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Electrochemical Sensing of Acetaminophen on Electrochemically Reduced Graphene Oxide-Nafion Composite Film Modified Electrode

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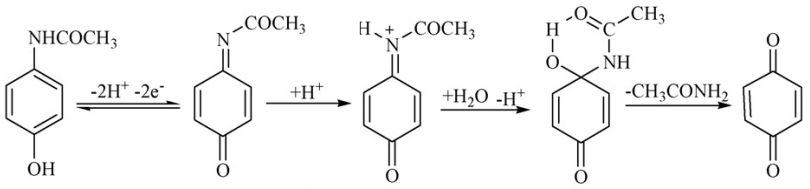
An electrochemical sensor based on graphene–Nafion nanocomposite film for voltammetric determination of acetaminophen (APAP) was presented. A Nafion graphene oxide-modified glassy carbon electrode was fabricated by a simple drop-casting method and then graphene oxide was reduced at the glassy carbon electrode surface by electrochemical method. The resulting electrode [electrochemically reduced graphene oxide (ER-GO)/Nafion glassy carbon electrode (GCE)] was used to determine acetaminophen. The electrochemical behaviors of acetaminophen on Nafion/ER-GO modified glassy carbon electrodes (GCEs) were investigated by cyclic voltammetry and square-wave voltammetry. The results obtained that the Nafion/ER-GO/GC modified electrode was exhibited excellent electrochemical activity on the oxidation of APAP. The calibration curve for APAP was shown two linear segments: the first linear segment increases from 0.4 to 1.0 and second linear segment increases up to 10 µM. The detection limit was determined as 0,025 µM (2.5×10^{-8} mol L^{-1}) using SWV. Finally, the proposed method was successfully used to determine APAP in pharmaceutical preparations and urine samples.

Keywords: Acetaminophen, Voltammetry, Graphene, Nafion, Urine Analysis, Pharmaceutical Analysis.

1. INTRODUCTION

Acetaminophen (4'-hydroxyacetanilide, *N*-acetyl *p*-aminophenol, paracetamol) (APAP) is a very popular analgesic and antipyretic drug, it is non carcinogenic and an effective substitute of aspirin for patients who cannot tolerate aspirin [1]. APAP is rapidly and extensively metabolized by

undergoing glucuronidation and sulfation to inactive metabolites which are eliminated in urine along with 5% of APAP being eliminated unchanged. At the recommended dosage, there are no side effects. However, overdoses cause liver and kidney damage. It is suspected that a metabolite of acetaminophen is the actual hepatotoxic agent [2].



Scheme 1. Electrooxidation of APAP in acidic media.

To date, a variety of methods such as high performance liquid chromatography (HPLC), spectrofluorimetry, liquid chromatography, electrospray mass spectrometry, spectrophotometry, have been developed for the determination of acetaminophen in pharmaceutical formulations and biological fluids[3]. Although these methods have advantages of sensitivity and accuracy, their high cost and complicated operation procedure limit their extensive application. Electrochemical methods are more and more widely used for the study of electroactive compounds in pharmaceutical forms and physiological fluids due to their simple, rapid, and economical properties[4]. As an electroactive substance, acetaminophen has also attracted much interest. Different electrochemical methods using various modified electrodes were also proposed for the oxidation of acetaminophen [5-11]. The cyclic voltammetric study concerning the electrochemical oxidation of acetaminophen was described in the works of Kissinger et al. [12,13]. APAP is an electrochemical oxidized in a pH-dependent two-electron, two-proton process to *N*-acetyl-*p*-quinone-imine (abbreviated NAPQI) (Scheme 1). Nematollahi et al. [13] demonstrated the electrochemical oxidation of acetaminophen in various pHs using cyclic voltammetry and controlled-potential coulometry. The rate constants were estimated by comparing the experimental cyclic voltammetric responses with the digital simulated results. The results indicated that electrochemically generated NAPQI participates in different type reactions based on solution's pH. It is hydrolyzed in strong acidic media (pH < 5) and hydroxylated in strong alkaline media (pH > 9) and also, it is dimerized in intermediate pHs (pH=5-9) [14]. Diagnostic criteria of cyclic voltammetry in accompanied by previously reported papers [15–18], indicated that the reaction mechanism of electrooxidation of acetaminophen in pHs > 9 is an ECECE mechanism. It has been reported that the stability of NAPQI was substantially affected by the sample pH [13, 14] with a highest stability in the pH range 4–9 and a half-life equal to 47 min at pH 7.4 [19,20].

