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Fabrication of highly sensitive nitrite electrochemical sensor in foodstuff using nanostructure sensor

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In this research, we introduced a powerful electrochemical sensor (based carbon paste electrode) for analysis of nitrite in foodstuff, using CdO decorated single wall carbon nanotube incorporated with 1-methyl-3-butylimidazolium bromide (CdO/SWCNTs/1-3-MBIB/CPE). Our results revealed that CdO/SWCNTs/1-3-MBIB/CPE shows excellent electro-catalytic activity towards electro-oxidation of nitrite. The obtained data illustrated an irreversible oxidation peak current at 0.92 V, pointing to the oxidation of nitrite. The CdO/SWCNTs/1-3-MBIB/CPE exhibited a linear response from 0.1 μM to 900.0 μM of nitrite with no interfering from other food compounds. The CdO/SWCNTs/1-3-MBIB/CPE has been used for determination of nitrite in real samples.

Keywords: Nitrite determination, CdO/SWCNTs, Sensor, Food analysis

1. INTRODUCTION

Nitrite can be prevents from bacterial growth and therefore it used for the meat curing. Also this compound is an important reducing agent, in a reaction with the meat's myoglobin. It has important role in food industrial because nitrite can interacts with amines to produce nitrosamines [1]. The high value of nitrite content in the human blood results a decrease in oxygen transportation by the human blood follow-on methemoglobinemia, "blue baby syndrome" which is the reaction of nitrite with Fe (II), resulting transformation of hemoglobin to methemoglobin (HbFe (III)) [2]. According to the above points, the assay of nitrite is vital in environmental safety. Therefore, analysis of nitrite have been suggested with spectrophotometry [3,4], chromatography [5-7] and electrochemical techniques [8–12]. In between, electrochemical methods have more attention for determination of components in environmental or biological samples due to fast response analysis [13-22]. On the other hand, electrochemical techniques specially by using chemically modified electrodes is reported to be easy, fast and cheap compared to the other methods [23-32]. Because of the nitrite electro-oxidation includes a comparatively high over-potential at the surface of bare working electrode, so the` utility of nitrite oxidation is limited by using unmodified bare electrode [8-12]. The chemically modified electrodes (CME) have been expanded to amplify the sensitivity as well as decrease the over-potential upon electro-oxidation [34-37]. Also, modification of electrode surfaces results extending in the dynamic range and associates decrease in the detection limit in analytical determinations [38-40]. Nanomaterials, organic ligands, inorganic complexes, DNA and ionic liquids are usual mediators for modification of bare electrodes for sensitive and selective analysis [41-50]

In this paper, we used different electrochemical techniques at pH 7.4 to demonstrate the electrochemical oxidation of nitrite on the CdO/SWCNTs/1-3-MBIB/CPE. The CdO/SWCNTs/1-3-MBIB/CPE showed the highly sensitivity activity compared to the other electrodes for analysis of nitrite. Finally, CdO/SWCNTs/1-3-MBIB/CPE was effectively used for the assay of nitrite in foodstuff.

2. EXPERIMENTAL

2.1. Chemicals

Sodium nitrite and graphite powder (<50 µm) were obtained from Merck. Other compounds such as cadmium acetate, phosphoric acid and single wall carbon nanotubes were purchased from Sigma-Aldrich. Single-walled carbon nanotubes were synthesized according to Cheraghi and Taher reported procedure [51].

2.2. Apparatus

All voltammetric and EIS investigations have been done by means of µ-Autolab, potentiostat/galvanostat (NOVA software), Metrohm Model 663 VA stand. Ag/AgCl/KCl$_{sat}$, CdO/SWCNTs/1-3-MBIB/CPE and Pt wire which are used as the reference, working and auxiliary electrodes, respectively.

2.3. Modified electrode fabrication

After each voltammetric run, a clean surface of electrode was acquired by polishing its surface. CdO/SWCNTs/1-3-MBIB/CPE was prepared by mixing of 0.25 g of ionic liquids binder, 0.80 g of the liquid paraffin, 0.15 g of CdO/SWCNTs, and 0.9 g of pure graphite powder. Then the mixture was shacked (by shaker) well for 3 h pending a homogeneously moistened paste was achieved. A part of the paste was packed definitely into one glass tube as explained above to get ready CdO/SWCNTs/1-3-MBIB/CPE.

