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Chemically Modified Screen-Printed Electrodes: An Electrochemical Interface for Nitrite Ions

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Abstract

A sensitive and selective nanostructured zinc oxide-based chemically modified screen-printed electrode as an electrochemical interface for the electroanalytical determination of nitrite ions at trace concentration level is demonstrated. Zinc oxide nanoparticles were synthesized through a green chemical approach. The resulting particles have been thoroughly analyzed using spectroscopic techniques. The aqueous colloidal solution of well-characterized NPs is prepared and subsequently applied in the chemical modification of screen-printed electrodes. Electrochemical techniques have been to explore the electrochemical nature of the chemically modified interface. Cyclic voltammetry was used to decipher activity for the electro-oxidation of nitrite and differential pulse voltammetry has been used to achieve lower detection limits. The developed sensor showed a dynamic range of up to 800 µM with a detection limit of 0.5 µM. Finally, the long-range analytical applicability has been validated by determining the nitrite levels from various real samples like borewell and sewage water, orange, sweet lemon, tomato, and egg samples. The sensor showed good stability with a deviation of ± 7 %.

Keywords: Cyclic Voltammetry, Nitrite, Real Samples, Sensor, Zinc Oxide

1.0 Introduction

Nitrite is considered as one of the most dangerous water pollutant which is well known to be widely distributed in water, fruits, vegetables, various foods, milk, dairy products, processed meat, and also in some food preservatives[1]. The presence of nitrate can form highly carcinogenic nitrosamine when it reacts with amines[2], it also irreversibly oxidizes hemoglobin present in blood[3]. Hence, the World Health Organization (WHO) has set a maximum permissible limit value of 3 ppb in the case of water samples. As a result of this, its accurate determination is highly desirable in water and other food-related products[4]. In view of this, various analytical techniques have been proposed including spectroscopic methods, chromatographic, capillary electrophoresis, fluorescence spectrum, and chemiluminescence techniques[5]. Even though these methods can determine the nitrite at reliable concentrations, these are in need of sophisticated instruments, skilled people to operate, time-consuming sample preparation, high detection limits, and demand to cover day-to-day maintenance costs. In addition to these, the most important and highly considerable drawback of these methods is, that they are laboratory-based based in otherworld's cannot be used for practical field analysis which is highly needed

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for nowadays industrial applications[5]. As a result of this, the development of field applicable, electrode - solution interface for the analytical quantification of nitrite at low concentration levels is of significant interest in the environmental as well as analytical chemistry protocols[2-5].

Electrochemical methods overcome the above problems associated with conventional methods. In the era of electroanalytical chemistry, various types of electrochemical interfaces have been developed for sensor applications[6]. A few of these include thin film electrodes, bulk-modified electrodes, carbon epoxy composites, sol-gel electrodes, solid electrodes, etc. Even though these electrodes have been well established and used, they require a prolonged experimental protocol for their preparation and pretreatment and more over less reproducible current-potential responses[7]. In order to bottleneck these issues, it is highly adequate to introduce a new class of electrochemical interfaces. One such is, the Screen-Printed Electrodes (SPEs). In recent years, these electrodes have prone to gained significant attention from electrochemists that to in electroanalysis due to their easy fabrication, portability, and possibility of doing in situ experiments. These SEPs have been used for many applications but face a lack of selectivity due to the absence of specific interaction between the interface and target analyte. In order to increase the selectivity of unmodified SEPs, it is highly desirable to tune its surface structure with modifier motifs that can interact with the analyte specifically[8-12]. A wide variety of modifiers have been used which include dyes, graphene, CNTs, graphite, metals, metal oxides, etc. Among the modifiers metal oxide based ZnO is less explored in the literature towards the analytical quantification of nitrite ions curing chemically modified screen-printed electrodes.

