

Determination of Folic Acid in Food by Differential Pulse Voltammetry with ZnO@GO Nanocomposites Modified Glassy Carbon Electrode

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The ZnO@GO nanocomposites/GCE for electrochemical determination of folic acid was conducted in this study. ZnO nanoparticles were synthesized by the sol-gel and homogeneously mixed with graphene oxide in a weight ratio of 0:1, 1:0, 1:2, 1:1 and 2:1 and applied for modification of the GCE surface. FESEM and XRD analyses were applied for structural studies and cyclic voltammetry and differential pulse voltammetry techniques were conducted for electrochemical studies of folic acid sensors. Results showed that high porous surface ZnO@GO nanocomposite included the zinc oxide nanoparticles which homogeneously anchoring on mesoporous graphene oxide and acts as a stabilizer between the graphene oxides sheets to prevent sheet aggregation. Results of electrochemical studies showed that ZnO@GO nanocomposites with a weight ratio of 1:1 demonstrated high stability, high sensitivity and the facility in charge transfer for determination of folic acid. Results also showed that the wide linear range, high sensitivity and low limit of detection were obtained 1 to 400 μM , 0.0637 $\mu\text{A}/\mu\text{M}$ and 0.04 μM for folic acid determination, respectively. Comparison of electrochemical activity ZnO-based sensors demonstrate that the prepared ZnO@GO nanocomposites/GCE illustrated better or superior linear range and sensitivity for determination of folic acid. Analytical applicability of the ZnO@GO nanocomposites/GCE to determine folic acid in apple juice indicated that the prepared electrode was the reliable folic acid sensor in food samples due to acceptable value of recovery (more than 94.60%) and RSD (less than 4.25%).

Keywords: Folic acid detection; Differential pulse voltammetry; Analytical applicability; Apple juice; Sensitivity

1. INTRODUCTION

Folic acid ($\text{C}_{19}\text{H}_{19}\text{N}_7\text{O}_6$, (2S)-2-[(4-[(2-amino-4-hydroxypteridin-6-yl) methyl] amino) phenyl] formamido] pentanedioic acid) as organic compound is a form of a water-soluble B₉ vitamin

which is the synthetic form of Folate. This compound is necessary for some important body functions such as cell division, synthesizing the protein and nucleotide monomers for DNA and RNA [1]. There are many pharmaceutical applications of folic acid such as prevention the low blood levels of folate, hyperhomocysteinemia, miscarriage, cancer, neural tube defects, psychiatric disorders, and serious birth defects such as spina bifida and anemia [2, 3]. Moreover, other pharmaceutical applications of folic acid are for treatment of depression, stroke and Alzheimer, and also for improvement of thinking skills in older people, palpitations, fatigue and dyspnea [4, 5].

Therefore, identification and use of the naturally high in folate foods is essential in a nourish schedule which are found in spinach, broccoli, lettuce, asparagus, bananas, melons, lemons, beans, yeast, mushrooms, meat, orange juice, and tomato juice. Accordingly, the folic acid has been added to dietary supplements and food production such as cereals and flour for prevention of folate deficiency effect [6].

In accordance with social requests for folic acid application, its identity and determination were investigated through several analytical methods such as chromatography, spectrophotometry, fluorescence, thermogravimetric, ultra-performance liquid chromatography–tandem mass spectrometry and electrochemical techniques [7-11]. Among them, electrochemical techniques as low cost, simple, and high accuracy and precision techniques have attracted more attention for detection of folic acid. Nevertheless, electrochemical techniques such as applicable determination methods require optimization of the electrode constituent materials and modification of the electrode surface with porous and nano-scale structures to improve the sensing properties including stability, sensitivity, selectivity and repeatability of electrochemical response [12, 13]. These strategies change in surface chemistry electrodes and improve the electron-transfer kinetics through the enhancement of the electrode effective surface area and decreasing the electron barrier potential [14], respectively.