3. RESULTS AND DISCUSSION

3.1 Characterization of synthesized CdO nanoparticles

CdO/SWCNTs morphology was characterized by TEM method that was showed in Fig. 1. As can be seen the CdO nanostructure grown on carbon nanotubes surface. Presence of a covalent bond between CdO nanoparticle and COOH positions at a surface of SWCNTs can be cause for preparation of CdO/SWCNTs.

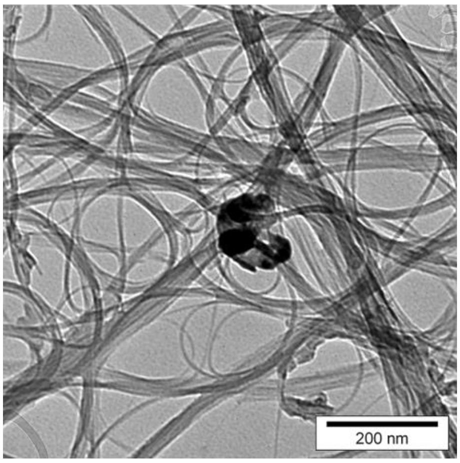

Figure 1. TEM image of CdO/SWCNTs

3.2 Electrochemical oxidation of nitrite

The active surface area determine by Randles–Sevcik equation. The results obtained were 0.18, 0.16, 0.14 and 0.12 cm^2 for CdO/SWCNTs/1-3-MBIB/CPE, 1-3-MBIB/CPE, CdO/SWCNTs/CPE and bare CPE (CPE), respectively.

Current density is a consequent of CVs of 900 μM nitrite (pH 7.4) at the surface of different propose sensors and has been shown in Figure 2 insert. The results confirm that the attendance of manufactured CdO/SWCNTs together with conductive binder (1-3-MBIB) cause increasing in the conductivity of electrode. The direct electrochemical oxidation of nitrite on the surface of different sensors was searched by the cyclic voltammetry method (Figure 2). CdO/SWCNTs/1-3-MBIB/CPE exhibited large oxidation current around 920 mV with the peak current of 39.6 μA (curve a). On the other hand, low electrochemical oxidation peak was observed at CPE (curve d) over the same condition. The nitrite oxidation peak potential at CdO/SWCNTs/CPE and at CPE observed around 960 and 1000 mV with the oxidation current of 28.5 and 22.3 μA, respectively. Also, at the surface of 1-3-MBIB/CPE, the oxidation peak potential can be seen at 940 mV with the oxidation peak current of

33.9 µA (curve b), which indicates enhancing in the oxidation peak currents in the present of conductive binder in CPE and also diminishing its over-potential. The results confirm that the presence of synthesized CdO/SWCNTs and 1-3-MBIB on CdO/SWCNTs/1-3-MBIB/CPE surface has large enhancement in electrochemical oxidation response of nitrite.

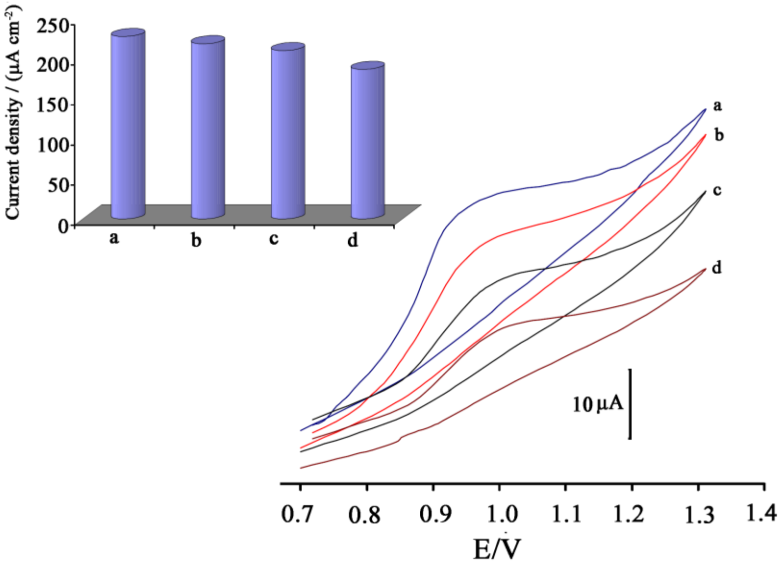

Figure 2. Cyclic voltammograms of (a) CdO/SWCNTs/1-3-MBIB/CPE, (b) 1-3-MBIB/CPE, (c) CdO/SWCNTs/CPE and (d) CPE in the presence of 900 µM nitrite at pH 7.4, respectively. Inset: the current density derived from cyclic voltammogram responses of 900 µM nitrite at pH 7.4 at the surface of different electrodes.

