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Amperometric Determination of Acetaminophen (paracetamol) Using Graphene Oxide Modified Glassy Carbon Electrode

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In this work, a simple, disposable electrochemical sensor based on graphene oxide (GO) modified glassy carbon electrode (GCE) was reported, which was used for the determination of pain reliever acetaminophen (AC). Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy were utilized to characterize the graphene oxide. In addition, the GO/GCE displayed excellent electrocatalytic activity towards the electrochemical determination of AC. Under the optimized conditions, the sensing platform showed wide linear responses for AC from 0.1 to 430 µM with detection limits (S/N=3) of 21 nM. The proposed sensor displayed good selectivity, sensitivity, and stability. Furthermore, GO modified electrode was also applied to detect practical samples with satisfactory results.

Keywords: painkiller, acetaminophen, graphene oxide, modified electrode, electrochemical sensor,

1. INTRODUCTION

Acetaminophen (paracetamol: N-acetyl-P-aminophenol) is widely used for fever and pain relief [1]. Since of its increasing use, expelled AC plus its dumping in sewages from engineering industries rises its existence in basis water systems. The existence of AC with concentration levels in little hundreds to some thousands of ng l^{-1} has been stated [2], thus creating it a fast-growing, emergent water problem global [3]. Additional, growing quantities of AC in intake water might cause augmented humanity, and heart, gastro-intestine, and kidney sicknesses [4]. Too, overeat and toxic-dose of AC cause not only hepato- and nephro-toxic things on healthiness [5], but also pollute water. Therefore,

the checking of AC in water is imperative. Nevertheless, observing is imperfect by the sensing assets and data, and it needs typical sampler with the present analytical methods. Surviving analytical methods to determine AC in pharmacological medicines and living fluids consist of titrametry [6], spectrophotometry [7], HPLC [8], GC [9], and electro-chemiluminescence [10] which need abstraction routes afore discovery. Although these systems compromise great sensitivity, selectivity and accuracy, their trouble in use, complex process and high-cost prevent their extensive application. The electrochemical methods are hopeful for the reason that the construction easiness and minor foot print of the sensors, great selectivity and sensitivity, rapidity, broad working range, and artless tester preparation [11–14]. Recently, numerous nanomaterials modified electrode were reported as an AC sensor including, metallic nanoparticles [14], conductive polymers [15] or their combinations [16].

During past decades carbon based nanomaterials, especially graphene fascinates plentiful courtesy in constructing electrochemical sensor and biosensors [17,18,19] owing to their exclusive properties including excellent thermal and mechanical properties, huge surface-to volume relation and great electrical conductivity. Graphene has been reported as an auspicious micro-environment for augmenting the DET of redox enzymes including glucose oxidase [20], cytochrome C [21] and hemoglobin [22]. Though, graphene is hydrophobic and tend to form unalterable agglomerates or even restack to form graphite over strong π-π stacking or Van-der Waals communication [23]. Graphene oxide (GO), oxidative derivative of graphene and it possesses worthy biocompatibility, great electronic structures and hydrophilic naturally different from other graphitic structures [24]. Comparatively, the originator for making the chemically reduced graphene, GO displays decent hydrophilicity and dispersibility in water since it comprises a huge number of hydrophilic functional groups, including -OH, -COOH and epoxides on the basal plane and the edges of the sheet. Though the electrical conductivity of GO is not equal to graphene, however GO is an appropriate nominee for biosensing examination because its nano-scale outcome, superficial properties and robust attraction with biomolecules [25]. For instance, based on its fashionable association with single-stranded DNA somewhat than double-stranded DNA, it has been browbeaten as fluorescent platform for DNA sensing [26]. Moreover, it has been functional as active nanocarriers to develop electrochemical biosensor [27] and immunosensor [28].

In the present work, we develop a facile, cost effective, sensitive and selective electrochemical AC sensor based on GO reformed glassy carbon electrode (GCE). The fabricated electrode is found that AC could be sensed from 0.1 to 430 μM with limit of detection of 21 mM. The developed sensing platform displayed great sensitivity, and selectivity with satisfactory reproducibility. The GO/GCE presented superb recovery grades for the determination of AC in human blood serum samples, enlightening its worthy sensibleness.

