 to 2.0 V in 0.1 M H₂SO₄ solution at a scan rate of 100 mV/s. Indium tin oxide (ITO) coated glass substrates were cleaned by using detergent, diluted hydrochloric acid and then finally rinsed with distilled water.

5 mg nano-TiO₂ particles suspension was added into 10 mL double-distilled water and then ultrasonicated for 10 min to create a suspension with a concentration of 0.5 mg mL⁻¹. The mixture of 10 μL nano-TiO₂ suspension was spread evenly onto the surface of the GC electrode which was left to evaporate the solvent under ambient conditions in the absence of light. The PAY/nano-TiO₂/GC electrode and alone PAY/GC coated electrode were fabricated in 0.5 mM AY monomer solution under the same conditions as those in the electrode activation procedure using nano-TiO₂/GC or GC electrodes. The as-prepared dark blue colored films were obtained and strongly adherent to the substrates. Subsequently, the modified electrodes were rinsed thoroughly with distilled water and then they were dried in air and used in electrochemical experiments.

3. Results and discussion

3.1. Electrochemical modification of electrode

The electrochemical polymerization was carried out onto nano-TiO₂ modified GC electrode as said condition in Section 2.2. Fig. 1

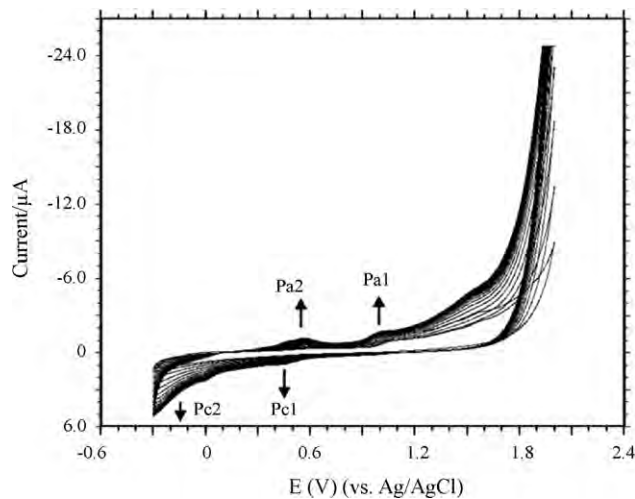


Fig. 1. CVs of the poly(AY) film growth on nano-TiO₂ coated GC electrode from electrolyte 0.1 M H₂SO₄ solution containing 0.5 mM AY monomers. Scan rate = 0.1 V/s.

shows the consecutive cyclic voltammograms (CVs) of polymer growth on the electrode surface. First scan started at -0.30 V, during the first scan an anodic peak observed at $+1.0$ V (P_{a1}), this peak ascribed to oxidation of $-NH_2$ group of acid yellow 9 (AY) monomer [41,51,52]. Upon continuous cycling, a new cathodic peak (at $+0.41$ V, P_{c1}) and a new anodic peak (at $+0.56$ V, P_{a2}) were developed which is indicated that the growth of the polymer film on nano-TiO₂/GC electrode. The polymerization mechanism may be described as follows AY was first oxidized to free radical (P_{a1}); the free radical combined together rapidly to hydrazobenzene sulfonic acid; then hydrazobenzene sulfonic acid was oxidized to azobenzene sulfonic acid (P_{a2}), and azobenzene sulfonic acid reduced to hydrazobenzene sulfonic acid (P_{c1}) (Scheme 1). After polymerization started, a broad cathodic peak (P_{c2}) centered at -0.24 V was developed. It may due to the insertion of anions (for example SO_4^{2-}) accompanied by the hydrogenation of polymer site and similar kinds of observations were reported for some azo and sulfonated aniline compounds at solid electrodes [41,53–56].

3.2. Electrochemical properties and surface characterizations

Typical CVs of PAY/nano-TiO₂/GC electrode in pH 1.5 H₂SO₄ solutions at different scan rates are shown in Fig. 2A. A pair of redox peak was obtained in each of the CVs at $E^{0'}$ $+0.31$ V and the peak currents of the PAY/nano-TiO₂/GC electrode are proportional to the scan rates up to 1000 mV/s, indicating the surface confined electrochemical redox process [57]. The anodic peak currents (I_{pa}) were linearly dependent of scan rate with the linear equation:

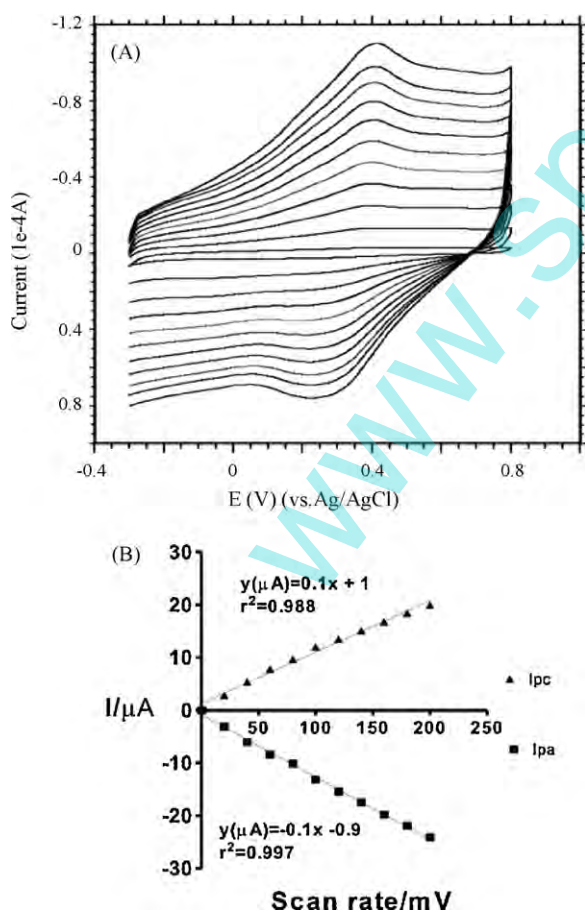


Fig. 2. (A) CVs of PAY/nano-TiO₂/GC modified electrode in pH 1.5 acidic solutions at different scan rates. The scan rates from inner to outer are 0.02, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 V/s, respectively. (B) Plot of I_{pa} and I_{pc} vs. scan rate.

I_p (μ A) = $-0.1x - 0.9$ ($r^2 = 0.997$), and the ratio of anodic peak current to cathodic peak current (I_{pa}/I_{pc}) was almost equal to unity (Fig. 2B). The peak to peak separation ($\Delta E_p = E_{pa} - E_{pc}$) was 75 mV at low scan rate (5–20 mV/s). According to the following equations

$$I_p = \frac{n^2 F^2 \Gamma v A}{4RT} \quad (1)$$

$$Q_r = nFA\Gamma \quad (2)$$

where n is the number of electrons, A is the working electrode area (0.0707 cm²), v scan rate (20 mV/s) and other parameters are usual meanings [57–59], the electron transfer number and the surface coverage were calculated to be 2.10 and 3.7×10^{-10} mol/cm², respectively. The cyclic voltammograms of PAY/nano-TiO₂/GC electrode was strongly affected by the solution pH (Fig. S1). An increase of solution pH caused a negative shift in $E^{0'}$ value with slopes of -58 mV/pH which is close to the expected value of -59 mV/pH for involving same number of electron and proton coupled electron transfer process (Scheme 1) [41].

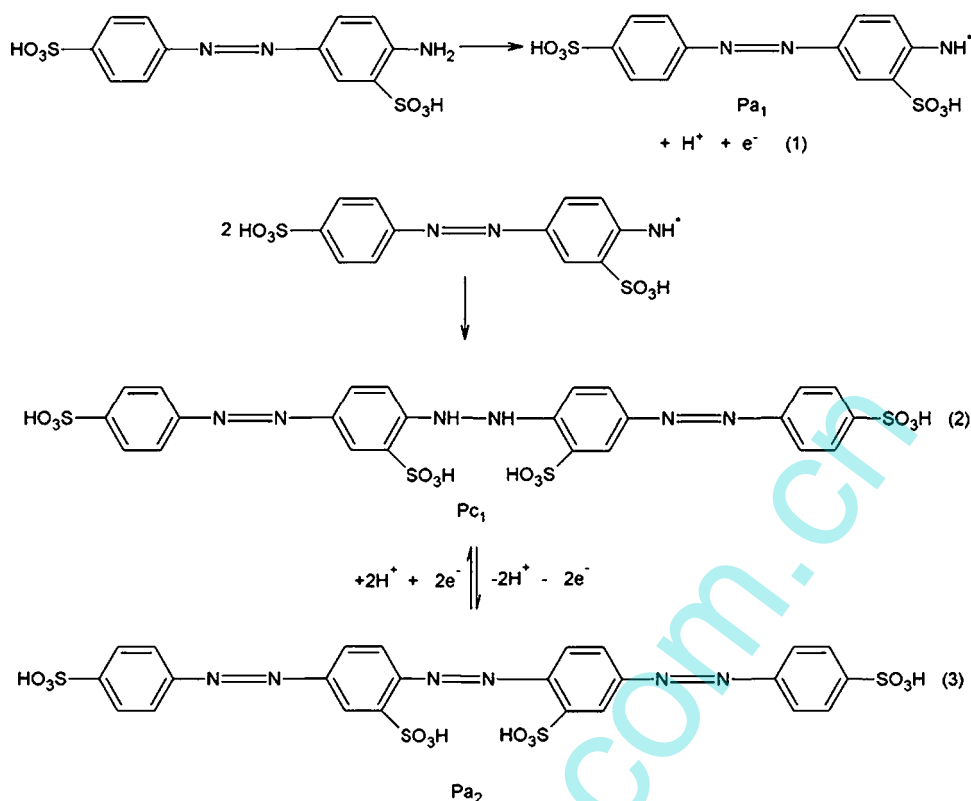
The surface characterizations of the TiO₂ and PAY/nano-TiO₂ coated electrodes were performed using SEM (a and b) and AFM (c and d) as shown in Fig. 3. The average particle size of the TiO₂ particles was found to be in the range of 100 ± 20 nm. SEM and AFM images of poly(AY) coated electrode confirmed that the polymer thin film deposition on electrode surface. The average roughness values found to be 17.7 and 171 nm for nano-TiO₂ and PAY/nano-TiO₂ coated electrode, respectively. It is confirmed that the poly(AY)/nano-TiO₂ hybrid films on the electrode surface. Thickness of the nano-TiO₂ and poly(AY)/nano-TiO₂ hybrid films were found to be 200 and 1391 nm, respectively. Film thickness and roughness values are higher for hybrid film modified electrodes; the high conductivity of nano-TiO₂ in the composite film increases the electrical properties of the redox processes of polymer and also provides a large surface area available for polymer intercalation.