In the present investigation, we have successfully synthesized ZnO nanoparticles using a green chemical approach and subsequently employed in the designing of chemically modified screen-printed electrodes[13]. Further, the practicality of the proposed sensor interface has been demonstrated by taking nitrite as a model analyte system and subsequently validated by using a naturally available sample.

2.0 Materials and Method

2.1 Chemicals and Reagents

AR Grade chemicals were used as received and procured from SD fine Chemicals.

2.2 Apparatus

Electrochemistry experiments have been carried out using CH-Instrument. Zinc oxide modified screen printed electrodes (ZnO SPEs) behave as working electrodes, Pt wire as the counter electrode and Ag/AgCl as a reference electrode. Buffer solutions were prepared by using Control Dynamics (model APX 175). IR has been recorded using a Shimadzu spectrometer (Model: 8400S, Shimadzu. XRD studies were carried out using the Bruker XS Model).

2.3 Synthesis of Green Chemical Extract and Zinc Oxide Nanoparticles through Green Chemical Approach

ZnO particles have been prepared using the following green chemical solution combustion approach. Briefly, 2.97g of $Zn(NO_3)_2.6H_2O$ was dissolved in 2 mL of green chemical extract containing of 3 - 4 mL of water and subjected to stirring for 8 minutes[14]. Further, the contents were kept in muffle furnace at ± 400^0C and subjected for the smoldering type of combustion reaction. The reaction mixture was completed within a minute which in turn results in the formation of ZnO in the form of a fine powder. For the sac of optimization, the reaction was repeated with different concentrations such as 4 mL and 6 mL keeping same concentration of nitrite and the resulted product was calcinated at 400 °C for 2 hours to remove any unwanted impurities and to obtain pure ZnO nanoparticles.

2.4 Fabrication of Screen-Printed Electrodes (SPE) and ZnO Modified Screen Printed Electrodes (MSPE)

SPE were prepared and well characterized and is previously reported literature by craig's graph[8-12].

2.5 ZnO Modified Screen Printed Electrodes (MSPE)

The SPEs were modified with as synthesized ZnO NPs through a drop casting method. 1 mg/mL aqueous colloidal suspension of ZnO was prepared and 10 µL of which has been coated on to the surface of SPE and dried it for about 1 hour.

2.6 Analytical Procedure

The electrochemistry of the sensor interface towards the determination of nitrite ion was investigated using

CV and DPV techniques. The CV were recorded at unmodified and ZnO modified SPEs in presence of nitrite in the potential window from 0.2 to 1.6 V at a scan rate of 50 mV/s in a phosphate buffer solution of pH 7. Similarly, Differential Pulse Anodic Voltammetry (DPAV) was performed under same experimental conditions in the potential range between 0.3 and 1.6 V.

2.7 Sample Preparation

Determination of nitrite in water samples: The bore well water is used as it is. The sewage water was soaked with activated charcoal and filtered in order to remove odor and impurities present in it.

Nitrite in sweet orange: sweet orange fruit juice is prepared by peeling and squeezing 100 gm of fruits using juice maker. Further it has been, filtered and made up to 100 mL with the help of standard volumetric using DI water. Finally, 5 mL of this was used for the analysis.

Nitrite in sweet lime fruits: sweet lime fruit is prepared by peeling and squeezing 100 gm of fruits using juice maker. Further it has been, filtered and made up to 100 mL with the help of standard volumetric using DI water. Finally, 5 mL of this was used for the analysis.

Nitrite in Sweet lemon: sweet lemon juice is prepared by peeling and squeezing 100 gm of fruits using juice maker. Further it has been, filtered and made up to 100 mL with the help of standard volumetric using DI water. Finally, 5 mL of this was used for the analysis.

Determination of nitrite in tomato: tomato juice is prepared by peeling and squeezing 100 gm of fruits using juice maker. Further it has been, filtered and made up to 100 mL with the help of standard volumetric using distilled water. Finally, 5 mL of this was used for the analysis.