Accordingly, many studies were conducted to improve folic acid electrochemical sensors. For example, Moghaddam et al. [15] modified carbon paste electrode by incorporation of graphene nanosheets and a ferrocene derivative which the modified electrode showed an excellent electrocatalytic effect on the oxidation of folic acid by square wave voltammetry (SVW) technique. Wang et al. [16] modified the glassy carbon electrode by single-wall carbon nanotubes and used the determination of the folic acid with cyclic voltammetry (CV) and linear sweep voltammetry (SVW) techniques. Their results illustrated that the carbon nanotube film on the electrode remarkably increased the sensitivity of folic acid sensor and led to elimination of interferences from some inorganic and organic species in the solution. Erkal-Aytemur et al. [17] also prepared nano-wrinkled layer carbonaceous electrode as folic acid sensor and electrochemical responses of sensor studied by CV, differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy. Their results showed high electrocatalytic activity for folic acid due to the presence of carbonaceous wrinkled layers which enhanced the surface area of the electrode. Therefore, this study was focused on electrochemical determination of folic acid in food through ZnO@graphene oxide nanocomposite.

2. MATERIALS AND METHODES

In order to synthesize the ZnO@GO nanocomposites, in the first step the ZnO nanoparticles were prepared by the sol-gel method as following process [18]: the mixture of 1 mM NaOH ($\geq 98\%$,

Sigma-Aldrich) solution, 10 mM zinc acetate dihydrate (99.99%, Sigma-Aldrich) solution and 1 wt% sodium alginate (medium viscosity, Sigma-Aldrich) was prepared in volume ratio of 1:2:5 under magnetic stirring at 70 °C for 60 minutes. Then, the mixture was centrifuged at 5000 rpm for 60 minutes. After then, the centrifuged suspension was washed with deionized water and ethanol (96%, Merck) for four times, respectively. The obtained suspension in ethanol was dried in oven at 120 °C for 120 minutes.

In the second, the dried powder of ZnO nanoparticles was added to graphene oxide (powder, 15-20 sheets, 4-10% edge-oxidized, Merck) in weight ratio of 0:1, 1:0, 1:2, 1:1 and 2:1 as denoted to S₀₁, S₁₀, S₁₁, S₁₂ and S₂₁, respectively and sonicated for 60 minutes in methanol (99.8%, Merck). The obtained black suspensions transferred to the oven at 70 °C for 1 hour and the resulting pastes transferred on GCE. Finally, the modified GCEs were dried in the oven at 90 °C for 4 hours.

For preparation of the apple juice sample as a real sample, apple juice was provided from local markets. The sample was centrifuged for 20 minutes at 1000 rpm and was filtered (Millipore™ Membrane Filter, 0.45 µm pore size, Merck, Germany) and then diluted in the PBS pH 7.0 and transferred into the electrochemical cell.

Scanning electron microscopy (SEM, S4700, Hitachi High-Technologies Co., Minato-ku, Japan) was applied to analyze the morphology of the prepared sample. Crystallinity and structure of samples were studied with Xpert Pro X-ray diffractometer (XRD, Philips X-pert-MPD, Malvern Panalytical Ltd., Worcestershire, UK) with 1.5404 Å (Cu K α) in wavelength at 40KV and 30 mA. Electrochemical measurements were performed in the three-electrode cell which contained Ag/AgCl/(sat KCl) as reference electrode, a Pt wire as the counter electrode and the bare and modified GCE as the working electrodes. CV and DPV electrochemical techniques were conducted in buffers with and without folic acid ($\geq 97\%$, Merck, Germany) solution. Autolab modular electrochemical system (Eco ChemieUltech) was applied to electrochemical studies. The electrolyte of electrochemical cell was 0.1 M phosphate buffer solutions (PBS) which were prepared from H₃PO₄ and NaH₂PO₄ at pH 7.0.

3. RESULT AND DISCUSSION

Figure 1a shows the SEM image of graphene oxide sheets which are rippled and entangled with each other. Figure 1c exhibits the SEM of ZnO@GO nanocomposite (S₁₁) which reveals that the prepared zinc oxide nanoparticles were homogeneously combined in graphene oxide texture. Anchoring of ZnO nanoparticles on mesoporous graphene oxide sheets lead to creation of a high porous surface and high surface-to-volume ratio on GCE. Introduction of the ZnO nanoparticles as a stabilizer between the graphene oxide sheets prevents sheet aggregation [19, 20].