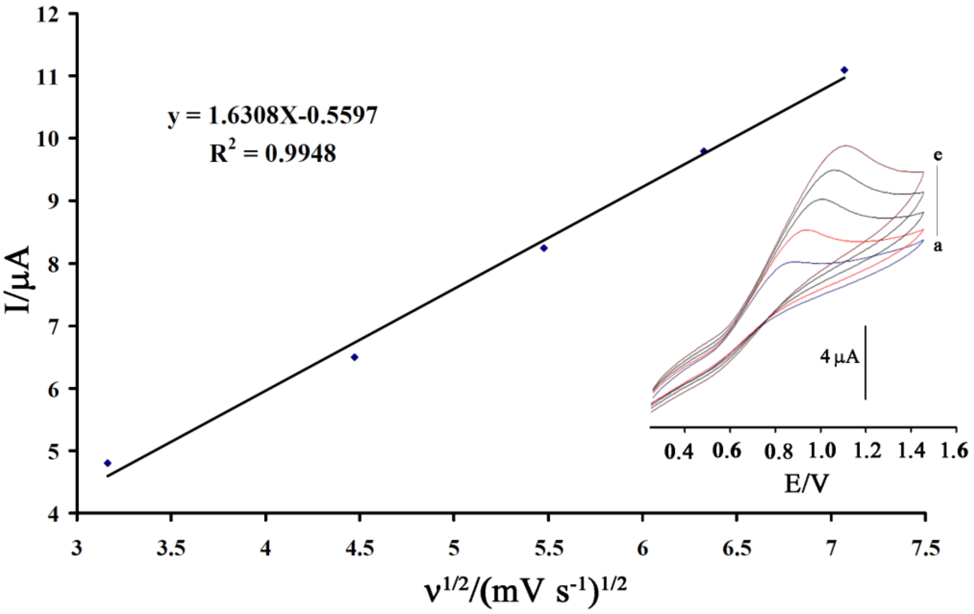

Figure 3. Plot of I_{pa} versus $\nu^{1/2}$ for the oxidation of nitrite at CdO/SWCNTs/1-3-MBIB/CPE. Inset shows cyclic voltammograms of nitrite at CdO/SWCNTs/1-3-MBIB/CPE at different scan rates (from inner to outer (a-e)) of 10, 20, 30, 40 and 50 mV s^{-1} in 0.1 M phosphate buffer, pH 7.4.

The dependence of the nitrite oxidation peak current response on the potential scan rate was evaluated by varying the scan rate during the electro-oxidation of nitrite at a surface of CdO/SWCNTs/1-3-MBIB/CPE (Fig. 3). The results revealed that the peaks current amplified linearly with raising the square root of scan rate that ranged from 10 to 50 mV s^{-1}, suggesting that the electro-oxidation of nitrite is controlled by the diffusion step [52-61]. Also the oxidation peak potential of nitrite shifts in negative direction by increasing the scan rate, pointing that the electrochemical reaction for nitrite is irreversible (Fig. 3 insert). The dependence of the peak potential (E_{pa}) and ln(ν) reveals a direct association with a regression eq. of:

$$E_{pa} = 0.1485 \ln(\nu) + 0.5093 \ (r^2=0.9902,\ E_p \text{ in V}, \nu \text{ in V } s^{-1}) \quad (1)$$

According to the Nicholson and Shain equation, the value of n(1–α) is calculated 0.2.

Electrochemical impedance spectroscopy (EIS) was also employed as a power surface investigation method to study the electro-oxidation of electroactive compounds [62-66]. Fig. 4 shows Nyquist diagrams of the EIS obtained at CPE (curve a); CdO/SWCNTs/CPE (curve b); 1-3-MBIB/CPE (curve c) and CdO/SWCNTs/1-3-MBIB/CPE (curve d) in the presence of 900 µM nitrite at pH 7.4, respectively. It can be seen that all of the electrodes exhibit an almost straight line that is characteristic of a diffusion limiting step of the electrochemical process. Nyquist diagrams trail the theoretical shapes and contain a compacted semicircle part, monitored at higher frequencies, which relates to the limit electron transfer. The respective semicircle diameters at the high frequency, corresponding to the electron transfer resistance at the electrode surface. The curve corresponding to bare CPE is a big semicircle plus a straight line (Fig. 4a). For CdO/SWCNTs/CPE, 1-3-MBIB/CPE, and CdO/SWCNTs/1-3-MBIB/CPE, R_{ct} decreases dramatically (Fig. 4 b–d), indicating that CdO/SWCNTs and 1-3-MBIB/CPE are high conductive mediator.

