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Electrochemical Sensor Based on CuO nanoparticles-modified graphene oxide (CuO@GO) nanocomposites for Determination of Zearalenone Mycotoxins in Food Samples

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This study reported the preparation of CuO nanoparticles-modified graphene oxide (CuO@GO) nanocomposites as an electrochemical sensor for determination of zearalenone mycotoxins in food samples. Hummer's method was used to synthesize the graphene oxide (GO) and CuO@GO nanocomposites on glassy carbon electrodes (GCE). Morphology and phase composition studies of the samples using SEM and XRD analysis showed that aggregated nanosheets of GO with graphitic carbon crystalline planes were synthesized. For CuO@GO nanocomposites, the CuO nanoparticles with monoclinic crystalline phase were uniformly dispersed and anchored on the GO nanosheets. The electrochemical characterization was done by CV and DPV techniques which revealed that limit of detection, sensitivity and linear range for determination of zearalenone on CuO@GO/GCE were obtained 0.012 ng ml⁻¹, 0.4895 µA/ng ml⁻¹ and 10 to 150 ng ml⁻¹, respectively. The response of the sensor was studied in presence of the interfering substances that showed acceptable, repeatable, stable and high selectivity responses of the prepared zearalenone sensor. The sensor performance for determination of zearalenone in milk samples were investigated that indicated the acceptable precision to determine zearalenone in real samples. As a result, modification of GO/GCE electrode with CuO nanoparticles improve the zearalenone sensing properties due to high electrical conductivity, the morphology of nanoparticles, and the synergistic effect of CuO nanoparticles and GO nanosheets.

Keywords: Electrochemical sensor; Graphene oxide; CuO nanoparticles; Nanocomposites; Zearalenone; Food samples

1. INTRODUCTION

Zearalenone (6-(10-hydroxy-6-oxo-trans-1-undecenyl)- β -resorcyclic acid lactone) is known as F-2 toxin *and* strong estrogenic *metabolite* that is produced by Fusarium graminearum and teleomorph Gibberellazeae. Studies showed that it acts as a G protein-coupled receptor 30 (GPR30) agonist due to binding to classical estrogen receptors and activating the receptors to generate a biological response. Therefore, zearalenone can block the agonist activity [1, 2].

Zearalenone as a heat-stable mycotoxin has been frequently detected in forages and cereal crops such as maize, corn, corn silage, barley, oats, wheat, rye, rice, and sorghum. Moreover, zearalenone can be transferred into the animal source foods such as milk, meat and egg [3]. Contamination of grains with zearalenone is reported low but its content can be increased more than 30% to 40% under high moisture conditions. Therefore, many studies were conducted to recognize the effect of human exposure to zearalenone through the diet which revealed the formation of some sexual disorders and early puberty in girls. It affects on reproductive system with clinical symptoms such as vaginal swelling, enhanced incidence of false pregnancy, fetal death and secretion of mammary gland [1, 3].

Therefore, many researchers have been performed to determination of zearalenone in food samples trough thin-layer chromatography, high-performance liquid chromatography, reversed-phase high-performance liquid chromatography, liquid chromatography with fluorescence, gas chromatography–mass spectrometry, UV spectroscopy and electrochemical [4, 5]. Studies showed the results of chromatography methods are not sufficient and these may oftentimes lead to false positive results [6, 7]. There are also high signal to noise ratios in recorded UV spectroscopy results [8, 9]. Between the methods, electrochemical techniques provide rapid response, high sensitivity due to modification of the electrode surfaces as an important element of sensors [10, 11].