Graphene represents a conceptually new class of materials that are only one atom thick, and, on this basis, offers new inroads into low-dimensional physics that has never ceased to surprise and continues to provide a fertile ground for applications [21-24]. Graphene-based modified electrodes can promote the electron transfer of analytes and enhance their response sensitivity. Kang's group investigated the electrochemical behaviors of acetaminophen on graphene modified glassy electrodes (GR/GCEs) by cyclic and square-wave voltammetry (SWV). The results showed that the GR/GCE

exhibited excellent electrocatalytic activity to APAP (pH=9.5)[25]. Bahramipur and Jalali investigated the electrochemical behavior of APAP at the graphene paste electrode [26]. Fan et al. described the Nafion/TiO_2/graphene nanocomposite (Nafion/TiO_2/GR) for APAP electrochemical sensing [27]. However, graphene films on electrodes in these researches are usually prepared by chemical reduction of graphene oxide (CR-GO) sheets [25–27]. Recently, electrochemical reduction of graphene oxide (GO) to graphene, which has been reported by several research groups [28-30], has arisen more interest due to its fast and green nature.

In this manuscript, a simple, economical, and *environmentally friendly method* was used to fabricate Nafion/ER-GO-modified glassy carbon electrode by applying a constant cathodic potential on GO/glassy carbon electrode (GCE), and a new electrochemical sensor for sensitive and selective determination of APAP using the Nafion/ER-GO-modified electrode was demonstrated.

2. EXPERIMENTAL

2.1. Apparatus

The voltammetric experiments were performed in an electrochemical assemble with a platinum wire as the counter electrode, a glassy carbon electrode (3 mm diameter) as working electrode and saturated calomel (SCE) reference electrode. Cyclic voltammetry (CV) experiments were carried out with a Gamry Reference 600 potentiostat (Gamry, USA). All experiments were performed at room temperature (25^{o}C). Before each experiment, the working electrode was polished with slurry containing 0.3 μm and then 0.05 μm sized aluminum oxide particles for 5 min. After each treatment the electrode was washed and ultrasonicated in distilled water for 5 min to remove retained aluminum oxide particles on the electrode surface. The pH values of the solutions were measured by a Hanna HI 221 pH-meter using the full range of 0-14.

2.2. Reagents and materials

All solvents and reagents were of analytical grade. Acetaminophen and *p*-aminophenol were purchased from Sigma (St. Louis, MO, USA), and they were all used as received. Aqueous stock solution of 1.0×10^{-2} mol L^{-1} acetaminophen (APAP) was used for further preparation of final solutions. A 1.0×10^{-2} mol L^{-1} *p*-aminophenol (4-AP) stock solution was prepared with ethanol. Both standard stock solutions were stored in a 4^0C refrigerator. Before use, all sample solutions were prepared by appropriate dilutions to the desired concentration with distilled water. A commercially available 5 wt% Nafion® solution in water-isopropanol was obtained from solution Technology Inc. (equivalent weight 1100 g/mol sulfonic acid groups). A 1.0 wt% Nafion solution was prepared by diluting the 5 wt% Nafion stock solution with isopropyl-alcohol. Graphene oxide was synthesized from graphite according to the Hummers and Offeman's method [31,32]. Scanning electron micrographs (SEMs) were obtained using an FEI - QUANTA FEG 450 SEM. Graphite and graphene oxide crystallographic structures were analyzed using a Rigaku D/max-2200 Ultima X-Ray

diffractometer with Cu Kα radiation. Graphene used in this experiment was prepared through electrochemically reduced graphene oxide (GO). All the measurements were carried out at *room temperature*.

2.3. Preparation of the graphene modified electrode

Prior to the surface coating, the bare GCE (*d* =3 mm) was polished with 0.05 μm gamma alumina powder, rinsed ultrasonically with ethanol:propanol (1:1) and distilled water, respectively, and dried at room temperature. Graphene oxide (GO) was dispersed in distilled water (1.0 mg mL^{-1}) with ultrasonication for 30 min or until fully dispersed. A 50 μL of 1 mg mL^{-1} GO solution was mixed with 10 μL of 1.0 wt % Nafion–isopropyl-alcohol solution by ultrasonication for ca. 30 min. Then, an aliquot of 10 μL of the mixture was coated on the glassy carbon electrode (GCE) to obtain the Nafion/GO/GCE electrode. The solvent was allowed to evaporate at room temperature for 2 h. After drying in air, a Nafion/GO/GCE was obtained. Subsequently, the Nafion/GO/GCE was immersed into 0.02 M KH_2PO_4 solution, and a cathodic potential of −0.7 V was applied to the GO/GCE by using potentiostat for about 10 min [33]. By this procedure, Nafion/ER-GO/GCE was prepared. Then, it can be used for electrochemical sensing of acetaminophen.

2.4. Experimental procedure

Under experimental conditions, various concentrations of APAP were investigated by CV and SWV in a ammonia buffer solution (pH 9.0). A three-electrode system was used, including a Nafion/GR/GCE as the working electrode, a platinum wire electrode as a counter electrode, and Ag/AgCl as a reference electrode. Cyclic voltammograms (CVs) and square-wave voltammograms (SWVs) of APAP were recorded. CV was performed at a scan rate of 50 mV s^{-1} in the potential range from 0.000 to 500 mV. Scan parameters were square-wave mode, step: 8 mV, amplitude: 50 mV, frequency: 50 Hz.