2. EXPERIMENTAL SECTION

2.1. Materials and methods

Pristine graphite, acetaminophen (paracetamol), dopamine, ascorbic acid, epinephrine, norepinephrine, nitric acid, and sulphuric acid, were purchased from Sigma- Aldrich. The phosphate

buffer solution (PBS) was prepared by using 0.05 M Na_2HPO_4 and NaH_2PO_4 solutions in doubly distilled water (DDW). All other chemicals were of analytical grade. Cyclic voltammetry (CV) and amperometric (i-t) measurements were carried out by CHI 750a electrochemical work station from CH Instruments. Scanning electron microscopy (SEM) was performed on a HITACHI S-3000H under an accelerating voltage of 20 kV. FT-IR spectra were recorded by using the model JASCO FT/IR-6600 spectrophotometer. Raman analysis were carried out at a Raman spectrometer (Dong Woo 500i, Korea). Electrochemical readings were realized in a conventional three electrode cell system using glassy carbon electrode, saturated Ag/AgCl platinum wire as a working electrode, counter electrode and reference electrode, respectively.

2.2 Synthesis of graphene oxide (GO) and preparation modified electrode

The water dispersible graphene oxide was synthesized through a modified Hummer's method [29]. 1 g graphite flakes and 0.5 g sodium nitrate were added to the 23 mL concentrated sulfuric acid and stirred for 30 min in an ice bath. Before, 3 g $KMnO_4$ was gradually added and allowed to stirring for 2 h. Later, it was heated to 35 $^{\circ}$C and sustained for 30 min, followed by gentle addition 46 mL water, rising the temperature upto 98 $^{\circ}$C and maintain it for 15 min to fully oxidize graphite. Next cooling with water-bath, the reaction mixture was dilute with 140 mL DDW and 3 mL H_2O_2 (30%). Afterward, the combination was cleaned by filter, multiple washing with 5% HCl and water, centrifugation and decanter, the precipitate was dialyzed for 7 days in DDW to remove the residual acid until the pH was close to 7. Lastly, the crude was dehydrated by vacuum overnight at room temperature. The standardized GO dispersion was attained by dissolving 2 mg GO in 2 mL DDW with ultrasonication for 30 min.

Before the electrode modification, glassy carbon electrode was successively refined with 0.1 and 0.05 µm alumina powder, washed with DDW and ethanol. Then the electrode was rinsed with DDW, followed by acetone. Subsequently 6 µL as-prepared GO dispersion was drop coated on the surface of the GCE, allowed drying at room temperature. Thus, GO/GCE was prepared, is used electrochemical experiments.

3. RESULTS AND DISCUSSION

3.1. Characterization of GO

The surface morphology of GO were examined by SEM and shown in Fig. 1A&B. As shown in Fig. 1A, GO appears that the random stacking structure of thin layer carbon characteristic of graphene oxide. It manifests homogeneous, flexible and wrinkled sheets. FT-IR spectroscopy was used to study the existing functional groups on the as-synthesized GO. Fig. 1C displays the FT-IR spectrum of GO. The spectrum of GO shows that the characteristic peaks were observed at 3500, 1635 cm^{-1}, which are corresponding to the stretching vibrations of the O-H bond and bending vibration of the water molecules. In addition, the peaks around 1734, 1410, 1216, 1046 cm^{-1} corresponding to the stretching

vibrations of C=O, C-O (epoxy), C-OH, C-O-C (alkoxy), respectively [16]. The characteristic peak at 790 cm^{-1} due to the absorption of epoxide group [11]. These findings confirmed the successful formation of GO. Raman spectroscopy is an extensively recommended technique to portray properties of the carbon nanomaterials by increase in Raman band strengths by the conjugated and C=C bonds. Fig. 1D displays the Raman spectrum of GO, the peaks at 1560 cm^{-1} and 1328 cm^{-1} agrees to the G band (E2g symmetry) of sp^2 hybridized graphitic structure and the D band of sp^3 disordered carbon, respectively [18]. This consequence obviously indicates that the pristine graphite were effectively converted to graphene oxide.