Nitrite in egg samples: These were collected from departmental stores in Tumakuru. The samples were prepared by extracting the liquid portion of the egg into 100 mL beaker and stirred till to get homogeneous mass. Further the protein was precipitated by treating it with 10% trichloroacetic acid and finally it has been removed by a simple filtration. 25 mL of this was transferred into a volumetric flask and make up to the mark. Finally, 5 mL of this was used for the analysis.

3.0 Conclusion

3.1 Characterization of Synthesized Zinc Oxide Nanoparticles

3.1.1 UV-Vis Spectroscopy (UV-Vis)

The formation of zinc oxide nanoparticles has been preliminarily confirmed with the aid of UV- vis technique. The spectrograms were recorded by using aqueous colloidal solution of ZnO and $Zn(NO_3)_2$ through ultrasonication. The spectrum (Figure 1) reveals a characteristic absorption peak for zinc oxide at a wavelength of 369 nm (Figure 1a) and Zinc nitrate hexahydrate at wavelength of 300 nm (Figure 1b). This shift is a characteristic indicative of nanoparticles formation and result is in good comparison with the reported literature[15,16].

3.1.2 Fourier Transform Infrared Spectroscopy (FT-IR)

Fourier transform (FTIR) measurements were employed to confirm the formation of the ZnO material. Spectrum of synthesized ZnO NPs has been obtained using KBr pellet method. The Figure 2a corresponds to spectrum of zinc nitrite hexahydrate and Figure 2b for synthesized ZnO NPs. Figure 2a confirms the presence of Zn-N bond through characteristic peak at 832cm^{-1}. Whereas Figure 2b shows a peak at 773 cm^{-1} which is a characteristic for Zn-O bond. This clearly confirms the formation of ZnO particles in the proposed method[17,18].

3.1.3 Surface Morphology of ZnO Particles

SEM is considered as one of the powerful tools to understand the surface morphological structure of the synthesized material. Figure 3 depicts the surface morphology of as synthesized ZnO particles at different magnification. From the micrographs, it is revelated that the as synthesized ZnO particles has narrow rods and spike like structure (Figure 3a) and plate like structures (Figure 3b)[19].

3.2 Electrochemistry of Nitrite at Modified Electrode

The electrochemical behavior of ZnO modified SPEs towards nitrite ion was studied by using cyclic voltametric technique in order to explore its electrochemical behavior and also the interacting ability of the as synthesized ZnO interface with nitrite ions. CV profile of bare and