3. RESULTS AND DISCUSSION

3.1. XRD and SEM characterization

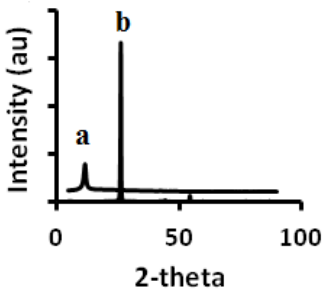


Figure 1. XRD pattern graphite (a) and grahene oxide (b).

XRD patterns of flake graphite and graphene oxide are shown in Figure 1, respectively. The characteristic peak patters of graphite has centered at 2θ=26.4° and 54.6°. The interlayer d-spacing of 2θ=26.4° is 3.37 Å. After oxidation the characteristic peak of graphite interlayers dissappared, but a new peak at 2θ=11.8° with d-spacing of 7.49 Å is observed. This indicates graphene oxide formation after oxidation of graphite [34-37].

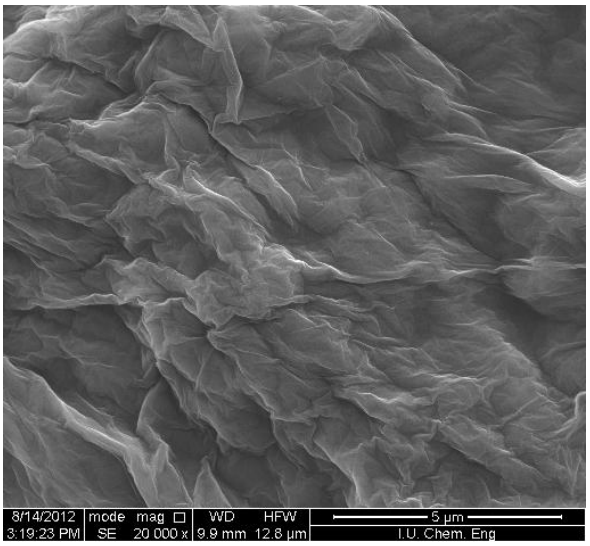


Figure 2A. SEM image of GO film on the GCE surface.

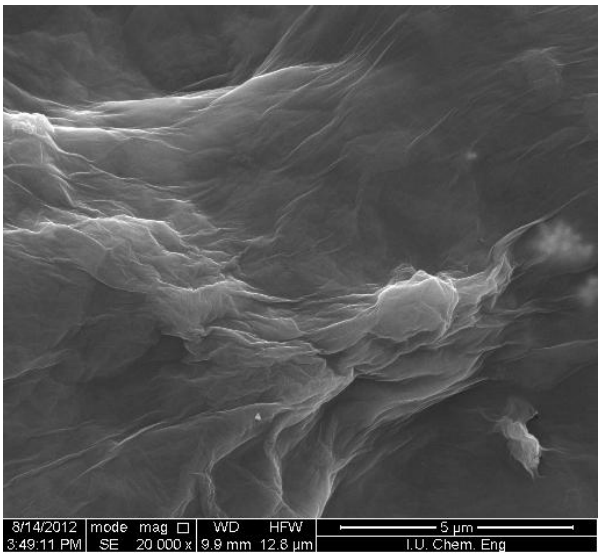


Figure 2B. SEM image of Nafion/ER-GO nanocomposite film on the GCE surface.

The morphologies of GR without Nafion and GR with Nafion (GR:Nafion=5:1) on electrode surface were observed by SEM. Fig. 2A and Fig 2B shows the SEM image of GO film and Nafion/ER-GO film on the surface of the GCE, revealing the typical crumpled and wrinkled graphene sheet

structure on the rough surface of the film. The prepared Nafion-graphene films possess smooth and homogeneous surfaces.

3.2. Effect of amount of Nafion graphene oxide mixture

We investigated GO-to-Nafion ratio ranging from 5:1 to 1:1 by CV. In this study, the best electrochemical behavior was obtained on the GO:Nafion surface with the ratio of 5:1. It was found that for GO-to-Nafion ratios higher than 5:1, the current peak signal of APAP was reduced gradually. Various Nafion/GO-modified GCE electrodes were prepared by placing different volumes of Nafion/GO solution in the range 2–10 μL at bare GCE surface. The peak current of Nafion/GO/GCE clearly increased as the amount of GO at a GCE from 5 to 10 μL increased. Further increase in the Nafion/GO volume decreased the current response slightly. The volume of homegenous suspension of Nafion/GO on the surface of the GCE was kept constant at 5 μL throughout of our experiments. It was proposed that the Nafion adsorbed onto the graphene by the hydrophobic interaction of its fluoro-backbones with the graphene layer and imparted stability by an electrosteric mechanism [38].