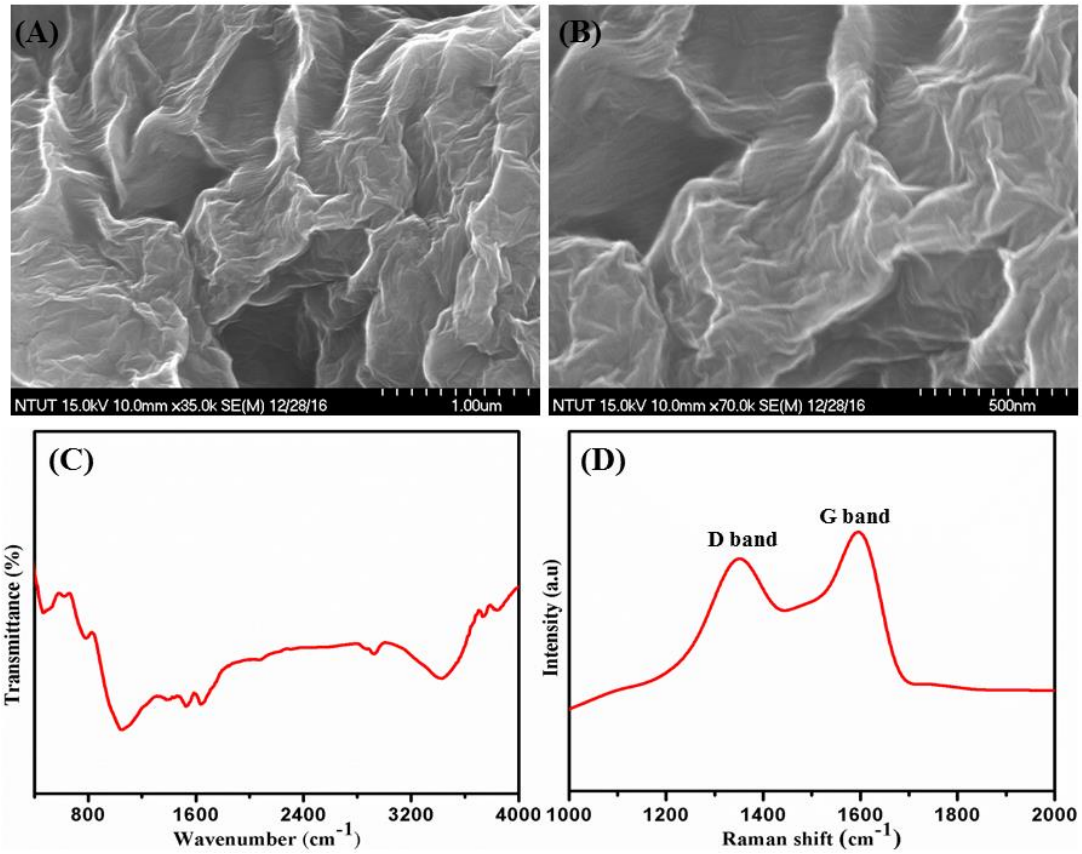


Figure 1. SEM images (A&B), FT-IR spectrum (C), Raman spectrum (D) of GO.

3.2. Electrochemical detection of AC at GO modified GCE

The electrochemical performance modified electrode toward AC detection were investigated by CV. Fig. 2A displays the CVs of bare GCE (a), and GO/GCE absence of 500 µM AC (b), GO/GCE in the presence 500 µM AC (c) in 0.05 M PB solution (pH 7.0) at a scan rate 50 mVs^{-1}. The bare GCE show a very weak oxidative behavior toward AC at a potential of 0.62 V with an anodic current of 7.8 µA. On the other hand, GO modified SPCE shows a well-defined oxidation peak for AC at a peak potential of 0.46 V. In addition, the oxidation peak current density observed at GO/GCE for 500 µM AC is about 23.8 µA, which is about 3-folds higher than that of those observed at bare GCE, indicates that the rapid electron-transfer process on GO/GCE. The outcomes confirmed that GO/GCE possesses

excellent electrocatalytic activity for AC compared to unmodified GCE. The mechanism of electrocatalytic oxidation of AC shown in Scheme. 1.