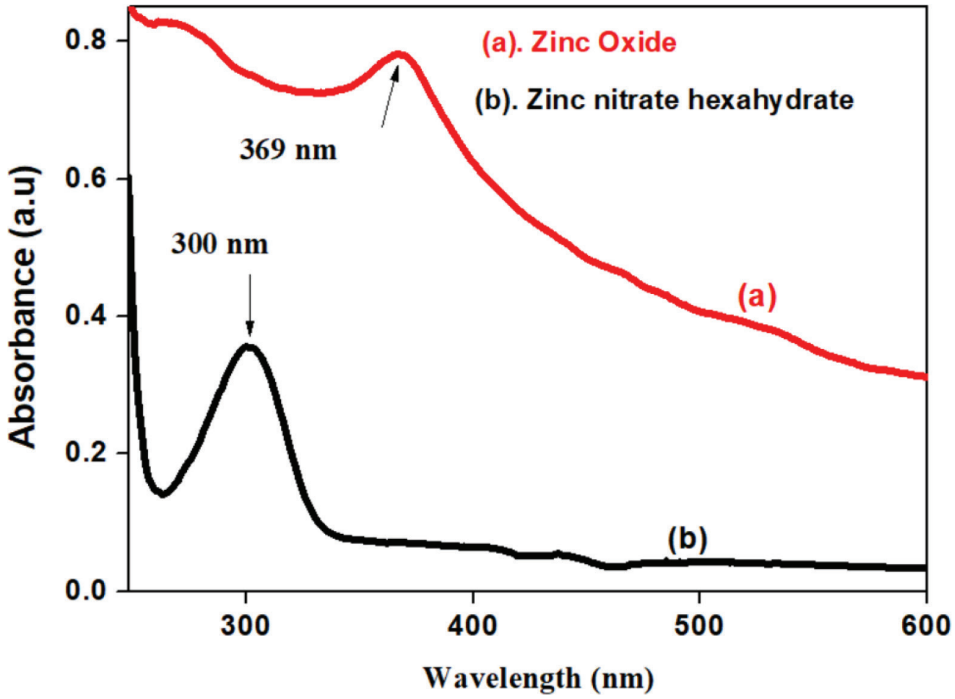


Figure 1. UV-Vis spectrum of: a) Synthesized ZnO nanoparticles b) Zinc nitrate hexahydrate.

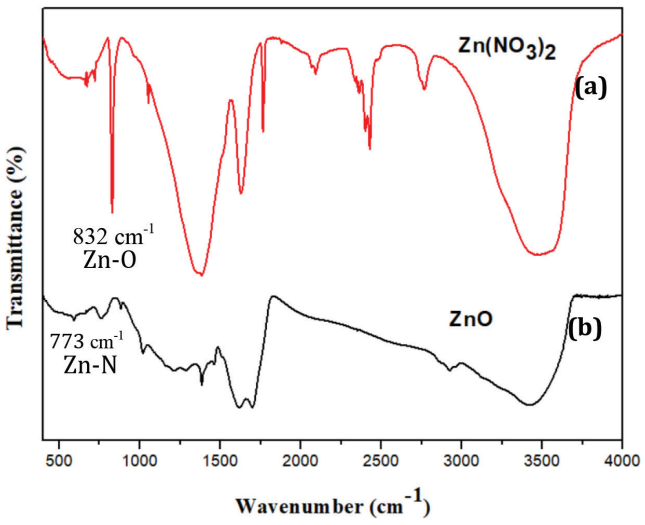
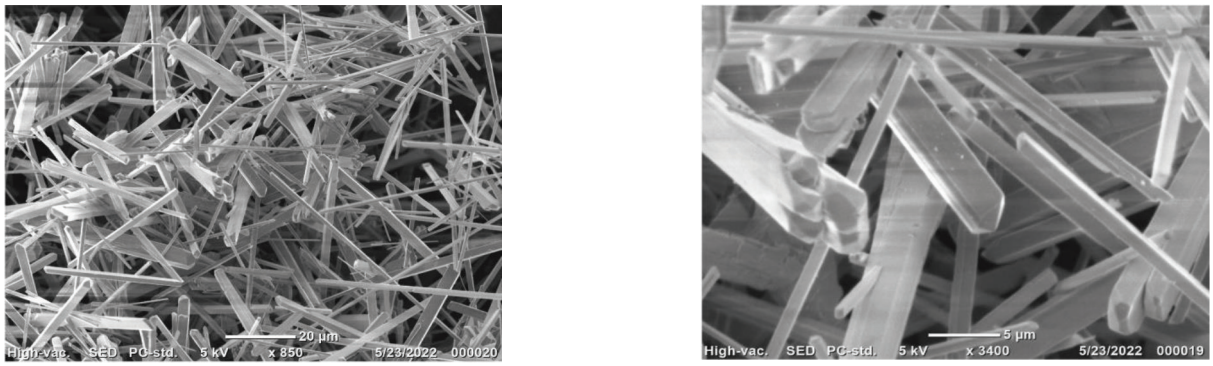


Figure 2. FTIR spectrum of a) zinc oxide nanoparticles and b) Zinc nitrate hexahydrate.