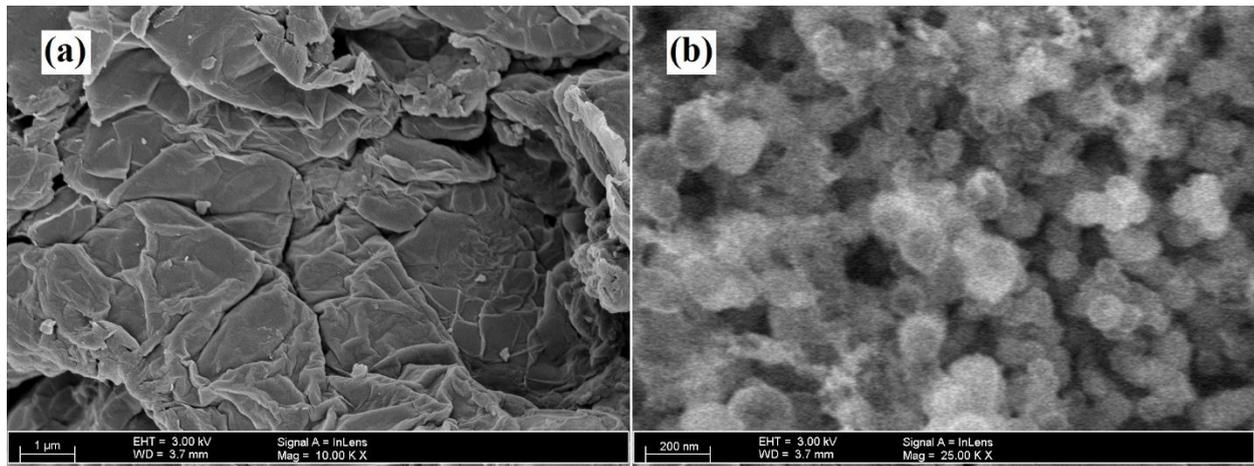


Figure 1. SEM images of (a) graphene oxide sheets and (b) ZnO@GO nanocomposite (S₁₁)

Figure 2 exhibits the XRD patterns of GO, ZnO nanoparticles, and ZnO@GO nanocomposite (S₁₁). As seen in Figure 2a, a diffraction peak at $2\theta = 26.55^\circ$ in XRD pattern of GO that reflects the presence of graphitic carbon plane of (002) according to JCPDS card No. 075-1621 [21]. The XRD pattern of synthesized ZnO nanoparticles in Figure 2b shows the diffraction peaks at $2\theta = 32.01^\circ, 34.58^\circ, 36.28^\circ, 47.29^\circ, 56.88^\circ, 62.95^\circ, 66.57^\circ, 68.28^\circ, 69.26^\circ, 72.81^\circ,$ and 77.18° which attributing to (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) planes, respectively accordance to JCPDS card No. 075-0576 that it confirms to growth of ZnO nanoparticles in wurtzite structure [22, 23]. The recorded XRD pattern of ZnO@GO nanocomposite in Figure 2c shows the formation of the planes of both of ZnO and GO which illustrate the introduction of the ZnO nanoparticles on GO nanosheets structures.

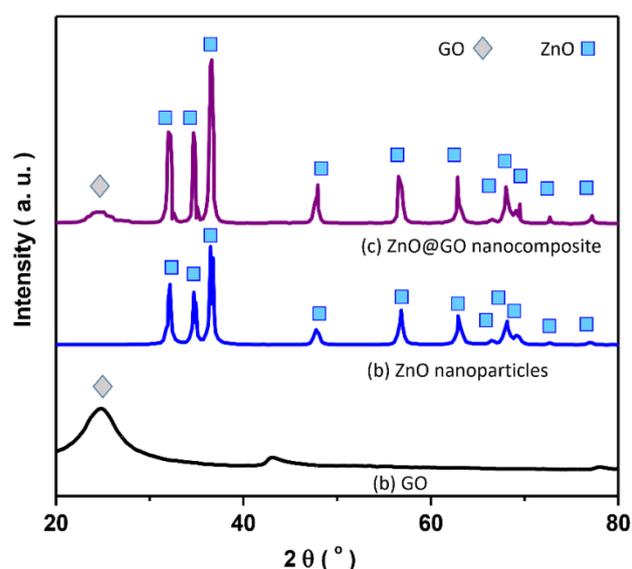


Figure 2. XRD patterns of (a) GO, (b) ZnO nanoparticles, and (c) ZnO@GO nanocomposite (S₁₁).