3.3. Electrocatalysis

The electrocatalytic ability of the PAY/nano-TiO₂/GC electrode towards DA was investigated. In order to assess the electrocatalytic activity of PAY/nano-TiO₂/GC electrode, its response to the oxidation of DA was studied. When 120 μ M DA was added to a pH 7.0 buffer solution, an increase in the oxidation peak at about 0.21 V could be seen with cathodic peak at 0.18 V (Fig. 4A, curve b). This new anodic peak results from DA oxidized to dopamine-*o*-quinone (DOQ), cathodic peak results from DOQ reduced to DA (Scheme 2, Eq. (4)) at PAY/nano-TiO₂/GC electrode. However, direct oxidation of DA on bare GC electrode results a highly ill-defined anodic peak potential at 0.23 V and a reduction peak potential at 0.16 V (curve c). For the oxidation of DA at PAY/nano-TiO₂/GC electrode, the separation of peak potentials (ΔE_p) was 30 mV, which was on accordance with a near Nernst reversible behavior and identified that the number of electrons involved in the reaction was about equal to 2. Intensive increase in DA peak current was also observed due to the improvement in the reversibility of electron transfer process on the larger surface area of the PAY/nano-TiO₂/GC electrode. All the above results suggested that an efficient oxidation reaction of DA at the PAY/nano-TiO₂/GC electrode.

3.4. Effect of scan rate and pH on DA oxidation

The effect of the scan rate on peak current of DA was investigated in pH 7.0 buffer solution containing 120 μ M DA. The anodic and cathodic peaks current were proportional to the scan rate over the range 10–100 mV/s. The linear regression equations were $i_{pa}(\mu A) = -0.249x - 0.612$ and $i_{pc}(\mu A) = 0.174x - 0.42$, with the



Scheme 1. Electrochemical polymerization mechanism of AY.

correlation coefficient, 0.999 and 0.999, respectively. Therefore, the electrochemical reaction of DA was surface confined electrode process. CVs were recorded in the presence of 120 μM DA in various pH supporting electrolyte solutions. The $E^{0'}$ of DA redox peak was shifted negatively with a slope of -60 mV/pH , it was indicated that same number of electrons and protons involved in the redox reaction of DA. In addition, we found that the maximum catalytic current was obtained for DA oxidation at PAY/nano-TiO₂/GC electrode in neutral buffer solutions (Fig. S2). Reasons for this phenomenon could be partly explained on the basis of the dissociation ability of $-\text{SO}_3^-$ (H) group of poly(AY) film in different pH environments. When the solution pH was neutral, the $-\text{SO}_3\text{H}$ group of poly(AY) film could dissociate favorably into a negative charge group $-\text{SO}_3^-$. Under this condition, the $-\text{NH}_2$ group of DA molecules (pK_a 8.9) [41,54] could obtain a proton and form the positive ion of DA. Therefore, the negative charge group $-\text{SO}_3^-$ on the surface of poly(AY) modified electrode had a well affinity to the DA positive ions and could catalyze and promote the oxidation of DA efficiently. Based on the above results the electrochemical oxidation reactions of DA is described in Scheme 3.

3.5. DA oxidation at PAY/GC and PAY/nano-TiO₂/GC modified electrodes

Fig. 4B shows the CVs of 120 μM DA oxidation by using PAY/GC (curve a) and PAY/nano-TiO₂/GC (curve b) modified electrodes. The I_{pa} of DA oxidation almost no change but a new hump is observed about -0.22 V at PAY/GC electrode. This is indicating that the oxidation product of DA was adsorbed on the electrode surface (curve a) [60,61]. By contrast this behavior is not observed in nano-TiO₂ coated electrode (curve b).