modified electrodes have been recorded in presence and in the absence of 1mM of nitrite ions in an aqueous PBS of pH 7 in the potential range from 0.2 to 1.6 V with a scan rate 50 mV/sec. and are shown in Figure 3. The ZnO modified SPEs did not show any voltametric peaks in the absence of nitrite ions (3a). This indicates that, the ZnO modified SPEs is not redox active and hence it can be used as a useful candidate in the designing of sensing platform for nitrite measurement in the given potential range. At the same time, the bare SPE showed a voltametric peak for nitrite oxidation (1mM) at a peak potential of 1.23 V with a peak current of 57 µA (Figure 3b). Whereas the ZnO modified SPEs showed a peak in the forward anodic sweep at a potential of 1.1 V with a peak current of 132 µA (Figure 3c) which is two to three times higher than that obtained at unmodified one. These observations revealed that the oxidation of nitrite ions is more favorable and also facile at ZnO modified SPEs with low over potential with enhanced peak current. Such response was not observed at bare SPEs. This clearly demonstrates that the observed response at the modified electrode is because of presence of ZnO nanoparticles. Hence, it is to conclude that, the ZnO modified SPEs showed potential affinity towards nitrite ions. The increased analytical response at modified interface is attributed to the porous nature of ZnO particles which inturn related to the catalytic and interacting ability of NPs towards nitrite ions. The observed response follows two electron oxidation of nitrite ions to through the following mechanism, which is in accordance with the reported literature[20,21].

$$2NO_2^- \rightarrow 2NO_2 + 2e^{-n}$$

$$2NO_2 + H_2O \rightarrow NO_3^- + NO_2^- + 2H^+$$



(a) (b)

Figure 3. SEM images of ZnO particles.

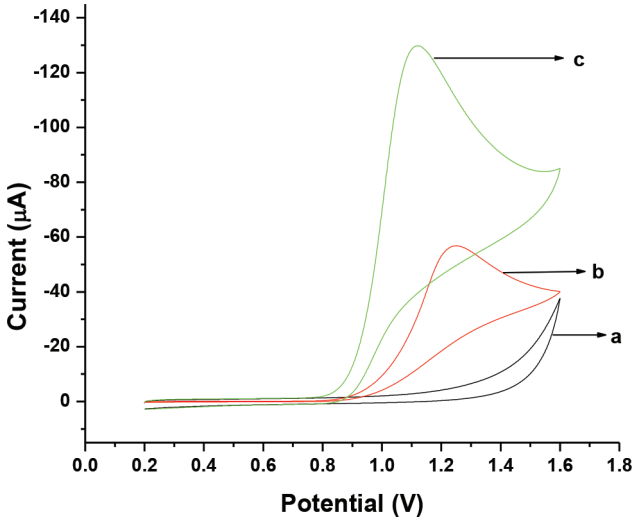


Figure 3. Cyclic voltammogram of 1 mM of nitrite ions in a PBS of pH 7 at 50 mV s^{-1}. a) M SPEs in the absence of nitrite, b) Bare SPEs in 1 mM of nitrite ions and c) modified electrode in 1 mM of nitrite ions.

With overall reaction being

$$2NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^{-n}$$

The oxidation of nitrite at modified interface is an irreversible process and more intensified or significant and hence the analysis was systematically studied by using DPV (Figure 4). For zero concentration of nitrite, the modified interface has not shown any peak (Figure 4a), but in its presence (10 µM) of a peak at 1.0 V for the oxidation of nitrite ion (Figure 4b). Hence the use of DPAV for quantification is expected to fine low detection limits.