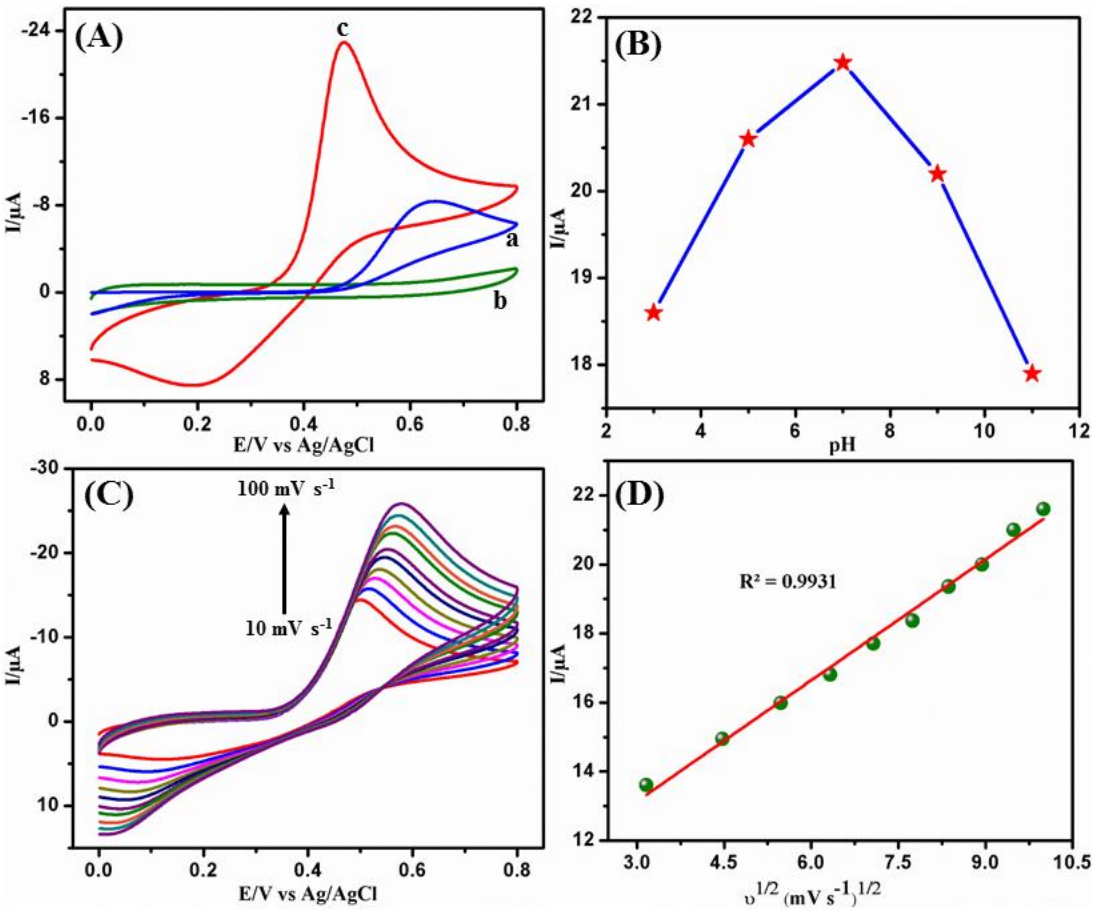
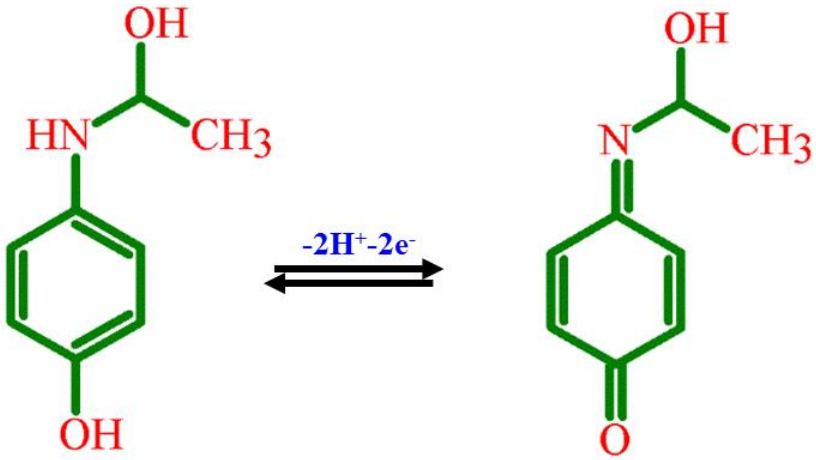