In order to study the electron transport properties of prepared electrodes, the CVs of modified electrodes were recorded in PBS pH 7.0 at scan rate 20 mV s^{-1} with $15 \text{ }\mu\text{M}$ folic acid. Figure 3 exhibits the current responses of bare and modified GCEs. As observed, there is no outstanding peak for GCE, while there are enhancement current electrocatalytic responses on the modified GCE with ZnO@GO nanocomposites. The well-defined electrochemical oxidation peaks of folic acid are observed at potentials, $0.723 \pm 0.005 \text{ V}$, $0.74 \pm 0.00\text{V}$, $0.635 \pm 0.004 \text{ V}$, $0.585 \pm 0.003 \text{ V}$, $0.585 \pm 0.004 \text{ V}$ and $0.624 \pm 0.003 \text{ V}$ at GCE, S₀₁, S₁₀, S₁₁, S₂₁ and S₂₁, respectively. Anodic peak current of folic acid oxidation at GCE, S₀₁, S₁₀, S₁₁, S₁₂ and S₂₁ are 1.20 ± 0.02 , 3.31 ± 0.04 , 4.82 ± 0.04 , 11.03 ± 0.04 , 9.37 ± 0.02 , and $6.62 \pm 0.04 \text{ }\mu\text{A}$, respectively. Therefore, S₁₁ shows the higher oxidation current and a shift toward a lower oxidation potential which indicating the promotion the charge transfer reaction at S₁₁ surface for electrocatalytic oxidation of folic acid due to the effective synergic effect of ZnO nanoparticles on GO nanosheets structures with weight ratio of 1:1. It is suggested the large specific surface area of mesoporous graphene oxide sheets can create more active electrocatalytic sites for redox reaction [24]. Therefore, the high surface-to-volume ratio of nanostructures provides significant sensitivity with excellent diffusivity paths for fast electron transfer [25, 26].

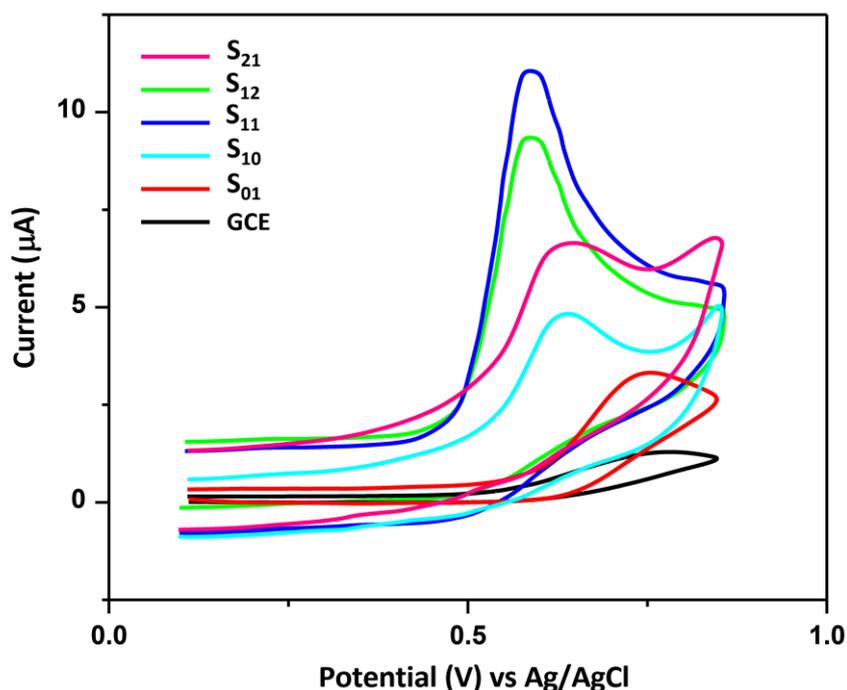


Figure 3. The recorded CVs in 0.1 M PBS pH 7.0 at a scan rate of 10 mV s^{-1} of bare GCE and modified GCE with ZnO@GO nanocomposites (S₀₁, S₁₀, S₁₁, S₁₂ and S₂₁) in present of $15 \text{ }\mu\text{M}$ folic acid.

The stability response of prepared electrodes was studied through the record CV in successive scans in PBS pH 7 at scan rate 10 mV s^{-1} with $15 \text{ }\mu\text{M}$ folic acid.