Modification of the surface has been performed to improve the physical and chemical properties, optimize the electrochemical reaction voltage, and enhance the electron transfer rate in the electrochemical process and effective surface area through the doping, composite and nanostructured materials [12-19]. For example, Andrés e al. [20] provided the sensitive method for detection of zearalenone in urine samples on CNTs/GCE by liquid chromatographic technique. He and Yan [21, 22] presented a voltammetric zearalenone sensor based on a composite of Pt nanotubes /Au nanoparticles and thiamine labeled GO which could improve the sensitivity of electrochemical sensors. Therefore, this study presented the preparation and electrochemical studies of modified CuO@GO/GCE as a sensor for determination of zearalenone mycotoxins in food samples.

2. EXPERIMENTAL

2.1. Synthesis of GO and CuO@GO nanocomposites

Hummer's method was applied to prepare the GO [23]. 10 g of graphite flakes (99.98%, Qingdao Weijie Graphite Co., Ltd., China) and 5 g of NaNO₃ (99.3%, Shandong Kaiteda Chemical Co., Ltd., China) were mixed in 200 ml H₂SO₄ (98%, Qingdao HiseaChem Co., Ltd., China) and stirred in ice bath for one hour. Then, 20 g of KMnO₄ (99%, Sigma-Aldrich) were steadily added to the mixture under temperature of 4°C. The resulting suspension was reacted under string in an ice bath for 2 hours. After then, the suspension was stirred at 30 °C for 24 hours. For synthesis of CuO@GO nanocomposites, 4g of copper oxide (99%, Hebei Henghe Import & Export Trading Co., Ltd., China)

were dispersed in suspension. Next, temperature was quickly increased to 95 °C for one hour while 200 ml deionized water was gradually added to suspension. After 30 minutes, 10 mL of H_2O_2 was added to brown suspension and the reaction product was centrifuged at 2000 rpm for 40 minutes and then washed with DI water and 10% HCl. The washed product was transferred on GCE and dried in an oven at 70°C to obtain GO.

2.2. Measurements techniques

The Surface and morphology of synthesized GO and CuO@GO nanocomposites were studied by scanning electron microscopy (SEM, Philips XL30. FEG; FEI, Eindhoven, the Netherlands). The phase composition and structures of prepared samples were studied with X-ray diffractometer (XRD, D8 Advance X-ray diffractometer, Bruker AXS GmbH, Germany) with Cu K α radiation (λ = 1.5418 Å) operated at 40 kV and 40 mA.

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) for electrochemical analyses of samples were carried out with potentiostat (AutoLab, Model PGSTAT 302, Metrohm USA Inc., Riverview, FL, USA) in standard three-electrode electrochemical cell using GCE, GO/GCE and CuO@GO/GCE as the working electrodes, a Pt wire as the counter electrode and an Ag/AgCl pseudo-reference electrode. Electrolyte in electrochemical cell was contained 0.1 M phosphate buffer solutions (PBS) which prepared of H₃PO₄ (\geq 85%, Henan Bright Commercial Co., Ltd., China) and NaH₂PO₄ (99%,Zhengzhou City Mantanghong Abrasives Co., Ltd., China).

3. RESULTS AND DISCUSSION

3.1. Structural characterizations

Figures 1 shows the SEM images of synthesized GO and CuO@GO nanocomposites. The SEM image of GO displayed aggregated nanosheets with distinct edges that the surfaces are wrinkled and folding.



Figure 1. SEM images of synthesized (a) GO and (b) CuO@GO nanocomposites.

With addition of CuO in GO texture for CuO@GO nanocomposites, the CuO nanoparticles are uniformly dispersed and mightily anchored on the GO nanosheets. Moreover, GO nanosheets serve as an appropriate substrate to homogeneously distribute CuO nanoparticles and prevent particle agglomeration. CuO nanoparticles act also as a stabilizer to growth of discrete GO nanosheets. Overall, CuO@GO nanocomposites surface was covered with more porous and higher density of nanostructured material toward the GO nanosheets which indicated the higher effective surface area and more absorption sites on the electrode surface.