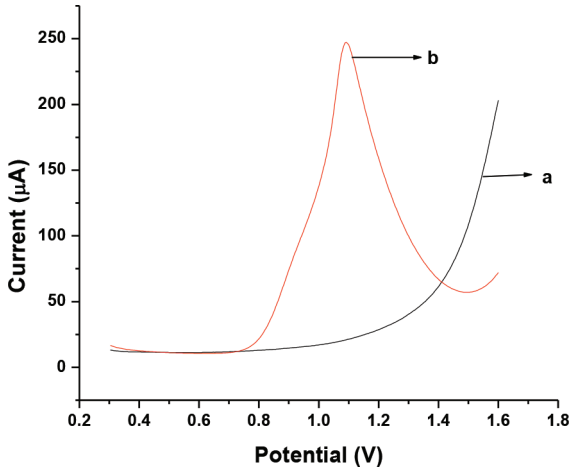


Figure 4. Differential pulse anodic voltammetry (DPAV) at ZnO modified SPEs in the absence (a) and in the presence of 10 µM of nitrite ion (b) in a PBS of pH 7.

3.3 Optimization Study

To obtain at most efficiency, the parameters which mainly decide the analytical response of nitrite ions at modified interface such as pH of buffer solution and thickness of the modifier molecule has been optimized and thoroughly discussed.

3.3.1 pH of Buffer Solution

The voltametric response in any I-V experiment depends on the pH value of the medium. Therefore, acetate buffer solution was prepared by the mixing of 0.1 M acetic acid and sodium acetate salt in the range from 3 to 6 and similarly, the phosphate buffer solution has been prepared by the uniform mixing of 0.1 M

sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate salts in the range between 6 to 9 (Figure 5). Cyclic voltammetry has been performed by using ZnO - SPEs in presence of 100 µM of nitrite ions in the buffer solutions in the pH range between 3 to 9. From the voltammogram it is revealed that the peak current increases from 3 to 7 and their after it starts to decrease. The peak current is considerably less at low pH value and is attributed to the instability of nitrite ions at acidic conditions and subsequently the current begins to increase as the pH of solution increases and becomes maximum at pH value of 7. This may be due to attainment of stability of nitrite ions at neutral pH. After pH 7, peak current begins to decrease may be due to the deficiency of protons for facile oxidation process[22-24]. As a result of this, phosphate buffer solution of pH 7 has been used as an optimized pH for all further investigations in the present study.

3.3.2 Thickness of Modifier

The determination of nitrite ion at modified sensor mainly due to the electrocatalytic activity of amount of catalyst particles present on the surface of GCE. Hence, it is adequate to optimize the volume of ZnO NPs by keeping the surface area of SPEs as constant. In this relation, the voltametric experiments have been carried out using 100 µM of nitrite ions in a PBS of pH 7 by varying the volume of colloidal solution of ZnO NPs from 2 to 14 mL. The variation of peak current for the nitrite oxidation respect to volume of modifier is shown in Figure 6. From the Figure, it is very clear that, the analytical response for the oxidation of nitrite ions from 2 mL to 14 mL increases and there after decreases may be due to surface saturation of SPEs. As a result of this, 10 µL of colloidal solution has been settled as an optimum modifier concentration for further analysis.

3.4 Analytical Merits

3.4.1 Calibration Plot

For practical applications, it is adequate to construct the range wherein the fabricated interface has to show the linear variation of peak current with respect to analyte concentration. Hence, calibration plot has been constructed with the help of DPAV curves by the successive addition of 50 µM of nitrite ions. The resource showed a linear response up to 800 µM (Figure 7) with a detection limit of 0.5 µM (3σ)[25]. As a result of this, the proposed interface has been validated by comparing it with reported results (Table 1). It signifies the importance of the proposed interface towards the detection of nitrite ions.

3.4.2 Interference Study

Accurate determination of nitrite from real sample is a difficult task due to the interference of common ions etc. which are normally encountered in the real sample. This was evaluated by taking 100 µM of nitrite ions with

