Food Anal. Methods (2012) 5:752–758
DOI 10.1007/s12161-011-9292-3

Sensitive determination of nitrite in food samples using voltammetric techniques

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Received: 10 May 2011 / Accepted: 18 August 2011 / Published online: 30 September 2011

Abstract A [5,10,15,20-tetrakis (4-methoxyphenyl) porphyrinato] manganese (III)chloride (TMOPPMn(III)Cl)-modified gold electrode sensor was developed for the determination of nitrite in food samples. The developed sensor showed an excellent catalytic activity and stability for nitrite oxidation. Under optimized conditions, nitrite concentration as low as 2.9×10^{-9} M can be determined in various food samples using the developed sensor. Effect of common interfering ions have been investigated in simulated mixtures containing high levels of interfering ions and the sensor was found to be tolerant against these ions. The determination of nitrite in food samples such as chicken ham, sausage and pickled vegetables with the proposed sensor was in good agreement with those obtained by standard spectrophotometric method.

Keywords Nitrite · Sensor · Modified electrode · Food samples

Introduction

Nitrite ion (NO_2^-) is one of the active intermediate species in the nitrogen cycle and a useful indicator of equilibrium state of the oxidative and reductive pathways of the nitrogen cycle. It is a simple oxy-anion of nitrogen with a pK_a of 3.2 at 20 °C (Braida and Ong 2000). Nitrites are both of environmental and biological importance. Nitrite is ubiquitous within environment, physiological systems, and commonly used in some food as preservatives (Badea et al. 2001; Casella and Gatta 2004). Nitrite is used in meat curing due to three functions; (a) antimicrobial action (as preservative), (b) gives characteristic flavour of cured meat and (c) gives characteristic pink colour to cured meat due to the formation of nitrosomyoglobin. Moreover, nitrite can be formed in the production of pickled vegetables because of the biodegradation of nitrate or other nitrogenous substances (Santos et al. 2009).

The health hazards caused by the build-up of high nitrite concentrations in food samples, considering their use as preservatives, are subjects under investigations (Aydin et al. 2005). Once NO_2^- is taken by the human body, it combines with blood pigments to produce meta-haemoglobin in which oxygen is no longer available to the tissues. In addition, nitrite can also react with amines and amides in stomach to produce carcinogenic *N*-nitrosamines (Fann and Kaneine 1993; Mirvish 1995). Therefore, the quantitative determination of nitrite concentrations is of great importance, especially for food quality control.

Various analytical methods have been used to determine nitrite ions, such as spectrophotometry (Fox 1979), gas chromatography (Helmke and Duncan 2007), ion chromatography (Butt et al. 2001) and chemiluminescence (Arthur et al. 2007). However, these methods proved to have limitations with respect to specificity, sensitivity, simplicity and analysis time. Even with the Griess assay (Fox 1979), the widely used classical method for the determination of nitrite is highly sensitive and specific; it is often associated with drawbacks such as longer coupling time, use of carcinogenic reagents and large sample volumes. Electrochemical techniques provide useful alternatives as they follow simple, cheap and safe analyses.

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Electrode modifications with deposition of various functional compounds have attracted much interest due to its potential application (Cardoso and Gushikem 2005; Caro et al. 2002). Electrodes modified by incorporating metalloporphyrins are prospecting candidates for various applications because they have excellent thermal, chemical, electrochemical and photochemical stability(Alves et al. 1992; Vijayanathan et al. 1993). Porphyrins have demonstrated good features as analytical sensors because they can electrocatalyze the electron-transfer reactions increasing the sensitivity of the electrode (Joseph and Girish Kumar 2010; Leena and Girish Kumar 2010). The coordinated metal, the peripheral substituents and the conformations of the macrocyclic skeleton influence the coordination and the related sensing properties of these compounds. In this context, a manganese(III) complex of a porphyrin, TMOPPMn(III)Cl (Fig.1), is used as an electrode modifier for the development of a sensor for nitrite in a gold electrode matrix.

Experimental

Apparatus

Voltammetric measurements were carried out with an Electrochemical analyzer (BAS Epsilon Bioanalytical System, USA) coupled to a PC. An electrochemical cell containing 10 ml of buffer solution with Ag/AgCl as reference electrode, a Pt wire as auxiliary electrode and the modified gold electrode (GE) as working electrode was used for all measurements. The pH measurements were carried out in a Metrohm pH meter.