Poly(AY) is negatively charged polymer, however, it has more affinity for positively charged species like DA (pK_a 8.9). As discussed in Section 3.4, poly(AY) coated electrode had potent electrocatalytic properties towards DA. However, our experimental results suggested that alone poly(AY) coated electrode get adsorbed by DA molecules after continuous measurements or dipping of poly(AY) electrode in pH 7 buffer solution containing 100 μM DA for an hour (Fig. 4C, curve a). This may be a usual behavior for opposite charged species but it is a limitation of modified electrodes. By contrast to PAY/GC modified electrode, PAY/nano-TiO₂/GC modified electrode was also immersed in 100 μM DA solution for an hour, thereafter, CVs were recorded, and the same voltammograms were observed before and after immersion. This result suggested that DA molecules were not adsorbed on the nano-TiO₂ incorporated electrode (Fig. 4C, curve b). This study clearly suggested that an application of nano-TiO₂ particles in this hybrid film modified electrode. In addition, after 50 measurements of 100 μM DA in pH 7.0 buffer solution using PAY/nano-TiO₂/GC modified electrode, there is no change in CVs before and after measurements which is indicated that nano-particles improved antifouling properties of electrode surface. For these reasons, we fabricated nano-TiO₂ coated polymer modified electrode and used in further studies throughout this work.

3.6. Electrocatalysis of AP

Cyclic voltammetry (CV) was used to investigate the electrochemical behavior of AP at the PAY/nano-TiO₂/GC coated electrode in 0.1 M PBS. As shown in Fig. 5, a reversible peak is observed at the PAY/nano-TiO₂/GC electrode. After addition of 120 μM AP, the PAY/nano-TiO₂/GC modified electrode gave a highly enhanced

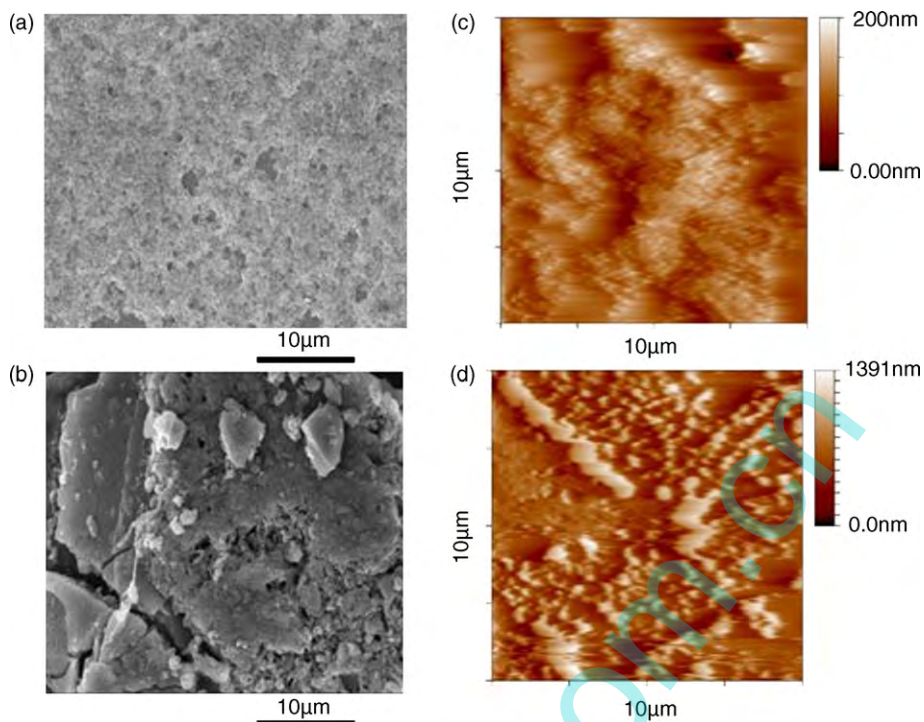


Fig. 3. SEM (a and b) and AFM (c and d) images of (a and c) Nano-TiO₂ film, (b and d) PAY/nano-TiO₂ hybrid film.

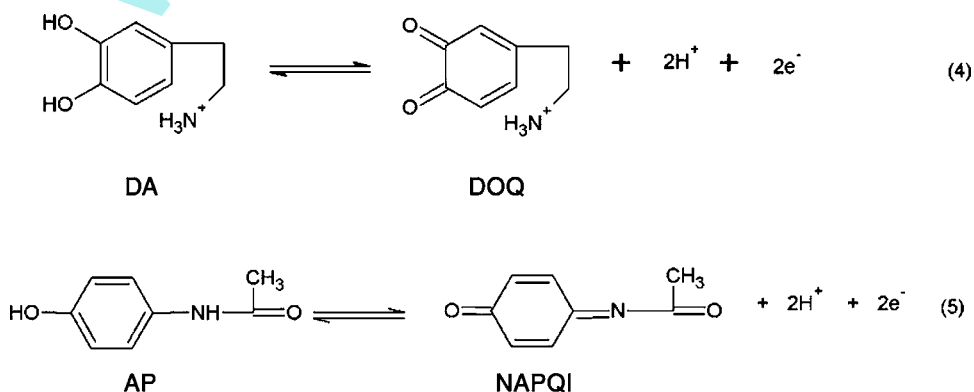
peak current and a more reversible electron transfer process to AP. A well-defined and stable redox wave of AP was observed with the anodic and cathodic peak potentials at 0.397 and 0.37 V, respectively (curve f). The separation of peak potentials at the PAY/nano-TiO₂/GC film modified electrode, ΔE_p was 27 mV, which was on accordance with a near Nernst reversible behavior and identified that the number of electrons involved in the reaction was about equal to 2.