3.3. Electrochemical behavior of APAP

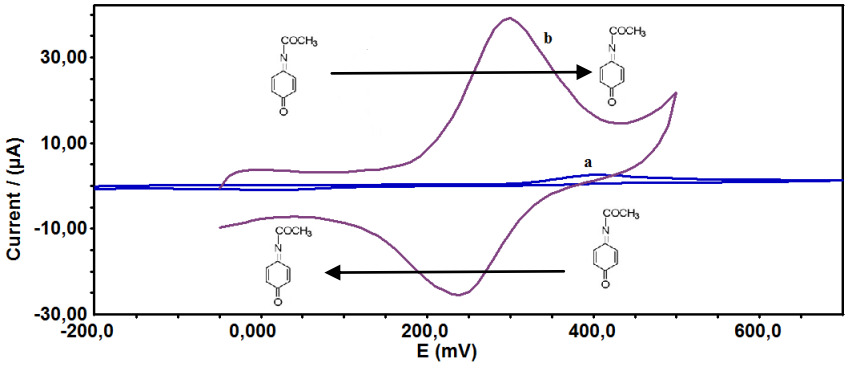


Figure 3. CVs of 0.1 mM parcetamol on the bare GCE(a), Nafion/ER-GO-GCE (b) in a 0.1 M ammonia buffer (pH 9.0), Scan rate: 50 mV s^{-1}

A cyclic voltammetry (CV) was used to investigate the electrochemical behavior of APAP on the bare GCE, Nafion/ER-GO/GCE in 0.1 M ammonia buffer solution at a scan rate of 50 mV s^{-1}, respectively. Fig. 3 depicts cyclic voltammograms of APAP on the bare GCE and Nafion/ER-GO/GCE in 0.10 M ammonia buffer solution (pH 9.0). At the bare GCE (Fig. 3a), acetaminophen shows an irreversible behavior with relatively weak redox current peaks at E_{pa} (anodic peak potential) = 0,400 V and E_{pc} (cathodic peak potential) = 0.013 V. The peak potential separation ($\Delta E_p = E_{pa} - E_{pc}$) was as large as 387 mV (Fig.3a). On the Nafion/ER-GO/GCE, the anodic and cathodic peak currents of APAP are significantly increased. The voltammogram shows an anodic peak in the positive-going scan and a cathodic counterpart peak in the negative-going scan which corresponds to the transformation of acetaminophen to N-acetyl-p-benzoquinone-imine (NAPQI) and vice-versa within a quasi-reversible

two-electron process [13,14]. According to the experimental results, the oxidation peak of APAP shifted negatively to 297 mV, and the reduction peak shifted positively to 238 mV at the Nafion/ ER-GO/GCE (Fig. 3b). The value of ΔE_p decreased to 59 mV, clearly indicating that the oxidation of APAP become more reversible at the Nafion/ER-GO/GCE. According to Nicholson's theory [39], the decrease of ΔE_p indicated that the electron transfer rate increased. So, significantly increased redox peak currents, reduced oxidation potential and greatly increased electron transfer rate of APAP at the Nafion/ER-GO/GCE. As can be seen in Fig 3, oxidation peak signal significantly increases to 36 μA, which is 9 (36:3=12) times higher than that on Nafion/ER-GO/GCE. These results demonstrated that the electrochemical reactivity of APAP is remarkably improved on the Nafion/ ER-GO/GCE.

3.4. Redox mechanism of APAP at the Nafion/ ER-GO /GCE

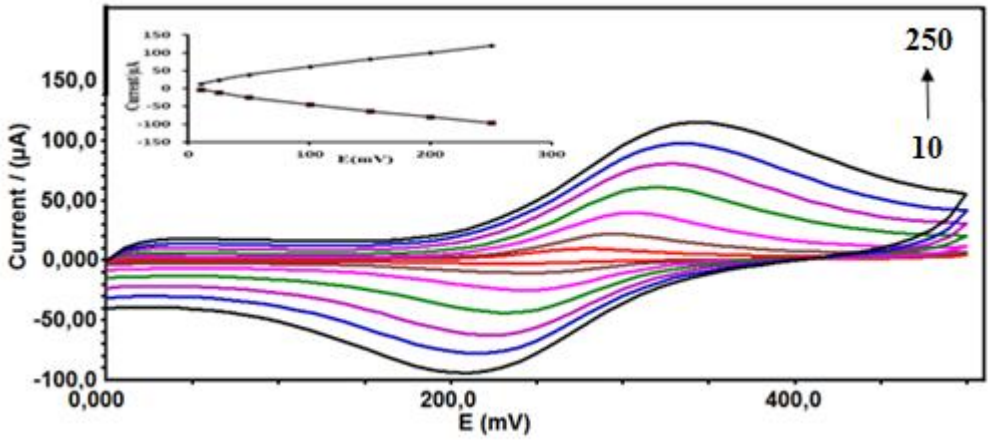


Figure 4. CVs of 0.1 mM PCT on Nafion/ER-GO/GCE at different scan rates from 10 to 250 mV/s. Inset is the plot of peak current vs. scan rate.