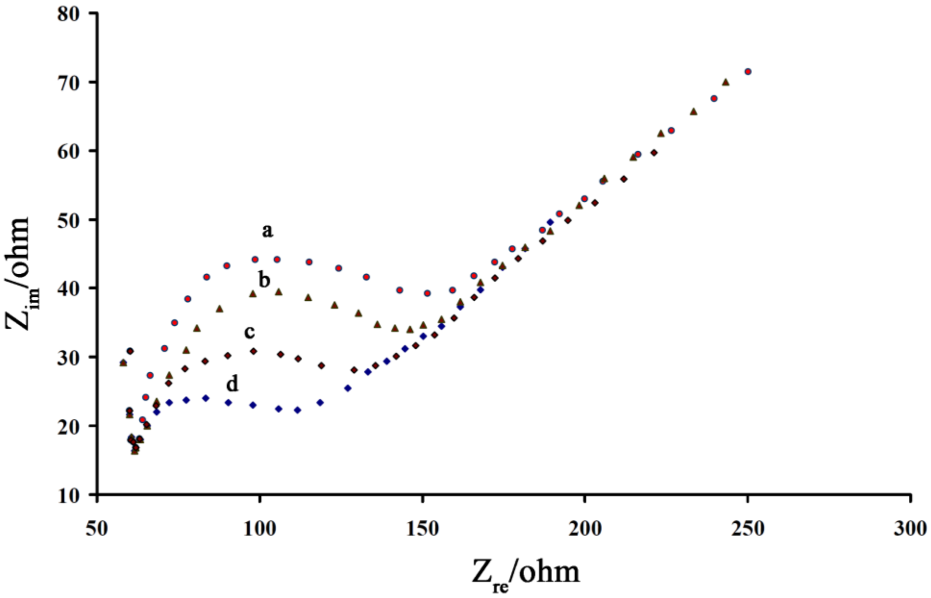

Figure 4. Nyquist plots of CPE (a), CdO/SWCNTs/CPE (b), 1-3-MBIB/CPE (c), and CdO/SWCNTs/1-3-MBIB/CPE (d) in the presence of 500 µM CPZ. Conditions: pH, 7.0; E_{dc}, +0.9 V vs. Ag/AgCl; E_{ac}, 5 mV; frequency range, 0.1–100,000 Hz.

Chrono-amperometric measurements of nitrite at CdO/SWCNTs/1-3-MBIB/CPE were carried out at a potential 1.5 V (Fig. 5A). The slopes of the consequential straight lines were then depicted *vs.* nitrite concentration (Fig. 5B). Using the obtained slopes and Cottrell equation the mean value of the diffusion (D) was calculated to be 7.37×10^{-6} $cm^2\,s^{-1}$.