Figure 2. (A) CV of bare GCE (a), GO/GCE absence of AC (b), GO/GCE in the presence 500 µM AC (c) in 0.05 M PBS (pH 7.0) at a scan rate of 50 mVs^{-1}. (B) Plot of oxidation peak current of AC vs. pH. (C) CV responses obtained from the GO/GCE in the presence of 500 µM AC in 0.05 M PBS (pH 7.0) at different scan rates from 10 to 100 mVs^{-1} (D) Ipa vs. square root of scan rate.



Scheme 1. Electrocatalytic oxidation mechanism of AC.

3.3 Effect of pH and scan rate

The influence of solution pH on the redox peak potentials of 500 µM AC at GO/GCE was investigated by CV and the result were presented in Fig. 2B. Additionally, the utmost current response for AC was detected at pH 7.0 compared other pH, these results consistent with the earlier reports [30]. Therefore, pH 7.0 was selected as an optimal condition for succeeding experimentations. Further, the effect of scan rate on the electrocatalytic performance of AC at GO/GCE was examined. Fig. 2C displays the CVs of GO/GCE in PBS containing 500 µM AC at various scan rates from 20 to 200 mV s^{-1}. The anodic peak currents of AC escalations with growing the scan rates scan rates from 10 to 100 mV s^{-1}. Furthermore, the anodic peak current (Ipa) of AC had a linear dependence with the square root of scan rates from 10 to 100 mV s^{-1} with the correlation coefficient of 0.9931 (Fig. 2D). The result demonstrates that the detection of AC at GO/GCE is controlled by a typical diffusion controlled electrochemical process [31].

3.4 Amperometric determination of AC

Amperometric i-t method used to evaluate the sensitivity of the GO/RDE toward AC detection. Fig. 3A displays the amperometric i-t response of the GO/RDE with cumulative additions of different concentrations of AC at even time breaks into a continuously agitated (1400 rpm) 0.05 M PBS (pH 7.0) at a applied potential of +0.45 V. The oxidation current growing quickly and getting 96% of the stable state current within 5 s, indicates that the rapid electron process and superb electrocatalytic activity of GO/RDE toward AC. The obtained oxidation current had a good linear relationship with the AC concentration. Fig. 3B shows the calibration plot of AC concentration vs amperometric current response. It shows a superb linear response to the AC concentration in the range from 0.1 to 430 µM with a limit of detection of 21 nM. Furthermore, the obtained analytical performance were compared with earlier reported AC sensors and shown in Table. 1.

