

*Full Paper*

## **Sensitive Determination of Hydroxylamine on ZnO Nanorods/Graphene Oxide Nanosheets Modified Graphite Screen Printed Electrode**

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*Received: 19 October 2016 / Received in revised form: 9 December 2016 /*

*Accepted: 14 December 2016 / Published online: 31 March 2017*

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**Abstract-** To date, the development of different modified electrodes has received much attention in electrochemistry. Hence, we report an electrochemical sensor for the determination of hydroxylamine using ZnO nanorods/graphene oxide nanosheets modified graphite screen printed electrode. Electrochemical determination of hydroxylamine reveals that the modified electrode showed lower detection limit of 0.5  $\mu\text{M}$  with a linear ranging from 1.0 to 100.0  $\mu\text{M}$  towards the hydroxylamine concentration via differential pulse voltammetry. The developed sensor also applied to the successfully determination of trace level hydroxylamine in real samples. In addition, the advantage of this type of electrode is simple, disposable and cost effective in electrochemical sensors.

**Keywords-** Hydroxylamine, ZnO nanorods, Graphene oxide nanosheets, Nanocomposite, Graphite screen printed electrode

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### **1. INTRODUCTION**

Hydroxylamine ( $\text{NH}_2\text{OH}$ , HA), a derivative of ammonium, and its salts are commonly used as reducing agents. They can also act as antioxidants for fatty acids. Furthermore,

hydroxylamine is a precursor of nitrous oxide as a side product during nitrification [1]. Also, hydroxylamine has a wide use in an industry a pharmacy process, where some of hydroxylamine derivatives make a great part of anticancer drugs. However, hydroxylamine well-known as a mutation agent, moderately toxic and harmful to human, animals, and even plants, even at modest level. Likewise, from the environmental, industrial and health viewpoints, development of a sensitive analytical method for determination of hydroxylamine in low level, is highly important [2-6]. Many of approaches developed and successfully applied for hydroxylamine determination such as: high performance liquid chromatography, gas chromatography, potentiometry, polarography, biamperometry and electrochemical methods [7-12]. Between these methods, electrochemical methods have special importance because of the simplicity, rapidity and inexpensiveness [13-23].

Screen-printed electrodes (SPEs) are an alternative material used instead of using the conventional electrodes based on economic substrate. Lately, SPE have been successfully used as the electrochemical sensor for various researches due to their disposability, simplicity of the apparatus, minimum sample preparation and obtaining of fast results. In addition, the main benefit of SPE is able to use only once and then is discarded [24].

But, hydroxylamine cannot be electrooxidized at bare SPE. Hence, to overcome this problem electrode modification is necessary [25-33]. Many materials have been considered as the electrode modification [34-42]. Among them, graphene, exhibiting many attractive advantages, such as high specific surface area and long cycling life stability. Unfortunately, the low energy storage capacity greatly hinders its extensive use. To overcome these shortcomings, many researches had focused on synthesis of graphene-based inorganic composites [43-47]. In graphene-based inorganic composites, graphene nano sheet can play a perfect role as a conductive support of metal oxides, while metal oxides can provide additional pseudocapacitance and prevent the restacking of graphene sheets, and lead to the formation of a new class of graphene-based materials. Among the metal oxides, zinc oxide (ZnO) nanostructures with many extraordinary properties, including wide direct band gap (3.37 eV at room temperature), large exciton binding energy (60 meV) and a high isoelectric point (IEP) (~9.5), nontoxicity, biological compatibility, chemical and photochemical stability, high electrochemical activities and easy preparation has been introduced as one of the most promising candidates to improve behaviors of graphene [48-55].

According to the previous points, it is important to create suitable conditions for the analysis of hydroxylamine in biological fluids. In this study, we describe application of novel ZnO/GR nanocomposite as a nanostructure sensor for voltammetric determination of hydroxylamine. The proposed sensor showed good electrocatalytic effect on hydroxylamine. ZnO/GR/SPE shows advantages in terms of selectivity, reproducibility, and sensitivity. Eventually, we evaluate the analytical performance of the suggestion sensor for hydroxylamine determination in water samples.

## 2. EXPERIMENTAL

### 2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. The screen-printed electrode (DropSens, DRP-110, Spain) consists of three main parts which are a graphite counter electrode, a silver pseudo-reference electrode and a graphite working electrode.