The effect of scan rate (v) on the oxidative and reductive peak currents of on the Nafion/ ER-GO /GCE was investigated by cyclic voltammetry. As shown in Fig. 4, the anodic and cathodic peak signals increased with the scan rate, which were linearly proportional to scan rate in the range from 10 to 250 mV s^{-1}. The anodic peak potentials shift in gradually positive direction and the cathodic peak potentials shift in negative direction. The linear relationship between the peak current and scan rate could be expressed by the linear regression equation as: I_{pa} (μA)= 0.4368 v/mV s^{-1}+ 13,443 (R^2 = 0.9928) and I_{pc} (μA)= -0.3849 v/mV s^{-1}- 0,3133 (R^2 = 0.9925). The results indicated that the electrochemical redox reaction of APAP on the Nafion/ ER-GO /GCE was a surface-controlled process. The logarithm of the scan rates were expressed as E_{pa}= 0.2417 + 0.0382 log v (R^2 = 0.9967) and E_{pc}= -0.2659-0.0137 log v (R^2 = 0.9959). According to Laviron's equation [40], the slope of the lines are equal to RT/(1−α) nF and −(RT/αnF) respectively. Therefore, the value of the charge-transfer coefficient (α) and the electron-transfer number (n) were estimeted to be 0.61 and 1.83, respectively.

3.5. Electrochemical characterizations of acetaminophen in different pH

The effect of solution pH on the electrochemical redox reaction of APAP on the Nafion/ ER-GO /GCE was investigated in the range of pH 2-13. Fig. 5 shows the cyclic voltammograms of acetaminophen in various pHs (2.0 to 13). Figure 6 in low pH (pH 2.0) response of oxidation process resulted in irreversible oxidation peak about 695 mV. This is expected because of the participation of proton(s) in the oxidation reaction of APAP to N-acetyl-p-benzoquinone-imine (NAPQI). Fig. 6 shows the cyclic voltammograms of Nafion/ ER-GO /GCE in acidic media (pH:2.0) containing 0.1mM APAP with different scan rates from 50 to 500 mV s^{-1}. At the scan rate of 50 mV s^{-1}, a cathodic peak current for the reduction of protonated NAPQI is not observed.

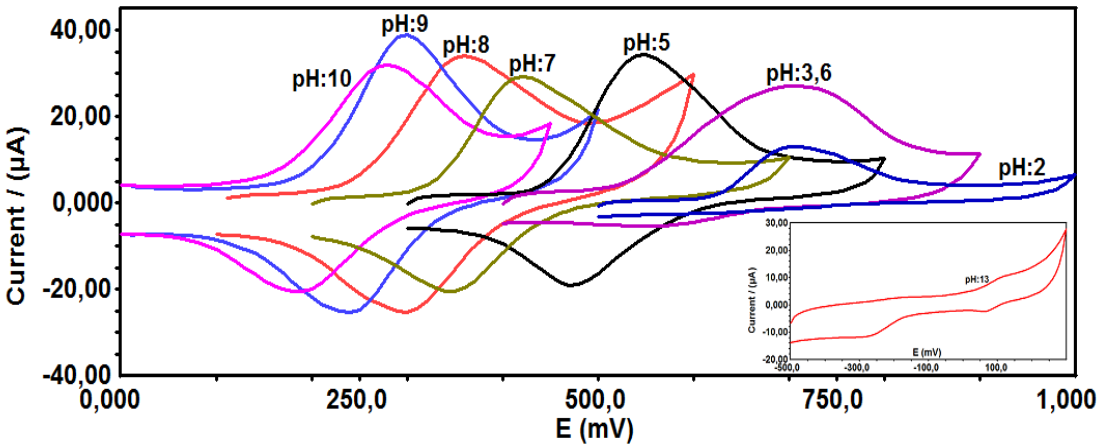


Figure 5. Cyclic voltammograms of 0.1mM acetaminophen at Nafion/ER-GO/GCE, in buffer solution with various pHs. pH from a to g are: 2.0, 3.6, 5.0, 7,0, 8.0, 9.0 and 10.0. Scan rate: 50 mV s^{-1}. The inset shows the corresponding CV of the Nafion/ER-GO/GCE towards APAP at pH=13.