XRD patterns of GO and CuO@GO nanocomposites are exhibited in Figure 2. For GO sample, XRD patterns show diffraction peaks at 14.49° and 31.68° correspond to the (004) and (002) planes of graphitic carbon and intercalated graphite (JCPDS card No. 46-0870) [24], respectively. For CuO@GO nanocomposites sample, XRD patterns displays diffraction peaks at of 35.48° ,38.81°,46.28°, 48.79°, 53.75°, 58.3°, 61.74°, 66.08° and 68.01° which associated with the (111), (200), (112), (202), (020), (021), (113),(311) and (220) planes which can be perfectly indexed to the monoclinic phase of CuO (JCPDS card No. 48-1548) [25], respectively. Moreover, the diffraction peaks at 14.50° correspond to the (004) plane Implied to GO in CuO@GO nanocomposites which implied the strong interaction of CuO particles with chemical functionalization of GO. The morphology and structure of CuO@GO nanocomposites can propose GO nanosheets film as an appropriate substrate for incorporation of CuO nanoparticles.



Figure 2. XRD patterns of synthesized (a) GO and (b) CuO@GO nanocomposites.

3.2. Electrochemical characterizations

The electrochemical response of GCE, GO/GCE and CuO@GO/GCEwere studied by using CV technique under potential ranges of -0.5 V to 1.0V in 0.1M PBS solution (pH 7.2) at scan rate of 20

 mVs^{-1} in presence of 15 ng mL⁻¹zearalenone in electrochemical cell. As can be observed from Figure 3a, there is one well-defined irreversible oxidation peak at 0.09 V with current of 5.19, 8.89 and 17.78 for GCE, GO/ GCE and CuO@GO/GCE, respectively. CuO@GO/GCE shows a noticeable increase of the peak current more than twice and three times higher than peak currents on GO/ GCE and GCE, respectively. The increase of electrocatalytic current on GO/GCE can be associated with the presence of oxygen functional groups on GO surface and larger electrochemical active surface area and excellent electron conductivity of GO nanosheets and higher active electron transfer sites [26]. The enhancement of electrocatalytic current on CuO@GO/GCE can be related to CuO nanoplates which enhanced electrocatalytic activity due to high electrical conductivity, the CuO nanoparticles morphology, and the synergistic effect of CuO nanoparticles and GO nanosheets[27].

Figure 3b shows the electrochemical CV response of CuO@GO/GCE to addition 15, 30, 45 and 60ng mL⁻¹ concentration of zearalenone under potential ranges from -0.5 to 0.5V in 0.1M PBS solution (pH 7.2) at 20 mVs⁻¹ scan rate. As seen, the current response of oxidation peak linearly increases with increasing the zearalenone solution in electrochemical cells which evidence to the high sensitivity and excellent stability of CuO@GO/GCE response to addition of zearalenone.



Figure 3. The electrochemical CV responses under potential ranges of -0.5 V to 1.0 V in 0.1M PBSsolution (pH 7.2) at 20 mVs⁻¹ scan rate for (a) GCE, GO/ GCE and CuO@GO/GCE in presence of 15ng mL⁻¹zearalenone, and (b)CuO@GO/GCE in addition of 15, 30, 45 and 60ng mL⁻¹ concentration of zearalenone in electrochemical cell.