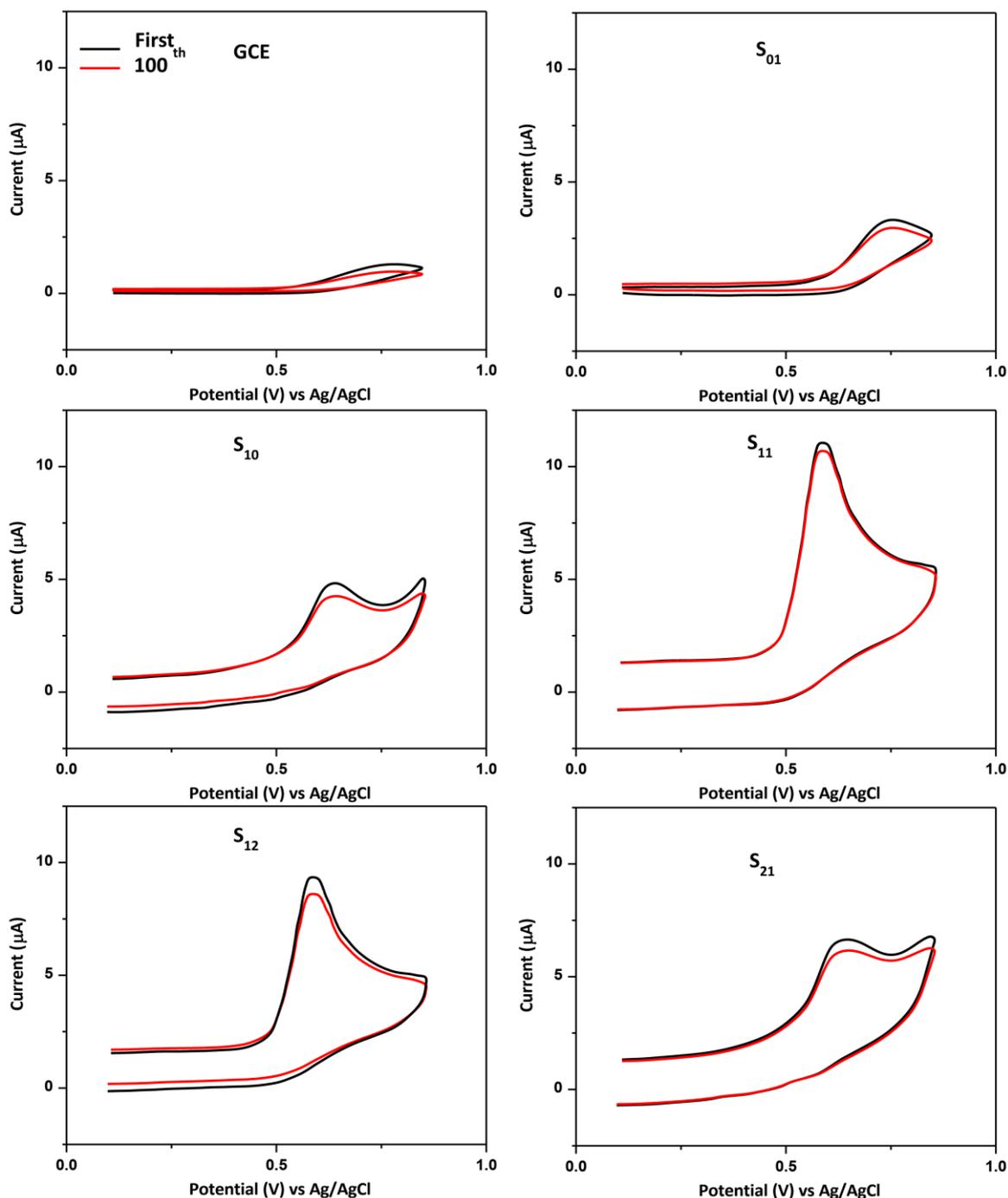


Figure 4. The recorded first and 100th CVs in 0.1 M PBS pH 7.0 at a scan rate of 10 mV s⁻¹ of bare GCE and modified GCE with ZnO@GO nanocomposites (S₀₁, S₁₀, S₁₂, S₁₁ and S₂₁) in present of 15 µM folic acid.

Figure 4 displays the initial and 100th recorded successive CVs which shows the changes of folic acid oxidation current after 100 scans for GCE, S₀₁, S₁₀, S₁₁, S₁₂ and S₂₁ are about ~ 30%, 12%, 13%, 4%, 8% and 7%, respectively. Therefore, the high stability responses were recorded for modified ZnO@GO nanocomposites because of concurrent participations of ZnO nanoparticles on GO

nanosheets prevent the self-aggregation and buffered the volume expansion of ZnO nanoparticles by preparation excess space during CV scans [27].