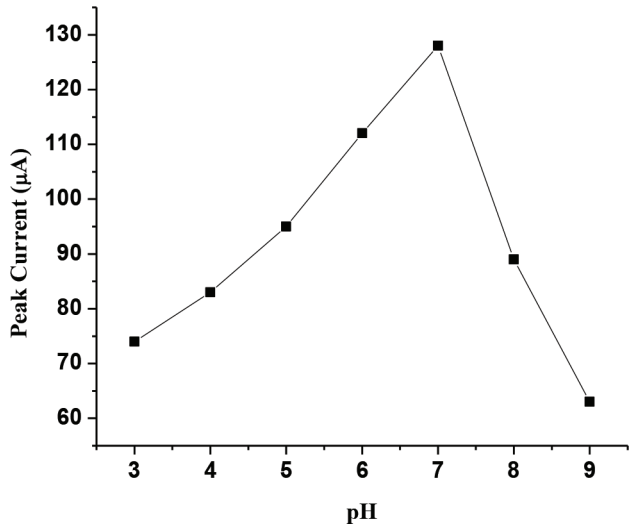


Figure 5. Variation of peak current with respect to pH of acetate (from pH 3 to 5) and phosphate (from pH 6 to 9) buffer solutions.

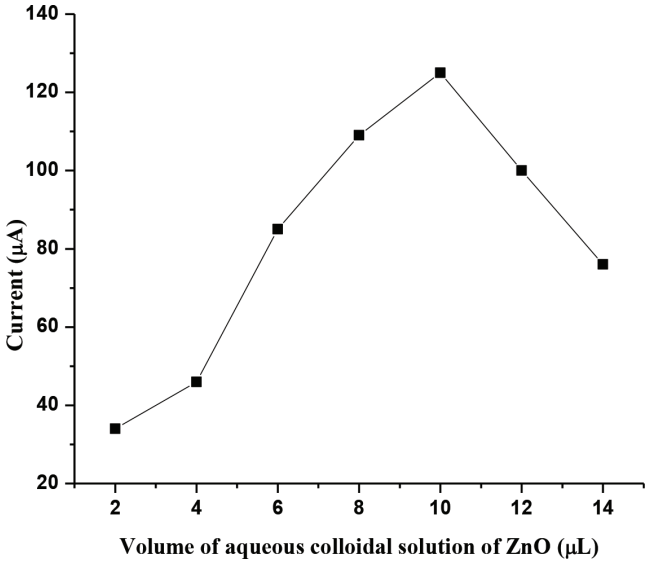


Figure 6. Effect of volume of modifier with respect to nitrate oxidation current.