All solutions were freshly prepared with double distilled water. Hydroxylamine and all other reagents were of analytical grade and were obtained from Merck chemical company (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-9.0.

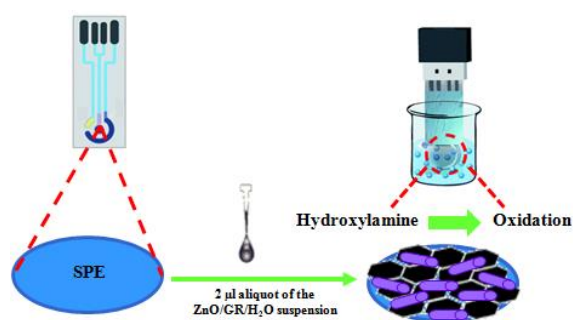
### 2.2. Synthesis of Graphene Oxide/ZnO Nanorods Nanocomposite

Graphene oxide nano sheets were synthesized from natural graphite flakes based on the modified Hummers and Offeman's method. In a typical synthesis process, 1.0 g of pristine graphite flakes was immersed in 50 mL of formic acid, and then sonicated for 2 h at room temperature. These resulting graphite plates were washed with acetone, and then dried in an oven at 95 °C for 12 h. Then, 100 mL H<sub>2</sub>SO<sub>4</sub> (95%) was added into a 500 mL flask, and cooled by immersion in an ice bath followed by stirring. About 1.0 g treated graphite powder and 0.5 g NaNO<sub>3</sub> were added under vigorous stirring to avoid agglomeration. After the graphite powder was well dispersed, 3 g KMnO<sub>4</sub> was added gradually under stirring and cooling so that the temperature of the mixture was maintained at below 10 °C. The mixture was stirred for 2 h and diluted with deionised double distilled water (in an ice bath). After that, 25 ml 15% H<sub>2</sub>O<sub>2</sub> was slowly added to the mixture until the color of the mixture changed to brilliant yellow, indicating fully oxidized graphite. The as-obtained graphite oxide slurry was re-dispersed in deionised double distilled water and then exfoliated to generate graphene oxide nano sheets by sonication for 2 h. Then, the solution was filtered and washed with diluted HCl solution to remove metal ions. Finally, the product was washed with deionised double distilled water until the solution became acid free, and dried under vacuum at 50 °C.

Then the reduced graphene oxide (0.096 g) was dispersed in 40 ml water and the solution was kept in ultrasonic bath for 1 h. The prepared solution was added to 40 ml of ZnCl<sub>2</sub> (0.04 M) solution. Final solution pH was set 11.7 by ammonia solution. The solution was kept at 95 °C for 4 h. The precipitate was gathered at 15000 rpm centrifugation for 15 min. Then it was washed by distilled water three times. Finally it was dried in oven at 45 °C for 4 h.

### 2.3. Preparation of modified electrode

The bare screen printed electrode was coated with ZnO/GR as follows. A stock solution of ZnO/GR in 1 mL aqueous solution was prepared by dispersing 1 mg ZnO/GR with ultrasonication for 1 h, and a 2  $\mu\text{L}$  aliquot of the ZnO/GR /H<sub>2</sub>O suspension solution was casted on the carbon working electrodes, and waiting until the solvent evaporation in room temperature (see scheme 1).



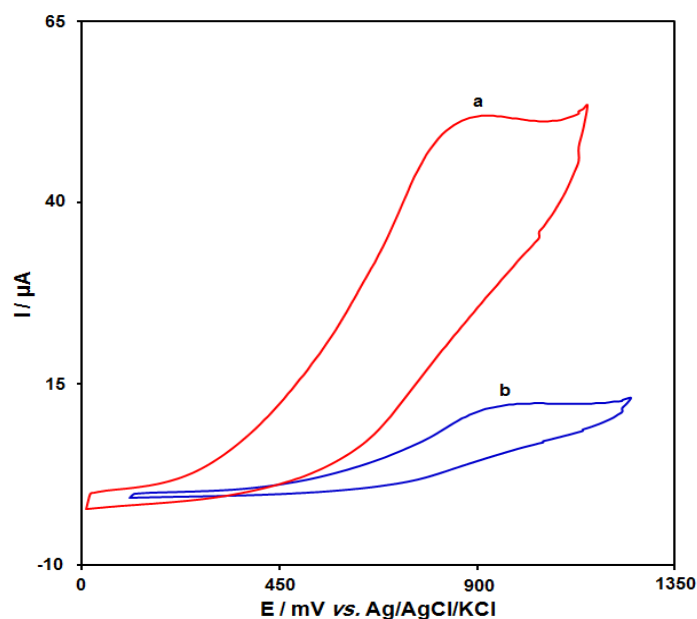
**Scheme 1.** Schematic diagrams illustrating the fabrication steps of modified electrode