Specifically, the most stable response of S₁₁ electrode showed that the unique nanostructure maintained well at an equal proportion of ZnO nanoparticles on GO nanosheets in the composite (weight ratio of 1:1). Therefore, S₁₁ electrode as ZnO@GO nanocomposites/GCE was selected for the following electrochemical study of folic acid because of its high sensitivity and stable response.

DPV technique was applied to study the response of S₁₁ electrode in successive injections of different concentrations of folic acid in 0.1 M PBS pH 7.0 at scan rate 20 mV s⁻¹. Figure 5 shows the recorded DPVs and calibration graphs of current against folic acid concentration (1-9 μM). As observed, with injection of folic acid concentration in each DPV, the oxidation current was increased and there is the linear relationship between oxidation current and folic acid concentration. Calibration graphs in Figure 6 for high concentration of folic acid (> 400 μM) shows the deviation because of filling of active sites on S₁₁ electrode surface which indicates linear range for determination of folic acid is 1 to 400 μM. Moreover, the sensitivity and limit of detection are obtained 0.0637 μA/μM and 0.04 μM, respectively.

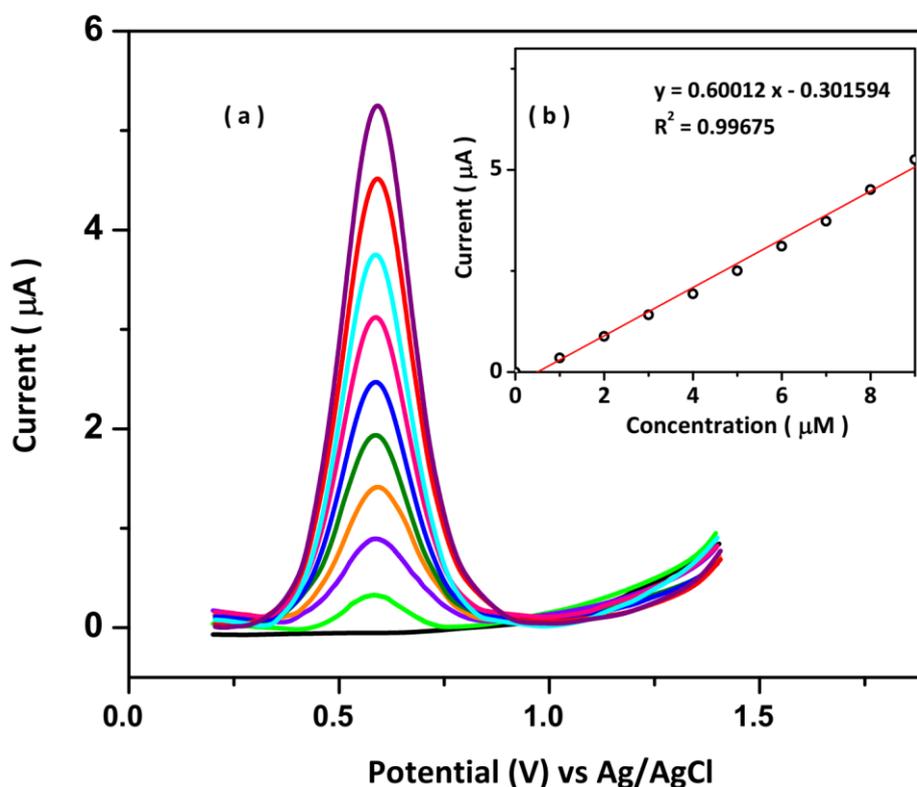


Figure 5. (a) The recorded DPVs of modified GCE with ZnO@GO nanocomposites (S₁₁) in successive injections of different concentration (1-9 μM) of folic acid in 0.1 M PBS pH 7.0 at scan rate 20 mV s⁻¹. (b) The calibration graphs of current against folic acid concentration.