The CV of 120 μ M AP at the bare GC electrode under identical conditions is given for comparison. As shown in Fig. 5, curve a', a highly irreversible oxidation peak is obtained at about 0.5 V, and no reduction peak appears in the reversal scan. The comparison of curves f and a' shows that the PAY/nano-TiO₂/GC film enhances the oxidation/reduction peak currents of acetaminophen remarkably and the electrode process turns more reversible, suggesting that PAY/nano-TiO₂/GC film can accelerate the electron transfer of AP and overpotential required for AP oxidation is reduced about 100 mV and increases I_{pa} four times higher than bare GC electrode.

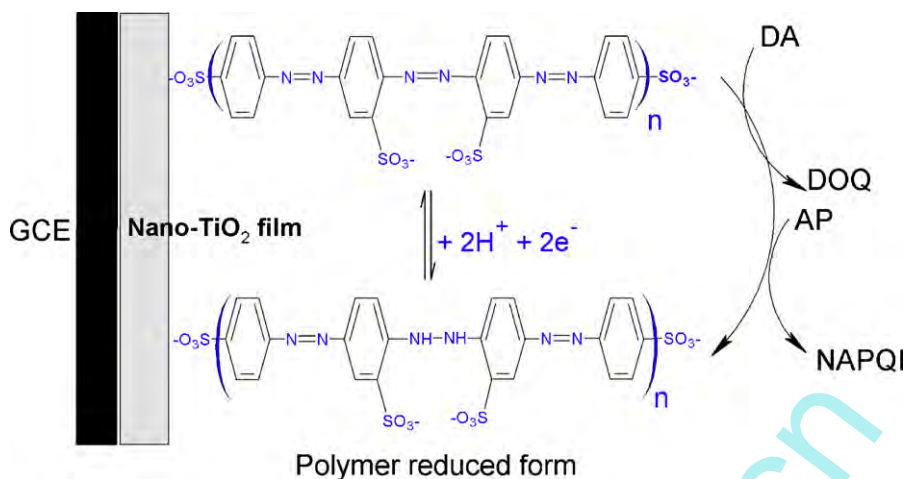
Electrochemical oxidation of AP at PAY/nano-TiO₂/GC electrode is two-electron transfer reaction, AP is oxidized to *N*-acetyl-*p*-benzoquinone imine (NAPQI) (Eq. (5) and Scheme 3) [62–64]. Further Fig. 5 (curve a–f) shows the voltammetric responses of the PAY/nano-TiO₂/GC modified electrode to the various concentrations of AP in pH 7.0 PBS. Distinct voltammetric peaks were obtained for concentrations as low as 20 μ M AP. The peak current values showed a strong linear correlation with AP concentration, $I_p (\mu A) = -0.06x - 0.5$, displaying an r^2 value of 0.994. The calibration curve exhibited a linear range up to 120 μ M, with a limit of detection (S/N = 3) for AP was 2 μ M.

3.7. Effect of interferences

In biological samples, ascorbic acid (AA), uric acid (UA) and reduced form of NADH are the most important interferences. Moreover, at bare electrodes, the oxidation of these molecules takes place at a potential close to that of DA and AP [29,37,38]. The voltam-



Scheme 2. Electrochemical oxidation of DA and AP.



Scheme 3. Electrochemical oxidation of DA and AP on nano-TiO₂/poly(AY) film.

metric responses were recorded by using PAY/nano-TiO₂/GC coated electrode in 0.1 M PBS before (curve a) and after successive additions of 10mM AA, 10mM UA and 15mM NADH (Fig. 6A curves b–d). There is no any change in anodic or cathodic peak currents of redox polymer due to above biomolecules oxidation reactions under the potential range used. It was concluded that these biomolecules are not interferences at this modified electrode. This behavior could be partly explained on the basis of the dissociation ability of –SO₃Na (H) group of poly(AY) film in different pH environments, in pH 7.0 PBS SO₃[–] groups are negatively charged and this properties makes polymer backbone to repel the negatively charged species, since AA, UA and NADH are negatively charged at pH 7 [16,18–22]. We concluded that, the PAY/nano-TiO₂/GC coated electrode can be applied for determination of DA and AP in the presences of AA, UA and NADH.