Following electrochemical measurements were performed by DPV technique for determination the sensitivity, linear range and detection limit of CuO@GO/GCE response to addition1ng mL⁻¹ of zearalenone solution under potential ranges from -0.5 to 0.5V in 0.1M PBS solution (pH 7.2) at 20 mVs⁻¹. Figures 4a and 4b present the recorded DPVs and calibration curve of CuO@GO/GCE to successive injection of 1 ng ml⁻¹zearalenone solution which signified to the limit of detection of 0.012 ng ml⁻¹ and sensitivity of 0.4895 μ A/ng ml⁻¹. Further electrochemical measurements to obtain the linear range of CuO@GO/GCE toward zearalenone were performed in successive addition of 10 ng ml⁻¹zearalenone solution in electrochemical cells. The plotted calibration curve is shown in Figures 5. It indicates that the obtained linear range for determination of zearalenone on CuO@GO/GCE is 10 to 150 ng ml⁻¹. Furthermore, the obtained sensitivity, linear range, detection limit of CuO@GO/GCE are compared with other sensors in the literature for determination of zearalenone. The comparison result indicates that the CuO@GO/GCE sensing properties are comparable or better than the reported electrochemical sensor in Table 1. Moreover, the broad linear range for determination of zearalenone obtained due to the high porosity, high effective surface area and effective active sites for CuO@GO nanocomposites [28].Studies showed combining of GO with CuO nanoparticles in electrochemical sensors lead to superb advantages such as enhance mass transport and sensitivity, reduce the detection limits and fast electron transfer kinetics in electrochemical reactions [29].



Figure 4. (a) The electrochemical DPV responses (b) calibration curve under potential ranges from - 0.5 to 0.5V in 0.1M PBS solution (pH 7.2) at 20 mVs⁻¹ for CuO@GO/GCE to addition 1ng mL⁻¹ of zearalenone solution.

CuO@GO/GCE



Figure 5. The calibration curve under potential ranges from -0.5 to 0.5V in 0.1M PBS solution (pH 7.2) at 20 mVs⁻¹ for CuO@GO/GCE to addition 10ng mL⁻¹ of zearalenone solution.

Electrode	Technique	Linear Range (ng mL ⁻¹)	Limit of detection (ng mL ⁻¹)	Ref.
CNT/ GCE	LC*	5 - 50	1.4	[20]
Au@AgPtcore/ shell particles	AMP**	0.005 - 15	0.0017	[30]
Ab***/ nanoporous gold films/ GCE	AMP	0.01-12	0.003	[31]
PtCo/ Ab/ thionine/ graphene nanosheets	CV	0.05 - 5.0	0.013	[32]
thin-layer MoS ₂ @thionin composite	SWV****	0.01 - 50	0.005	[33]
Au nanoparticles/ MWCNTs/ poly (vinylpyridine)/ screen-printed carbon	SWV	0.05 – 50	0.016	[34]
Pdnanoparticles@conductive polymeric ionic liquid/ GCE	SWV	0.03 –35	0.01	[35]
MWCNTs/ GCE	DPV	2.0 - 50.0	0.58	[36]
AuPt nanoparticles/ CNTs/ GCE	DPV	0.005 - 50	0.0015	[37]

Table 1. Comparison between electrochemical sensing results of CuO@GO/GCE with other sensors in the literature for determination of zearalenone.

*LC:Liquid chromatography; **AMP:Amperometry, ***Ab: Zearalanol antibody, ***SWV: Square wave voltammetry

DPV

10 - 150

0.012

This work

In order to study the selectivity, repeatability and stability of the electrochemical zearalenone sensor, the electrochemical response of CuO@GO/GCE for addition of the interfering substances which contained different concentrations of the possible organic and inorganic solutions in food simulant were investigated. Figure 6 shows the electrochemical DPV responses of CuO@GO/GCE for

Ing mL⁻¹addition of zearalenone and sequential injections 0.5ng mL⁻¹ of Cu²⁺, Ni²⁺, Fe²⁺, K⁺, Mn²⁺, Zn²⁺, Co²⁺, lactic acid, ascorbic acid, vitamin B12, vitamin C and L-Cysteine under potential ranges from -0.5 to 0.5V in 0.1M PBS solution (pH 7.2) at 20 mVs⁻¹. The ratio between the concentration of zearalenone and the interfering substances was picked out according to their concentrations in milk-based infant formula and foods [38]. As observed from Figure 6, when the first zearalenone solutions were added to the electrochemical cell, the electrocatalytic current signal was recorded of 0.81 μ A. After sequential addition of the interference solutions the recorded electrocatalytic current by the sensor was not affected. Therefore, the CuO@GO/GCE shows the selective behavior to identify zearalenone in food simulant. As seen in Figure 6, the addition of zearalenone solution between the interfering solutions and the final injections of experiment shows the increase about 0.79 μ A electrocatalytic current which indicated to excellent ability of prepared sensor to repeatable and stable determination zearalenone in the food simulant.