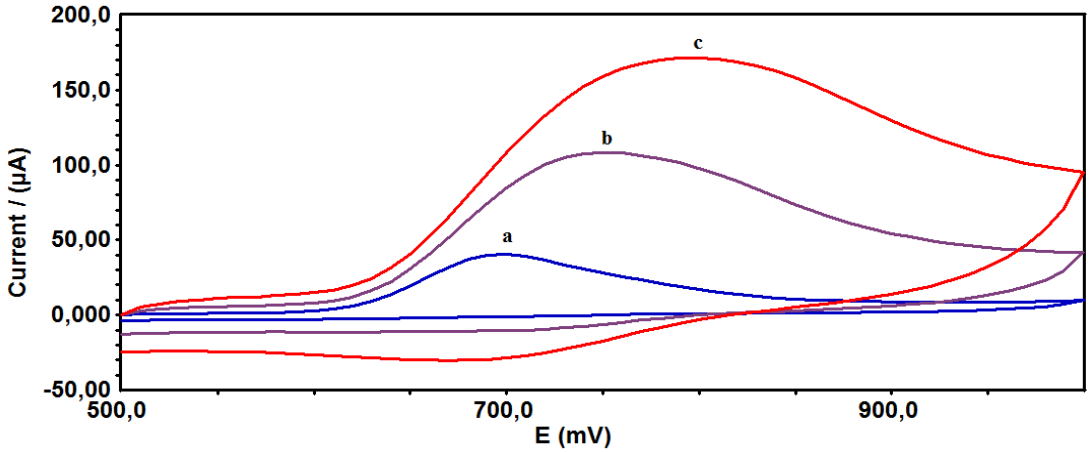
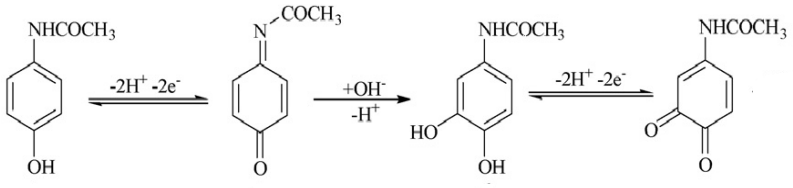


Figure 6. Cyclic voltammograms of 0.1 mM APAP at Nafion/ER-GO/GCE, in phosphate buffer solution (pH = 2.0). Scan rate: (a) 50 mVs^{-1}, (b) 250 mVs^{-1} and (c) 500 mVs^{-1}.

A small cathodic peak current due to the reduction of protonated NAPQI is evident when the scan rate of 250 mV s^{-1} is employed. The presence of the cathodic peak I_{pc} strongly depends on the potential scan rate. In this experimental condition, NAPQI product under protonation reaction changed to NAPQI-hydrate. Because the hydrated NAPQI is electrochemically inactive within the potential range studied, it is not possible to convert the hydrated NAPQI back to APAP by reducing it. A poorly defined cathodic peak for the reduction of *p*-benzoquinone is observed when scan rate of 250 mV s^{-1} is employed. The cathodic peak is broad because the formation of *p*-benzoquinone from hydrated NAPQI occurs during the reverse scan. The mechanism presented in Scheme 1 for the electrooxidation of APAP in acidic media. Under these experimental conditions, the peak current ratio (I_{pc}/I_{pa} , 50 mV s^{-1}) is about 0.0, while, in higher scan rate (250 mV $s^{-1}$and 500 mV s^{-1}) this value reaches to 0.10 and 0.18. This result indicates the reactivity of electrochemically produced NAPQI. The result obtained is in agreement with the results of Kissinger et al. [13], Nematollahi et al. [14] and Li and Chen [41]. It was found that the peak potentials for peak I_{pa} shifted to the negative potentials by increasing pH (3.6 to 10) (Fig. 5). Cyclic voltammetry of APAP (0.1mM) in aqueous solution containing 0.1 M phosphate buffer (pH=7.0) shows one anodic and the corresponding cathodic peak, which corresponds to the transformation of APAP to NAPQI and vice-versa within a quasi-reversible two-electron process (Fig. 5) [13,14]. Under these experimental conditions, the peak current ratio (I_{pc}/I_{pa}, 50 mV s^{-1}) is about 0.57. The quinoneimine oxidation product of APAP, NAPQI, is a highly *reactive electrophile*. In general, NAPQI has two possible reaction sites, namely, the 2- and 3-substitution positions. NAPQI is hydroxylated in strong alkaline media. When pH was higher than 9.0, the *current* value of the *peak current* of APAP decreased with the increasing of pH. Fig. 5 (inset) shows cyclic voltammograms of acetaminophen (0.1mM) in alkaline solution (pH = 13.0). In scan rate 50 mV s^{-1}, the voltammogram exhibits one anodic peak in the positive-going scan and two cathodic peaks (69 mV and –261 mV versus Ag/AgCl).



Scheme 2. Electrooxidation of APAP in basic media.