3.8. Simultaneous detection of DA and AP

The next attempt was taken to detect DA and AP simultaneously by using the PAY/nano-TiO₂/GC. The linear sweep voltammetry (LSV) results show that the simultaneous determination of DA and AP with well-distinguished two anodic peaks corresponding to their oxidation could be possible at the PAY/nano-TiO₂/GC. Fig. 6B shows the LSVs obtained at the modified electrode when the concentrations of DA and AP were simultaneously changed. The presence of PAY/nano-TiO₂/GC film resolved the mixed voltammetric response into two well-defined voltammetric peaks at potentials 0.202 and 0.39 V, corresponding to the oxidation of DA and AP, respectively. The separation between the two peak potentials is sufficient enough for the simultaneous determination of DA and AP. In addition, a substantial increase in peak currents was also observed due to the improvements in the reversibility of the electron transfer processes. LSV results show that the calibration curves for DA and AP were linear for the whole concentrations range investigated (12–120 μM for DA and 12–120 μM for AP) with good correlation coefficients. The linear regression equations for DA and AP were $I_p (\mu A) = -0.04x - 0.006$ ($r^2 = 0.963$) and $I_p (\mu A) = -0.05x + 0.09$ ($r^2 = 0.992$), respectively. Experimental results showed that the detection limit was 1.0 μM for DA and 2.0 μM for AP (S/N=3). We have tested the linear detection of DA using PAY/nano-TiO₂/GC electrode as shown in Fig. 7. PAY/nano-TiO₂/GC electrode responds with the concentration of added amount of DA. The perfect linear regression equation ($y = -x$) was found with correlation coefficient value of $r^2 = 1$. This r^2 value is very good than other electroanalytical

methods reported for detection of DA [39–45]. We believe our proposed modified electrode can be applied for selective detection of DA too.

3.9. Stability, reproducibility and repeatability of the modified electrode

Stability test for PAY/nano-TiO₂/GC electrode was tested by keeping the electrode in pH 7 buffer solutions about 1 month, after a month the CVs were recorded and compared with CVs obtained before immersion, the results indicated that 2% peak current was decreased for PAY/nano-TiO₂/GC electrode. It was concluded that PAY/nano-TiO₂/GC electrode have good stability and higher shelf-life. Good stability of the PAY/nano-TiO₂/GC electrode can be attributed to the irreversible deposition and the strong adherent of poly(AY) onto nano-TiO₂ modified GC electrodes.

A relative standard deviation (R.S.D.) of 2.3% for nine measurements of 120 μM AP ($n = 9$) suggested that the PAY/nano-TiO₂/GC electrode have good reproducibility. Six hybrid film electrodes fabricated independently, were used to determine 120 μM AP, and the R.S.D. was 3.9%, revealing an excellent repeatability of the electrode preparation procedure. The R.S.D. of 15 successive detections of 100 μM DA using a same PAY/nano-TiO₂/GC modified electrode was 1.2%, and the R.S.D. of 8 different modified electrodes based on the same fabrication procedure and determination was 2.34%. These results indicated that the PAY/nano-TiO₂/GC electrode has good reproducibility. To ascertain the repeatability of the electrode, measurement of 100 μM AP were done using the same modified electrode for 21 times at the intervals of 2 h. The R.S.D. value was found to be 2.8% which is indicated that PAY/nano-TiO₂/GC electrode has good repeatability.

3.10. Determination of AP in real samples

To ascertain the applicability of the method presented above was applied to the determination of AP in PACIN™ 650 tablets (John-

Table 1
Determination of AP in tablets

Samples	Labeled (mg/tablet)	Found ^a (mg/tablet)	R.S.D. (%)	Recovery (%)
Tablet-1	650	656.83	3.84	101.05
Tablet-2	650	662.00	2.33	101.85
Tablet-3	650	666.33	1.52	102.51

^a Average value of three measurements.