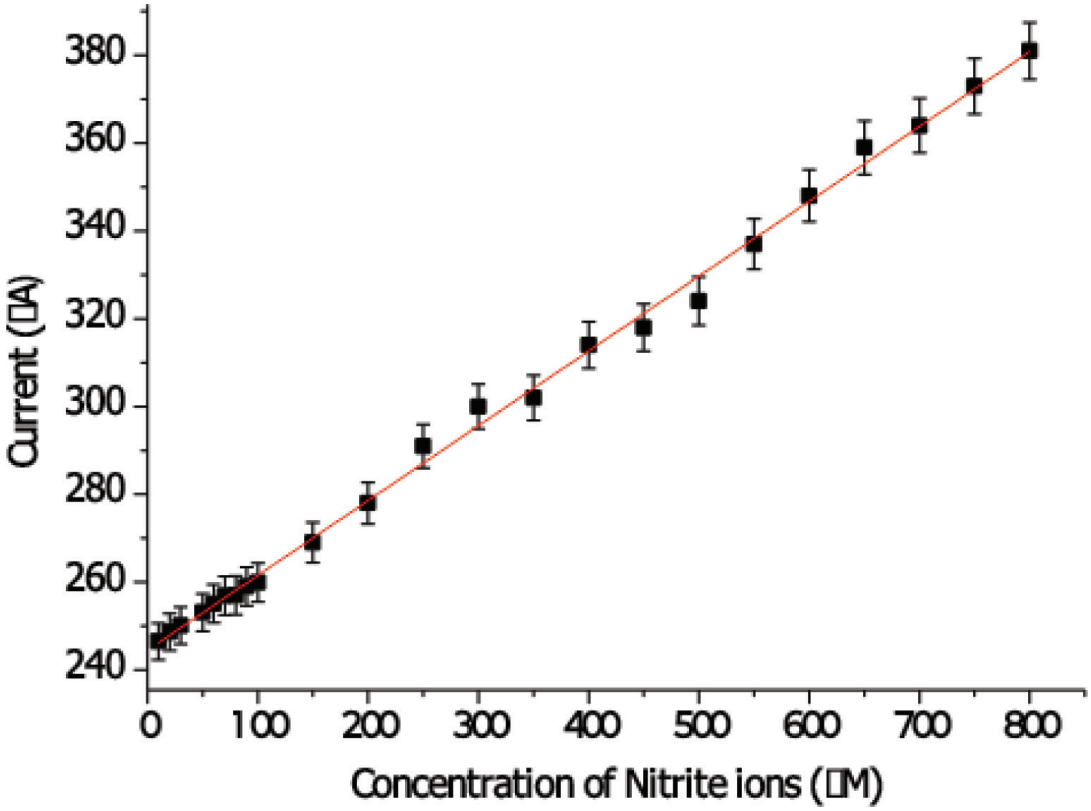


Figure 7. Calibration plot in nM concentration levels.

Table 1. Comparison table

Modified electrode	Linear range (µM)	Detection limit (µM)	Refer ence
Zr based porphyrin (MOF 525)	20 - 800	2.1	[26]
Au nanorods /Nafion	3 - 6000	0.6	[27]
AuNPs/MOF-5	5 - 65000	1.0	[28]
MOF-525/GNRs-50	100 - 2500	0.75	[29]
rGO/PANI/AsM	25 - 7500	0.803	[30]
AgNPs/CS/GSPE	30 - 1140	1.84	[31]
ZnO - SPEs	**Upto 800**	**0.5**	**Present work**

Table 2. Interference Study

Foreign ions	Tolerance limit (µM)
NH_4^+, Ba^{2+}, Mg^{2+}, Ca^{2+}, PO_4^{3-}, SO_4^{2-}, K^+, Mn^{2+}, CH_3COO^-, $C_2O_4^{2-}$, CO_3^{2-}.	10000
Hg^{2+}, Bi^{2+}, Sn^{2+}, Fe^{3+}, Pb^{2+}, Na^+ Ca^+	900
Cu^{2+}, Ni^{2+} SiO_3^{2-}.	500

different ions (Table 2). The results of the table convey the least interference at modified electrode, and this may be due to specific and selective interaction of the ZnO modified GCE with nitrite ions.

3.4.3 Application Study

The practical use of modified interface has been evaluated by the measuring the amount of nitrite present in natural samples. Each 5 mL of sample was taken in an electrochemical cell containing 5 mL of buffer solution. The recovery was done by taking three successive measurements (n=3) (Table 3).

4.0 Reproducibility of the Results

The reproducibility of the proposed sensor interface has been checked by taking the same electrode for 10 cumulative measurements in a PBS of pH 7 containing 100 µM of nitrite ions under identical conditions. The relative standard deviation was found to be ± 7 %.

(ND = not declared)

Table 3. Determination of nitrite ions from various real samples

Sl. No.	Sample	Original (µM)	Spiked (µM)	Total (µM)	Recovery (%)
1	Borewell water	ND	100	103	101
2	Sewage water	ND	100	102	102
3	Sweet orange - 1	ND	100	105	105
4	Sweet orange - 2	ND	100	106	106
5	Sweet lime fruit - 1	ND	100	104	104
6	Sweet lime fruit - 2	ND	100	104	104
7	Sweet lemon - 1	ND	100	102	102
8	Sweet lemon - 2	ND	100	107	107
9	Tamoto - 1	ND	100	105	105
10	Tamoto - 2	ND	100	104	104
11	Egg - 1	ND	100	108	108
12	Egg - 2	ND	100	107	107

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