Figure 6. The electrochemical DPV responses under potential ranges from -0.5 to 0.5V in 0.1M PBS solution (pH 7.2) at 20 mVs⁻¹ for CuO@GO/GCE to addition 1ng mL⁻¹ of zearalenone solutions and sequential injections 0.5ng mL⁻¹ of interference solutions.

In order to validate the application performance of the CuO@GO/GCE as electrochemical sensor for determination of zearalenone in real samples, the content of zearalenone in milk samples were determined by addition of skimmed milk from commercial sources. The skimmed milk sample was provided from the local market and 1 ml of provided sample was dried. The obtained powder was added to 0.1 M PBS. Figure 7 shows the electrochemical DPV responses and calibration curve of CuO@GO/GCE to successive addition 1 ng ml⁻¹ of zearalenone solutions under potential ranges from -

0.5 to 0.5V in prepared 0.1M PBS solution with real sample at 20 mVs⁻¹ which referred the zearalenone concentration in real sample solution is obtained 1.04 ng ml⁻¹, that is close to obtained results by Prelusky et al. [39]. The results of recoveries and relative standard deviations (RSD) are presented in Table 2. The analytical results implies the recovery range is from 84.4% to 97.0% with the RSD values below 1.34% which implies the prepared sensor with acceptable precision can be applied to determine zearalenone in practical samples.



Figure 7. (a) The electrochemical DPV responses and (b) the calibration curve of CuO@GO/GCE for addition of the prepared real samples solution under potential ranges from -0.5 to 0.5V at 20 mVs^{-1} scan rate in prepared 0.1 M PBS solution with real sample.

Table 2. Results of zearalenone detection in real samples using CuO@GO/GCE as electrochemical sensor (n = 4).

Sample	Added	Found	Recovery (%)	RSD (%)
	concentrations	concentrations(ng		
	$(ng mL^{-1})$	$\mathbf{m}\mathbf{L}^{-1}$)		
skimmed	1.00	0.93	93.1	1.04
milk				
	2.00	1.68	84.4	1.34
	3.00	2.89	96.3	1.01
	4.00	3.88	97.0	1.05

4. CONCLUSION

This study presented the preparation and electrochemical study of modified CuO@GO/GCE as a sensor for determination of zearalenone mycotoxins in food samples. Hummer's method was applied to prepare the GO and CuO@GO nanocomposites on GCE. The study of morphology and phase composition using SEM and XRD showed that aggregated nanosheets of GO with graphitic carbon planes were synthesized and after addition of CuO in GO texture, the CuO nanoparticles with monoclinic crystalline phase were uniformly dispersed and mightily anchored on the GO nanosheets for CuO@GO nanocomposites . The electrochemical characterization of the samples by CV and DPV techniques revealed that the limit of detection, sensitivity and linear range for determination of zearalenone on CuO@GO/GCE were obtained 0.012 ng ml⁻¹, 0.4895 µA/ng ml⁻¹ and 10 to 150 ng ml⁻¹ ¹, respectively. Acceptable, repeatable, stable and high selectivity responses of the prepared zearalenone sensor were recorded in presence of the interfering substances. The performance of CuO@GO/GCE for determination of zearalenone in milk samples as real samples were investigated. The analytical results implied that the prepared sensor with acceptable precision can be applied to determine zearalenone in real samples. Results indicated the sensing enhancement of the CuO@GO/GCE can be related to CuO nanoparticles which improved electrocatalytic activity due to high electrical conductivity, the morphology of nanoparticles, and the synergistic effect of CuO nanoparticles and GO nanosheets.

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