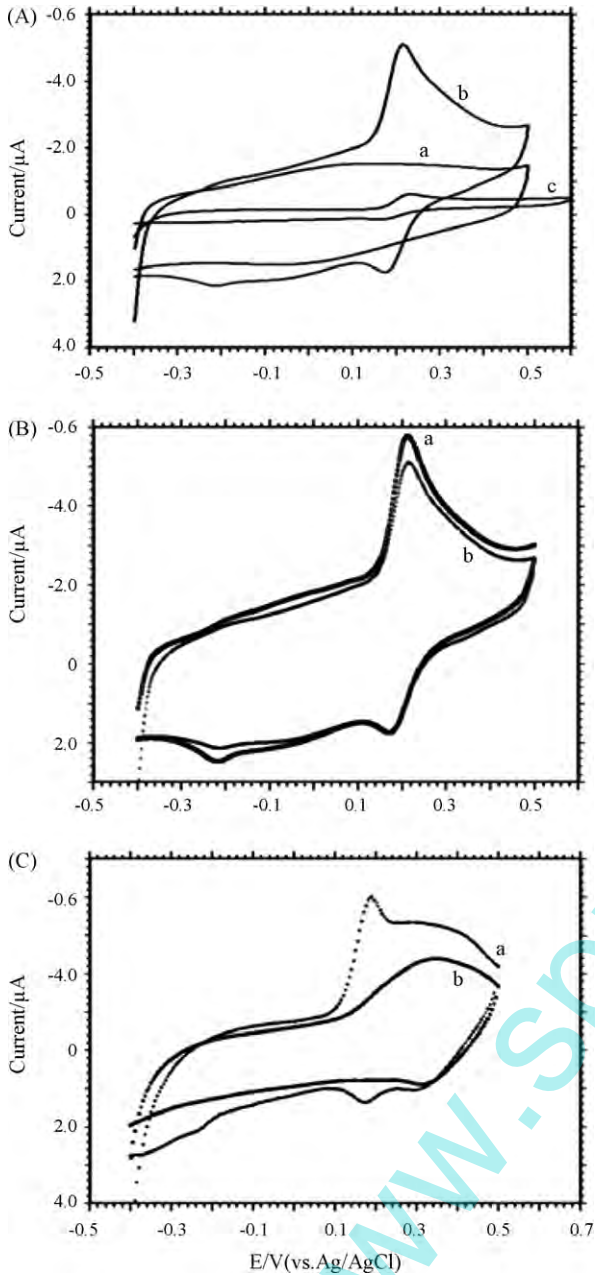


Fig. 4. (A) CVs recorded using PAY/nano-TiO₂/GC modified electrode in pH 7.0 buffer solutions in the presences of (a) 0.0 μM DA, (b) 120 μM DA and (c) at bare GC electrode. (B) CVs recorded using (a) PAY/GC and (b) PAY/nano-TiO₂/GC modified electrode in pH 7.0 buffer solutions containing 120 μM DA. (C) CVs were recorded in pH 7.0 PBS after immersion of (a) PAY/GC and (b) PAY/nano-TiO₂/GC modified electrode in 0.5 mM DA solution for an hour.

son & Smith Co., Bangalore, India). The results obtained are listed in Table 1. In our experiments, the concentration of AP was calculated using standard additions method. The R.S.D. of each sample for three time's parallel detections is less than 3.84%. In addition, the recovered ratio on the basis of this method was investigated and the value is between 102.51 and 101.05%. The recovered ratio indicates that the determination of AP using PAY/nano-TiO₂/GC electrode is effective and can be applied for detection of AP in real samples.

The linear range, detection limit, oxidation potential and pH used for detection of AP were compared with the earlier reports in Table 2. From the data's shown in Table 2, this new method could be

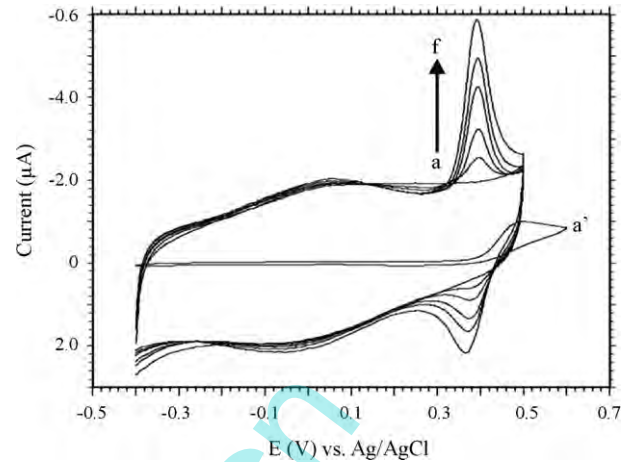


Fig. 5. CVs recorded in pH 7 PBS after each addition of acetaminophen using PAY/nano-TiO₂/GC modified electrode. (a) 0.0 μM, (b) 20 μM, (c) 40 μM, (d) 60 μM, (e) 80 μM, (f) 120 μM and (a') bare GC electrode with 120 μM AP.