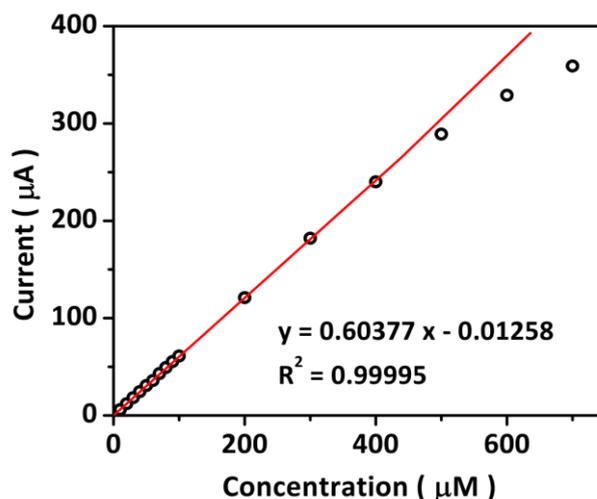


Figure 6. The calibration graphs of current against folic acid concentration for high concentrations (1-700 μM) for modified GCE with ZnO@GO nanocomposites (S₁₁) in 0.1 M PBS pH 7.0 at scan rate 20 mV s^{-1} .

Table 1 presents a comparison of electrochemical activity ZnO-based sensors for determination of folic acid. The results demonstrate the prepared ZnO@GO nanocomposites/GCE has a linear range and sensitivity are better or superior to the other reported ZnO based electrochemical sensors because of effective synergic effect of ZnO and GO nanostructures.

Table 1. Comparison of electrochemical activity ZnO-based sensors for determination of folic acid.

Electrodes	Technique	detection limit (μM)	Linear range (μM)	Sensitivity ($\mu\text{A}/\mu\text{M}$)	Ref
ZnO@GO nanocomposites/GCE	DPV	0.04	1 to 400	0.0637	This work
ZrO ₂ /ZnO nanocomposite	DPV	0.29	2– 480	0.047	[28]
ZnO-CuO nanoplates/ carbon paste electrode	SWV	0.025	0.05 – 200	0.0172	[29]
ZnO nanoparticle/ionic liquid/ carbon paste electrode	SWV	0.01	0.05 – 1.5	0.033	[30]

To study the selectivity of ZnO@GO nanocomposites/GCE for determination of folic acid, the DPV responses of successive injections of 1 μM folic acid and 10 μM of other analytes in 0.1 M PBS pH 7.0 at scan rate 20 mV s^{-1} were recorded. Table 2 shows the outstanding and selective response of ZnO@GO nanocomposites/GCE for injection of 1 μM of folic acid and no significant responses for the injections of other analytes. Therefore, the modified electrode did not show any interference effect with detection of folic acid in presence of the analytes which were presented in Table 2.

Table 2. Study the interference effect on determination of 1 μM folic acid on ZnO@GO nanocomposites/GCE surface.

Analyte	Added (μM)	Electrocatalytic current (μA)	RSD (%)
Folic acid	1	0.69	± 0.06
Ca^{2+}	10	0.08	± 0.01
CO_3^{2-}	10	0.10	± 0.02
K^+	10	0.09	± 0.02
Na^+	10	0.08	± 0.02
PO_4^{3-}	10	0.08	± 0.01
SO_4^{2-}	10	0.06	± 0.01
Na^+	10	0.11	± 0.05
ascorbic acid	10	0.07	± 0.01
uric acid	10	0.09	± 0.05
citric acid	10	0.12	± 0.02
glucose	10	0.08	± 0.03
Glutamic acid	10	0.09	± 0.02
lactose	10	0.13	± 0.01
fructose	10	0.11	± 0.03
Gallic acid	10	0.09	± 0.03

Analytical applicability of the ZnO@GO nanocomposites/GCE also was evaluated to determine folic acid in apple juice. The DPV technique and the standard addition method was recorded in 0.1 M PBS of pH 7.0 at scan rate 20 mV s^{-1} . The results of this study are illustrated in Table 3 which illustrates the results of recovery (more than 94.60%) and RSD (less than 4.25%) values are acceptable. Therefore, the results indicate the reliable application of ZnO@GO nanocomposites for the determination of folic acid in food samples.