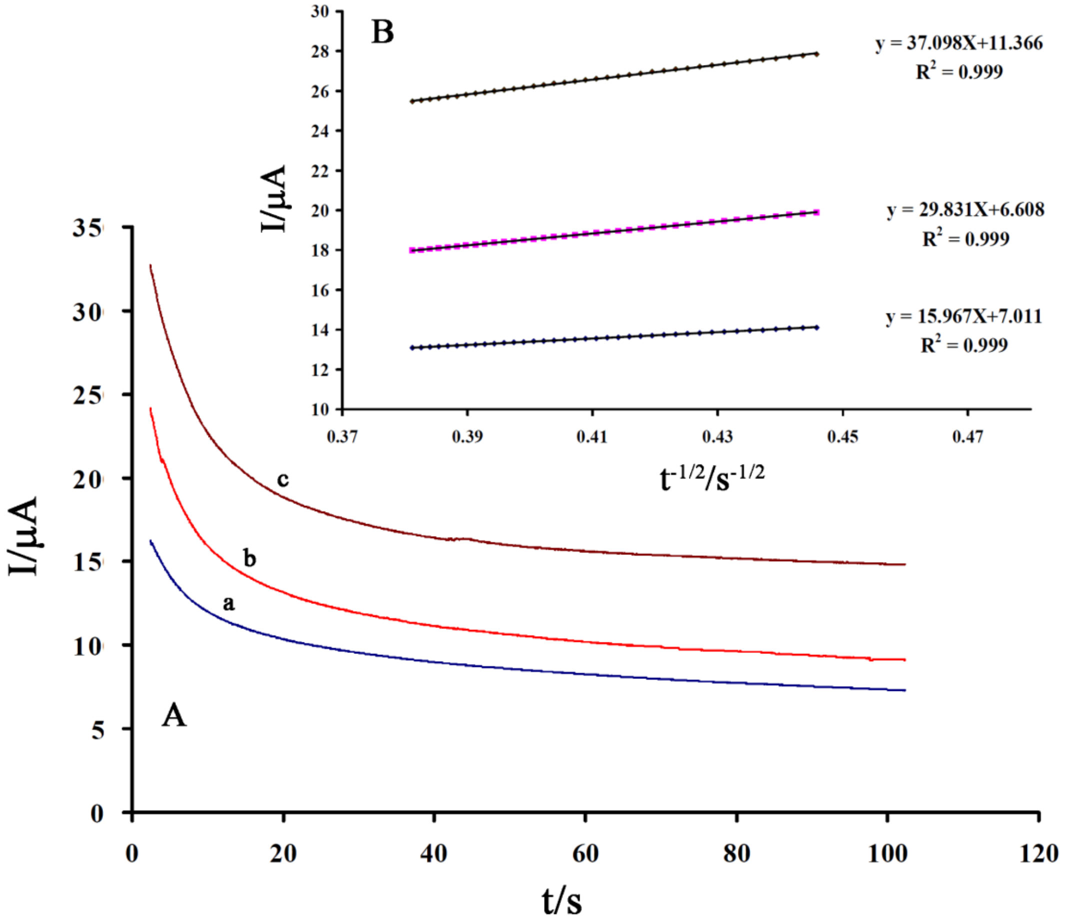

Figure 5. A) Chronoamperograms obtained at the CdO/SWCNTs/1-3-MBIB/CPE in the presence of a) 300; b) 500 and c) 800 µM nitrite in the buffer solution (pH 7.4). B) Cottrell's plot for the data from the chronoamperograms.

3.3 Calibration plot and detection limit

It is well-known that square wave voltammetry (SWV) has superior current sensitivity [67-70] compared to the linear sweep voltammetry (LSV) (Fig. 6). The nitrite shows a linear dynamic range 0.1–900 µM by square wave voltammetric method (see Fig. 6). The detection limit has been acquired at 0.07 µM nitrite with regarding the definition of $Y_{LOD} = Y_B + 3\sigma$. This dynamic range and limit of detection for nitrite compare to previous published paper in table 1.