## 3. RESULTS AND DISCUSSION

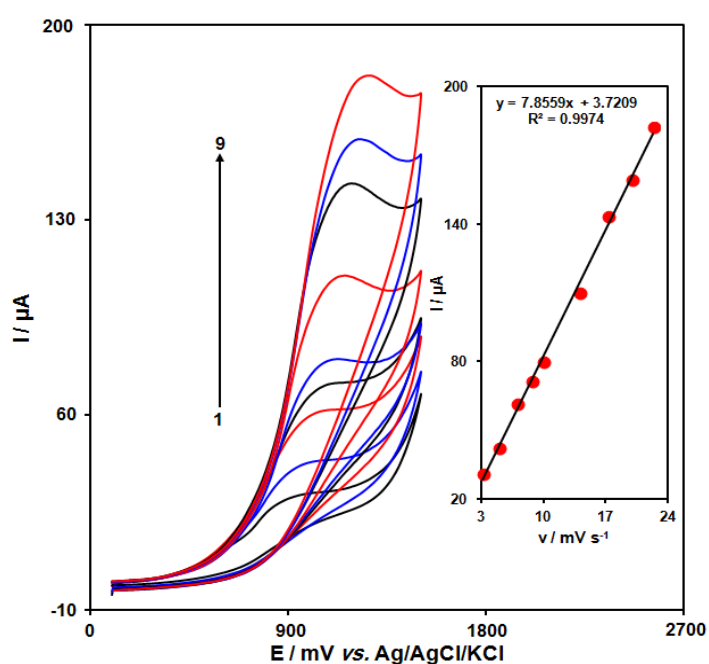
### 3.1. Electrocatalytic oxidation of hydroxylamine at a ZnO/GR/SPE

The electrochemical behavior of hydroxylamine is dependent on the pH value of the aqueous solution. Therefore, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of hydroxylamine. Thus the electrochemical behavior of hydroxylamine was studied in 0.1 M PBS in different pH values ( $2.0 < \text{pH} < 9.0$ ) at the surface of ZnO/GR/SPE by CV. It was found that the electrocatalytic oxidation of hydroxylamine at the surface of ZnO/GR/SPE was more favored under neutral conditions than in acidic or basic medium. Thus, the pH 7.0 was chosen as the optimum pH for electrocatalysis of hydroxylamine oxidation at the surface of ZnO/GR/SPE.

Fig. 1 depicts the cyclic voltammetric responses for the electrochemical oxidation of 100.0  $\mu\text{M}$  hydroxylamine at ZnO/GR/SPE (curve a) and bare SPE (curve b). The anodic peak potential for the oxidation of hydroxylamine at ZnO/GR/SPE (curve a) is about 900 mV compared with 990 mV for that on the bare SPE (curve b). Similarly, when the oxidation of hydroxylamine at the ZnO/GR/SPE (curve a) and bare SPE (curve b) are compared, an extensive enhancement of the anodic peak current at ZnO/GR/SPE relative to the value obtained at the bare SPE (curve b) is observed. In other words, the results clearly indicate that the combination of graphene and ZnO nanocomposites improve the hydroxylamine oxidation signal.