Table 3. Results of practical determination of folic acid in apple juice samples (n = 4)

Samples	Added (μM)	Fund (μM)	Recovery (%)	RSD (%)
apple juice	5.00	4.73	94.60	3.21
	10.00	9.58	95.80	2.52
	20.00	19.85	99.25	1.97
	30.00	29.35	97.83	4.25
	40.00	38.08	95.20	1.88

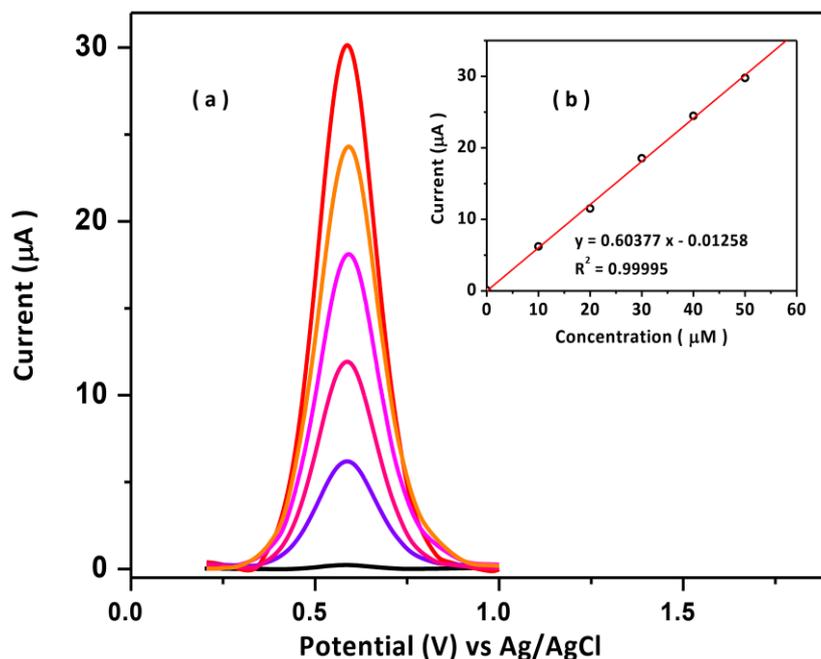


Figure 7. (a) The recorded DPVs of ZnO@GO nanocomposites/GCE in prepared real sample with 0.1 M PBS pH 7.0 at scan rate 20 mV s^{-1} in successive injections of $10 \text{ } \mu\text{M}$ of folic acid; (b) The calibration graphs of current against folic acid concentration.

Further study for determination of amount folic acid in apple juice as real sample, the DPVs of ZnO@GO nanocomposites/GCE at the standard injections of $10 \text{ } \mu\text{M}$ of in folic acid at scan rate 20 mV s^{-1} in electrochemical cell were recorded. Therefore, 1 ml of the filtered apple juice with 1 ml of 0.1 M PBS of pH 7.0 used as initial electrolyte. The recorded DPV responses and the calibration plots are shown in Figure 7. In accordance with the equation of calibration curve, the obtained folic acid in electrochemical cell and apple juice are $0.0691 \text{ } \mu\text{M}$ and $0.138 \text{ } \mu\text{M}$, respectively which is in agreement with the report of Aidoo et al. [31].

4. CONCLUSIONS

This study investigated the electrochemical properties of ZnO@GO nanocomposites/GCE as a folic acid electrochemical sensor. ZnO nanoparticles were synthesized by the sol-gel and homogeneously added to graphene oxide in a weight ratio of 0:1, 1:0, 1:2, 1:1 and 2:1. The resulting pastes applied for modification of the GCE surface. Structural studies showed that high porous surface ZnO@GO nanocomposite included the zinc oxide nanoparticles which homogeneously anchoring on mesoporous graphene oxide and acts as a stabilizer between the graphene oxides sheets to prevent of sheets aggregation. The electrochemical studies showed that ZnO@GO nanocomposites with weight ratio of 1:1 exhibited high stability, high sensitivity and the improvement in charge transfer for determination of folic acid due to the effective synergic effect of ZnO nanoparticles on GO nanosheet structures and large specific surface area of mesoporous graphene oxide sheets. DPV studies showed

that the wide linear range, high sensitivity and low limit of detection were obtained 1 to 400 μM , 0.0637 $\mu\text{A}/\mu\text{M}$ and 0.04 μM , respectively. Comparison of electrochemical activity ZnO-based sensors demonstrate that the prepared ZnO@GO nanocomposites/GCE illustrated better or superior linear range and sensitivity for determination of folic acid. Selectivity of modified electrodes to determine folic acid in present of other analytes were studied. Analytical applicability of the ZnO@GO nanocomposites/GCE also was evaluated to the determination of folic acid in apple juice. The results indicate the reliable application of ZnO@GO nanocomposites for the determination of folic acid in food samples due to acceptable value of recovery and RSD.

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