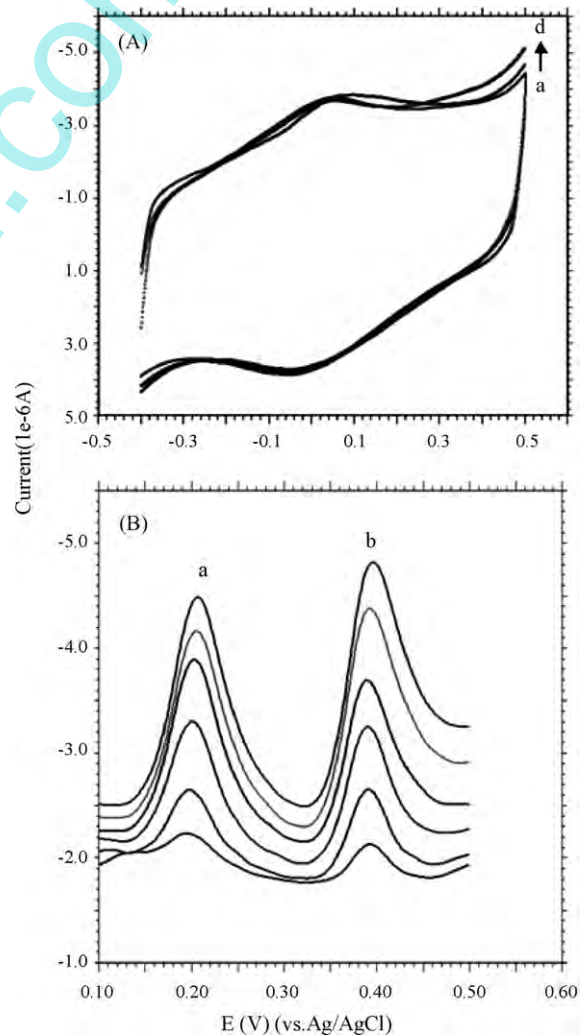


Fig. 6. (A) Typical CVs obtained with a PAY/nano-TiO₂/GC modified electrode in 0.1 M PBS (pH 7.0) with successive additions of (a) blank, (b) 10 mM ascorbic acid, (c) 10 mM uric acid and (d) 15 mM NADH solutions. (B) LSVs of simultaneous detection of DA and AP at PAY/nano-TiO₂/GC modified electrode (each additions increased 20 μM).

Table 2
Comparisons of various electroanalytical methods proposed for detection of acetaminophen

Modified electrodes	pH used	Linear range	Detection limit	Oxidation potential (V)	References
(PANI–MWCNTs) composite modified electrode	5.5	1.0×10^{-6} to 1.0×10^{-4} mol/L	2.5×10^{-7} mol/L	0.436	[64]
Tetra (<i>p</i> -aminophenyl)porphyrin nickel film electrode	Basic medium (0.5 M NaOH)	1×10^{-6} to 2×10^{-4} M	–	0.43	[62]
Multi-wall carbon nano-tube composite film modified glassy carbon electrode	5.0	5.0×10^{-7} to 1.0×10^{-4} mol/L	5.0×10^{-8} mol/L	0.4	[63]
Cu(II)-conducting polymer complex modified electrode	7.0	2.0×10^{-5} and 5.0×10^{-3} M	5.0×10^{-6} M	–	[65]
PAY/nano-TiO ₂ /GC electrode	7.0	1.2×10^{-5} to 1.2×10^{-4} M	2.0×10^{-6} M	0.397	Proposed method

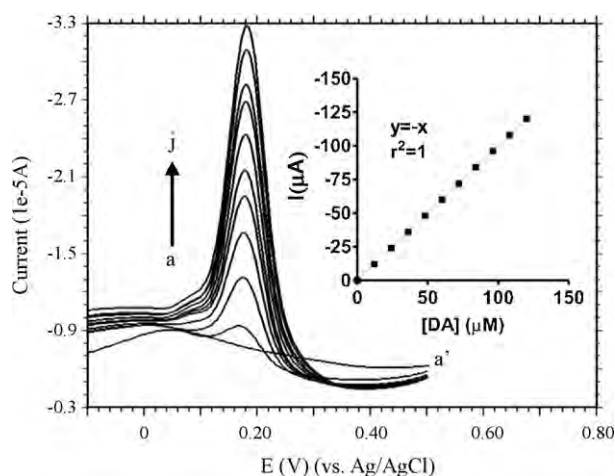


Fig. 7. Differential pulse voltammograms recorded in 0.1 M PBS at different concentration of DA using PAY/nano-TiO₂/GC modified electrode, (a') 0.0 μM, (a) 12 μM, (b) 24 μM, (c) 36 μM, (d) 48 μM, (e) 60 μM, (f) 72 μM, (g) 84 μM, (h) 96 μM, (i) 108 μM and (j) 120 μM DA.

applied for detection of AP in neutral buffer solution (pH 7) at lower oxidation potential. For the first time we demonstrated simultaneous detection of dopamine and acetaminophen in neutral solution. The major biological compounds ascorbic acid, NADH and uric acid are not interferences at this method. From our experimental data's we conclude that our proposed method can be applied for selective detection AP in the presence of DA, ascorbic acid, NADH and uric acid.

4. Conclusions

Herein, we reported a new PAY/nano-TiO₂/GC hybrid film modified GC electrode fabrication, characterizations and its applications for detection of DA and AP by cyclic voltammetry and LSV. It was found that the peak current responses of DA and AP were improved significantly and the oxidation peak shifted towards more negative potential in the presence of PAY/nano-TiO₂ hybrid film. The results indicated that the hybrid film provided a good platform for determination of DA and AP. The enhanced electrocatalytic activity of PAY/nano-TiO₂/GC towards DA and AP mainly came from the combined properties of organic polymer/inorganic nano-TiO₂ particles such as strong adsorptive ability, larger specific area. Finally, the hybrid film electrode was successfully employed for the voltammetric determination of DA and AP in neutral buffer solution. We also demonstrated that the application of PAY/nano-

TiO₂/GC for detection of AP in commercial tablets with satisfactory results.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2008.04.057.

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