There is a strong relation between basicity and instability of NAPQI. This is expected because of the participation of hydroxide ions in reaction mechanism (EC mechanism or ECE mechanism) [13,41]. The mechanism presented in Scheme 2 for the electrooxidation of APAP in basic media. On the other hand, the formal potential $E^{0'}$ changed linearly as a function of solution pH in the range of pH 7.0–9.0 with the linear equation as: $E^{0'}/V = -0.0596\ pH + 0.8122$ (R = 0.9933). Based on the equation $dE_p/dpH = 0.059\ \chi/\alpha n$, the proton number ($\chi$) was estimated about 1. Thus, the redox reaction of APAP on the Nafion/ER-GO/GCE is a one-proton and two-electron process. Our experimental results were in

agreement with the *literature reports* [17,18, 20,41]. In this work, ammonia buffer solution (0.1 mol L^{-1}, pH=9.0) was used as supporting electrolyte for further experiments.

3.6. Voltammetric determination of acetaminophen

In order to obtain higher sensitivity, square-wave voltammetry (SWV) was used to determine acetaminophen. Figure 7 depicted the SWV curves of different concentration of acetaminophen at Nafion/ ER-GO /GC modified electrode. As can be seen, the SWV peak current increased linearly with acetaminophen concentration. The calibration curve for APAP shows two linear segments: the first linear segment increases from 0.4 to 1.0 μM with linear regression equation of I_p/μA=19.976 C (μM) - 2.875 (R^2=0.9982), and second linear segment increases up to 10 μM with linear regression equation of I_p/μA=4.8828 C (μM) + 12.953 (R^2=0.9959). The limit of detection, defined as $C_L=3S_{y/x}/b$ [42] (where $S_{y/x}$ is the standard deviation of y-residuals and b is the slope of the calibration plot) was 0.025 μM, which was lower than that at GR modified GCE (0.032 μM) [25], GR modified carbon paste electrode (0.6 μM) [26], Nafion/TiO_2-GR/GCE (0.21 μM) [27]. Compared with the pure graphene modified GCE [25,26] and Nafion/TiO_2-GR/GCE [27], the electrocatalytic activity of the Nafion-graphene hybrid material was further improved possibly due to the enhanced electron transfer in the Nafion-graphene hybrid system.

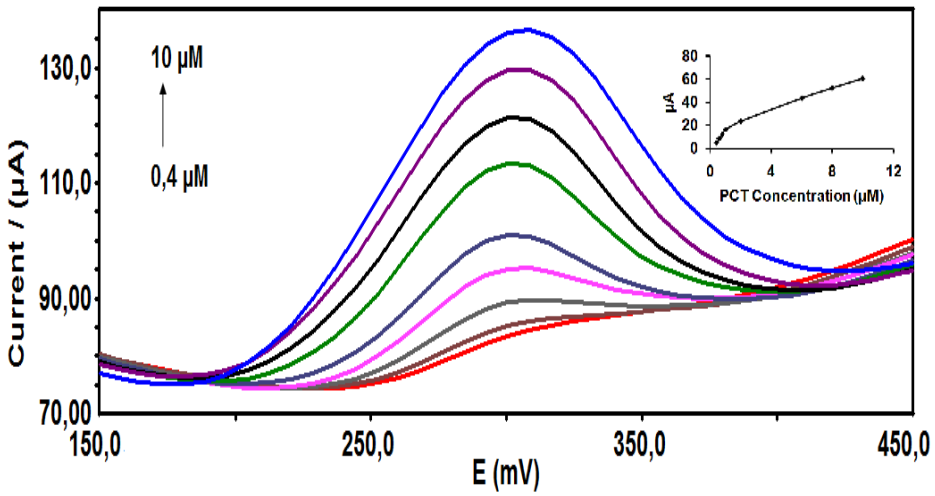


Figure 7. SWV on Nafion/ER-GO/GCE for different acetaminophen concentrations: 0.4, 0.6, 0.8, 1.0, 2.0, 4.0, 6.0, 8.0, and 10 μM in 0.1 M ammonia buffer. Inset is the relationship of current responses to acetaminophen concentration.

3.7. Interference experiments

The electrochemical behaviors of the coexisting electroactive species, which often cause serious interference with the determination of APAP, such as *p*-aminophenol, ascorbic acid, glucose, urea, dopamin, were investigated by SWV. The tolerance limit was defined as the concentration ratio

of additive APAP causing less than ±5.0% relative error. The modified electrode could virtually eliminate the interference of *p*-aminophenol, ascorbic acid, glucose, dopamin and uric acid at 500-fold concentration of APAP (at 1.0 μM), and it has been satisfactorily used for the voltammetric determination of APAP. The influence of some inorganic ions on the determination of APAP was studied. The result showed that 500-fold of Zn, Mn, Co, Cu Al, Cr did not interfere with the oxidation signal of 10 μM APAP (peak current change ±5.0%).