**Fig. 1.** Cyclic voltammograms of (a) ZnO/GR/SPE and (b) bare SPE in 0.1 M PBS (pH 7.0) in the presence of 100.0  $\mu\text{M}$  hydroxylamine at the scan rate  $50 \text{ mVs}^{-1}$



**Fig. 2.** Cyclic voltammograms of ZnO/GR/SPE in 0.1 M PBS (pH 7.0) containing 150.0  $\mu\text{M}$  hydroxylamine at various scan rates; numbers 1-9 correspond to 10, 25, 50, 75, 100, 200, 300, 400 and 500  $\text{mV s}^{-1}$ , respectively. Inset: Variation of anodic peak current vs. square root of scan rate

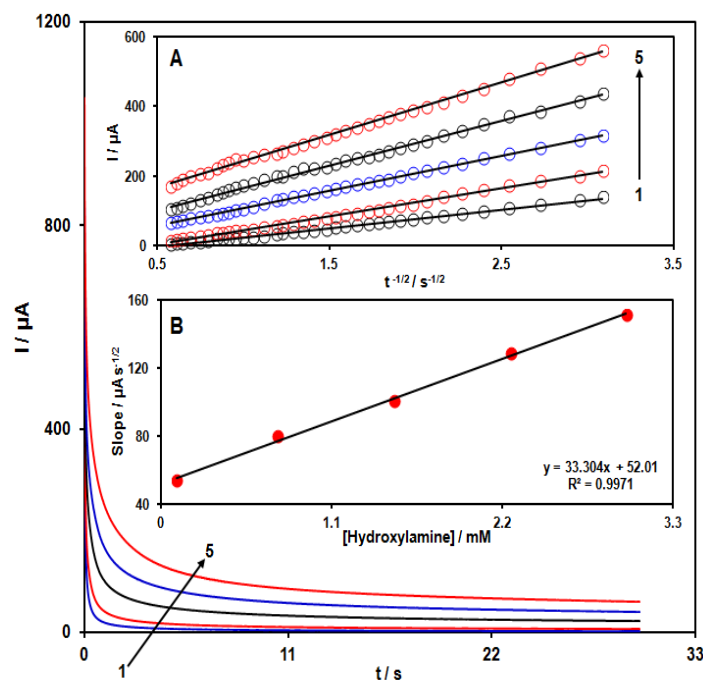
The effect of potential scan rates on the oxidation current of hydroxylamine has been studied (Fig. 2). The results showed that increasing in the potential scan rate induced an

increase in the peak current. In addition, the oxidation process is diffusion controlled as deduced from the linear dependence of the anodic peak current ( $I_p$ ) on the square root of the potential scan rate ( $v^{1/2}$ ) over a wide range from 10 to 500  $\text{mV s}^{-1}$ .

### 3.2. Chronoamperometric measurements

Chronoamperometric measurements of hydroxylamine at ZnO/GR/SPE were carried out by setting the working electrode potential at 0.95 V for the various concentration of hydroxylamine in PBS (pH 7.0) (Fig. 3). For an electroactive material (hydroxylamine in this case) with a diffusion coefficient of  $D$ , the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [56].

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \quad (1)$$

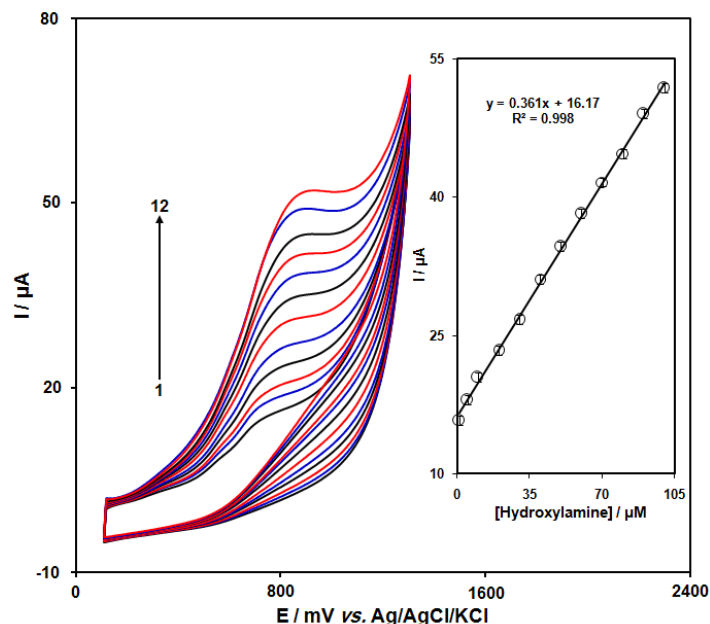


**Fig. 3.** Chronoamperograms obtained at ZnO/GR/SPE in 0.1 M PBS (pH 7.0) for different concentration of hydroxylamine. The numbers 1–5 correspond to 0.1, 0.75, 1.5, 2.25 and 3.0 mM of hydroxylamine. Insets: (A) Plots of  $I$  vs.  $t^{-1/2}$  obtained from chronoamperograms 1–5; (B) Plot of the slope of the straight lines against hydroxylamine concentration