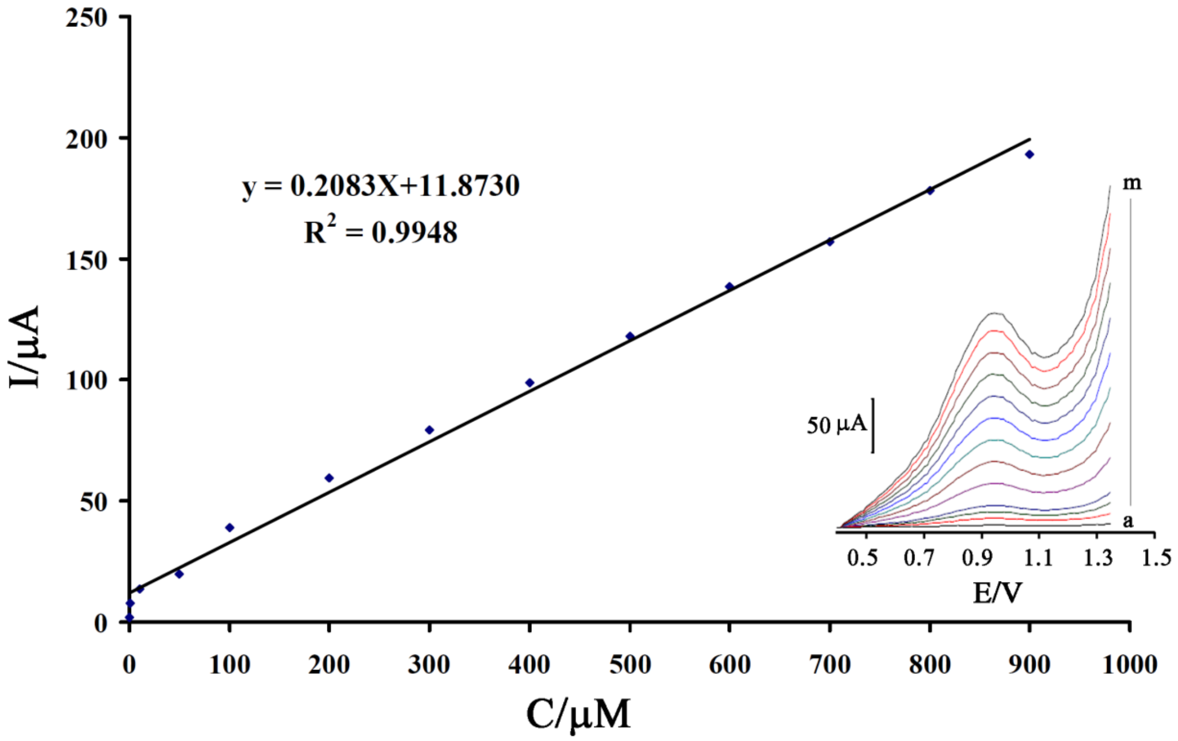

Figure 6. The plots of dynamic range for nitrite concentration. Inset shows the SWVs of CdO/SWCNTs/1-3-MBIB/CPE in the presence of different concentrations of nitrite (0.1, 1.0, 10.0, 50.0, 100.0, 200.0, 300.0, 400.0, 500.0, 600.0, 700.0, 800.0 and 900.0 µM; from inner to outer).

3.4 Steadiness and repeatability assay

The steadiness as well as repeatability of CdO/SWCNTs/1-3-MBIB/CPE was investigated by SWV (square wave voltammetry) measurements of 20.0 µM nitrite. The RSD % for ten sequential analyses was 1.7%. By utilizing five diverse electrodes, the RSD % for ten measurements calculated to be 2.9 %. By storing the electrode in the laboratory, the customized electrode keeps about 96.8 % of its initial response after two week and 91.8% after 45 days. These consequences show that CdO/SWCNTs/1-3-MBIB/CPE has worthy steadiness as well as reproducibility, and could be used for nitrite determination.

3.5 Interference study

The effect of different substances as potentially interfering compounds towards assessment of nitrite was investigated under the optimum conditions: 5.0 µM of nitrite at pH 7.4. The consequences reveal that 1000-fold of glucose, Mg^{2+}, Na^{+}, Ca^{2+}, Al^{3+}, Cl^{-}, F^{-}, SO_4^{2-}, K^{+}, Br^{-}, fructose, lactose, sucrose, 500-fold of methionine, phenyl alanine, L-theronine, alanine, histidine, glycine and ascorbic acid (After addition of 1 mmol L^{-1} ascorbate oxidase) did not affect the selectivity. Nor did saturation of starch solution influence the assessment of nitrite.

Table 1. Comparison of the efficiency of CdO/SWCNTs/1-3-MBIB/CPE with some published papers

Electrode	Method	LDR (μM)	LOD (μM)	Ref.
Carbon paste	Cyclic voltammetry	3.9-405	0.613	[11]
Glassy Carbon	Differential pulse volatmmetry	0.5-7.5	0.1	[8]
Glassy Carbon	Chronoamperometry	20-640	0.4	[10]
Carbon paste	SWV	0.1-900	0.07	This work

3.6 Real sample analysis

Towards evaluation of practical effectiveness of the propose sensor, nitrite was determined in different food samples, using the standard addition method. These results are compared to those resulted by using the Griess assay method (See Table 2) [71]. These data point to that the determination of nitrite using the CdO/SWCNTs/1-3-MBIB/CPE was successful and insightful.

Table 2. Determination of nitrite in foodstuff.

Sample	Founded (mg kg^{-1})	Griess assay (mg kg^{-1})	F_{ex}	F_{tab}	t_{ex}	t_{tab}
Sausage	15.32±0.44	14.98±0.59	8.2	19.0	2.8	3.8
Pickled vegetable	5.67±0.40	5.55±0.55	7.8	19.0	1.7	3.8

4. CONCLUSION

A powerful vomltammetric amplified sensor (CdO/SWCNTs/1-3-MBIB/CPE) was developed as a new voltammetric sensor for nitrite analysis in foodstuff. CdO/SWCNTs/1-3-MBIB/CPE decreased the nitrite overpotential and increased its sensitivity ≈2 fold. The result showed a linear dynamic range in the range of 0.1–900 μM with the detection limit of 0.07 μM for nitrite analysis.

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