3.8. Repeatability and stability

The repeatability and stability of this assay were also investigated. The repeatability of the Nafion/GR/GCE was evaluated as the following process. The repeatability of experiments were performed in 0.1 M pH 9.0 ammonia buffer solution containing 1.0 μM acetaminophen. Ten-time measurement of peak current was carried out using the same electrode that was refreshed before each measurement. The process for refreshing the electrode can be described as follows: continual CV scans were preformed in a blank pH 9.0 ammonia buffer until no redox peak current of acetaminophen can be observed. The relative standard deviation (RSD) was calculated to be 3.4%, indicating that the as-prepared Nafion/ER-GO/GC electrode has a good repeatability. Furthermore, when the modified electrode was stored in refrigerator, no significant change in the response was observed for more than two weeks.

3.9. Determination of APAP in tablets

Table 1. Determination results of APAP in drug formulations by SWV (n=5).

Sample Proprietary name	Composition	Added (mg/tablet)	Proposed method* Found (mg/tablet)	Referance method* Found (mg/tablet)	Recovery (%)
Termalgin (Novartis)	Acetaminophen (500 mg) Cafein (30 mg)	- 50 100	498±3.3 546±4.0 601±2.9	501±3.8 $F_{exp.}$=1.33 $t_{exp.}$=1.88	 99 100
Minoset (Roche)	Acetaminophen (500 mg) Propifenazon (150 mg) Cafein (50 mg)	- 50 100	505±2.4 557±3.5 603±3.0	503±2.9 $F_{exp.}$=1.46 $t_{exp.}$=1.37	- 101 101

Theoretical value for F is 6.39 (P = 0.05) and for t is 2.31 (P = 0.05).
*The 95 % confidence limits of the mean (n = 5).

The developed method was applied to the analysis of two different commercial acetaminophen tablets. The tablets were weighed, ground into powder. Each weighed sample was extracted with ethanol for 15 min in an ultrasonic bath, the suspension was centrifuged for 5 min, and then the

collected samples were filtered through 0.45-micrometer cellulose membranes. All the sample solutions were transferred to 100-mL flask and diluted with ammonia buffer (pH 9.0) so that the concentration of APAP lies in the range of the calibration plot, and then appropriate amounts of these diluted samples were transferred to the electrochemical cell for the determination of each species using SWV. The recovery of the SWV method was also studied to evaluate the accuracy of the method, and the results are listed in Table 1. The recoveries of the tests were in the range from 99 % to 101 %. Additionally, the obtained results of the analyses of tablets were compared statistically (by means of the Student *t*-test and the variance ratio *F*-test) with those obtained by a reference UV-vis *spectrophotometric method* [43]. The experimental results are given in Table 1.

3.10. Determination of APAP in urine samples

Recovery tests were carried out by adding certain amounts of APAP standard solutions into the two diluted urine samples of healthy specimen. Each 2.0 mL of fresh sample was taken and diluted to 10 mL with 0.1 M ammonia buffer. *The obtained results* were summarized and *presented in Table* 2.

Table 2. Determination results of APAP in the urine samples by SWV (n=5).

Urine sample	Added (μM)	Found (μM)	Recovery (%)	RSD (%)
Urine A	-	LOD<	-	-
	2	2.02	101.0	3.45
	4	3.96	99.0	2.54
Urine B	-	LOD<	-	-
	5	5.05	101.0	3.77
	10	10.09	100.1	2.97

As can be seen, the recovery for the determination of APAP added to urine samples was good. This showed that the proposed method was suitable for the determination of APAP in urine samples, too. Table 2, the recoveries of APAP at Nafion/ER-GO/GCE are in the range from 99 % to 101%, declaring that this method is effective and reliable. These results indicate that the sensor developed in this work has high sensitivity and selectivity for detecting acetaminophen in commercial tablets and urine samples.

4. CONCLUSIONS

We have demonstrated application of the Nafion/Graphene modified glassy carbon electrode for determination of acetaminophen. The resulted graphene-modified electrode also showed a better performance than pure graphene modified GCE and Nafion/TiO_2-GR/GCE [14-16]. The prepared Nafion/GR/GCE exhibited best performance as a sensor for the determination of trace APAP based on the square-wave voltammetry (SWV). Graphene hybrid material can be used as an advanced carbon-

based electrode material for the sensitive determination of APAP. Nafion/ER-GO/GCE showed a good performance for detecting APAP due to the unique properties of graphene, which increased the active surface area of electrode and accelerated the electron transfer. The composite film modified electrode was successfully employed for the voltammetric determination of acetaminophen with low detection limit, wide linear range and good selectivity. The developed method can be used for the detection of APAP and *p*-aminophenol simultaneously without interference of each other.

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