where  $D$  and  $C_b$  are the diffusion coefficient ( $\text{cm}^2 \text{s}^{-1}$ ) and the bulk concentration ( $\text{mol cm}^{-3}$ ), respectively. Experimental plots of  $I$  vs.  $t^{-1/2}$  were employed, with the best fits for different concentrations of hydroxylamine (Fig. 3A). The slopes of the resulting straight lines were then plotted vs. hydroxylamine concentration (Fig. 3B). From the resulting slope and Cottrell equation the mean value of the  $D$  was found to be  $9.4 \times 10^{-5} \text{ cm}^2/\text{s}$ .

### 3.3. Calibration plot and limit of detection

The peak current of hydroxylamine oxidation at the surface of the modified electrode can be used for determination of hydroxylamine in solution.



**Fig. 4.** CVs of ZnO/GR/SPE in 0.1 M PBS (pH 7.0) containing different concentrations of hydroxylamine. Numbers 1-12 correspond to 1.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0 and 100.0  $\mu\text{M}$  of hydroxylamine. Inset shows the plot of the peak current as a function of hydroxylamine concentration in the range of 1.0-100.0  $\mu\text{M}$

**Table 1.** Comparison of the efficiency of some modified electrodes used in the electro-oxidation of hydroxylamine

Electrode	Method	LOD (M)	LDR (M)	Ref.
Carbon paste	Voltammetry	$1.0 \times 10^{-7}$	$9.0 \times 10^{-7} - 4.0 \times 10^{-4}$	[23]
Carbon paste	Voltammetry	$1.5 \times 10^{-8}$	$5.0 \times 10^{-8} - 5.0 \times 10^{-4}$	[57]
Glassy carbon	Amperometry	$1.0 \times 10^{-6}$	$1.0 \times 10^{-6} - 8.17 \times 10^{-5}$	[58]
Carbon paste	Amperometry	$8.0 \times 10^{-7}$	$2.5 \times 10^{-6} - 4.0 \times 10^{-4}$	[59]
Graphite screen printed	Voltammetry	$5.0 \times 10^{-7}$	$1.0 \times 10^{-6} - 1.0 \times 10^{-4}$	This Work

Therefore, CV experiments were done for different concentrations of hydroxylamine (Fig. 4). The oxidation peak currents of hydroxylamine at the surface of a modified electrode were

proportional to the concentration of the hydroxylamine within the ranges 1.0 to 100.0  $\mu\text{M}$ . The detection limit ( $3\sigma$ ) of hydroxylamine was found to be  $5.0 \times 10^{-7}$  M. These values are comparable with values reported by other research groups for determination of hydroxylamine (see Table 1).

### 3.4. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of hydroxylamine in water samples. The results for determination of hydroxylamine in water samples are given in Table 2. Satisfactory recovery of the experimental results was found for hydroxylamine. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

**Table 2.** The application of ZnO/GR/SPE for determination of hydroxylamine in water samples (n=5). All concentrations are in  $\mu\text{M}$

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
Tap Water	0	-	-	-
	5.0	4.9	98.0	3.3
	1.0	10.1	101.0	2.1
River Water	0	-	-	-
	7.5	7.6	101.3	2.4
	12.5	12.2	97.6	2.9

## 4. CONCLUSIONS

In this work, we reported the fabrication of modified graphite screen printed electrode and its application for the electrochemical detection of hydroxylamine. The fabricated hydroxylamine sensor exhibited excellent features such as low oxidation potential, low detection limit (0.5  $\mu\text{M}$ ) and wide linear range (1.0–100  $\mu\text{M}$ ) towards the voltammetric determination of hydroxylamine in neutral buffer solution. The disposable electrochemical sensor also showed good selectivity, reproducibility and stability. In addition, the ZnO/GR/SPE could be applied for detection of hydroxylamine in water samples, and therefore, the fabricated electrode will be an attractive candidate for practical applications. The several advantageous features together with the very easy fabrication and easy



regeneration of electrode surface (no need for any polishing), makes it useful for the electrochemical determination of hydroxylamine.

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