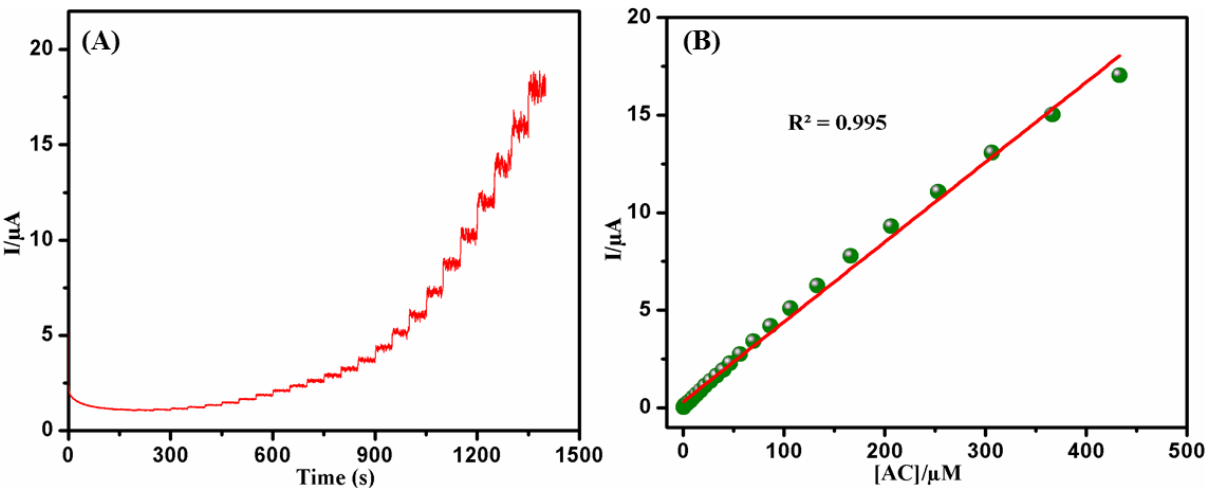


Figure 3. (A) Amperometric current response of different amount of AC at GO modified electrode, (B) calibration plot of I_{pa} vs [AC].

Table 1. Performance comparison of the fabricated electrode for AC detection with other electrodes

Sensor	**Linear range (μM)**	**LOD (nM)**	**ref**
MWCNT/GO/GCE	0.5–400	47	32
$CoFe_2O_4$/Gr/CPE	0.03–12.0	25	33
Fe_3O_4-PDDA-G/GCE	0.1–100	37	34
SWCNT–GNS/GCE	0.05–64.5	38	35
DMBQ-MCNTPE	5.0–500	1000	36
SDS-LDH/GCE	0.5–400	130	37
GO/GCE	0.1–430	21	This work

3.5. Selectivity, reproducibility, stability and real sample analysis

The anti-interference ability of the proposed sensor were studied in the presence of compounds those oxidized at near AC oxidation potential. Therefore, the selectivity of the sensor was tested by following compounds including uric acid (UA), ascorbic acid (AA), dopamine (DA), norepinephrine (NEP) and epinephrine (EP). The obtained results demonstrate that the additions of UA, AA, DA, NEP, and EP does not show any current response and did not influence the current response of AC. The consequences designate the good anti-interference of the invented sensor for the detection of AC.

The reproducibility of the proposed sensor was assessed by the detection of 500 μM AC. The relative standard deviation for detection of AC by three dissimilar electrodes was estimated to be 2.53 %, designates the applicable reproducibility of the proposed AC sensor based on GO/GCE. The long-term stability of the developed sensor was inspected occasionally up to 4 weeks. The sensor was tipped in pH 7 and stored at 5 °C, when not in use. The oxidation peak current response of 500 μM AC was observed by CV. The sensor recollects 97.8 and 93.8 % of original current response to AC after 1 and 4 weeks storage in pH 7. The outcome designates the applicable long-term stability of the sensor.

Table 2. Determination of AC in human blood serum samples using GO modified GCE.

sample	Added (μM)	Found (μM)	RR (%)
1	5	4.98	99.6
2	10	9.99	99.9

The fabricated sensor was used for the determination of AC in human blood serum samples using the standard addition method. The obtained recovery results of AC in human blood serum samples are shown in Table 2. The recovery of AC in human blood serum samples was found in the ranging from 99.6 to 99.9 %, indicates that the suitable practicality of the urbanized AC sensor.

4. CONCLUSIONS

Herein, we have reported a simple, highly sensitive and selective acetaminophen sensor using a glassy carbon electrode modified with graphene oxide. Scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy were used to characterize the GO. The GO/GCE showed an outstanding analytical performance towards the detection of AC, in terms of broad dynamic range (0.1-430 µM), very low LOD (21 nM) together with suitable long-term stability. Besides, the GO based sensor exhibited good practical applicability for the detection of AC in human blood serum samples, validates that the proposed sensor can be used for reliable determination of AC in real samples.

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