Reagents

Doubly distilled water was used for preparing all aqueous solutions; using analytical grade reagent. Sodium nitrite ($NaNO_2$), monosodium dihydogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), zinc acetate ($Zn(CH_3CO_2)_2$), manganese acetate. Tetrahydrate ($Mn(CH_3CO_2)_2 \cdot 4H_2O$) were purchased from SD Fine Chemicals (Mumbai, India). Porphyrin and metalloporphyrin (TMOPPMn(III)Cl) were prepared as previously reported (Adler et al. 1967; Gaugham et al. 1975). Pyrrole and anisaldehyde were obtained from Sisco Research Laboratories Ltd (India) and purified prior to use. Nafion (5%) was purchased from Sigma Aldrich.

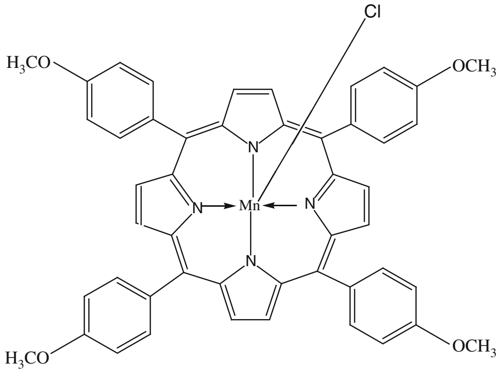

Fig. 1 Structure of TMOPPMn(III)Cl

Standards and sample solutions

Standard stock solutions

A standard stock solution of (1×10^{-1} M) was prepared by dissolving sodium nitrite in double distilled water. Working standard solutions were obtained by dilution of the stock solution.

Sample preparation for electrochemical assay

Samples of chicken ham, sausages and pickled vegetables were purchased at local stores and were treated according to the literature (Santos et al. 2009). Briefly, food samples were treated with saturated borax solution and zinc acetate (5%) solution was added to precipitate proteins. The mixture was diluted with water and filtered. The resulting filtrate solution was refrigerated under 4 °C for further studies. The nitrite concentration in real samples was determined using differential pulse voltammetric (DPV) technique and compared with those obtained with the standard spectrophotometric method.

Fabrication and characterization of working electrode

Gold electrode (GE), acquired from BAS Epsilon Bioanalytical System (USA), was used for sensor fabrication. Prior to modification, the electrode surface was mechanically polished with alumina slurries, rinsed with double distilled water and ultrasonicated successively in ethanol and water for 3 min each. The electrode was subjected to an electrochemical cleaning with 0.05 M sulphuric acid, after which it was rinsed with water and allowed to air dry. Gold electrode was modified by dropping 3 µl of 2.5mg ml^{-1} of TMOPPMn(III)Cl solution onto the clean electrode surface and evaporating the solvent at room temperature. TMOPPMn(III)Cl solution was prepared by dissolving 2.5 mg of TMOPPMn(III)Cl in a mixture of 3 ml nafion and 2 ml ethanol.

Surface morphological observations of bare GE and TMOPPMn(III)Cl-modified GE (TMOPPMn(III)Cl/GE) were carried out by scanning electron microscopy (SEM).

Comparison of SEM images of the bare GE and TMOPPMn(III)Cl/GE points to the effective modification of bare GE (Fig. 2a and b).

Surface area study

The microscopic areas of the bare GE and TMOPPMn (III)Cl/GE were obtained by cyclic voltammetry (Fig. 3a and b) using 1.0×10^{-3} M $K_3Fe(CN)_6$ as a redox probe containing 0.1 M KCl at different scan rates (Xu and Wang 2005). For a reversible process, the anodic peak current ip is linear to $\nu^{1/2}$ as follows:

$$\mathrm{ip} = \left(2.69\times10^5\right)n^{3/2}\mathrm{AC}_0 D_{\mathrm{R}}{}^{1/2}\nu^{1/2},$$

where ip refers to the anodic peak current, A the surface area of the electrode, C_o concentration of $K_3Fe(CN)_6$ and ν the scan rate. For 1.0×10^{-3} M $K_3Fe(CN)_6$, the electron transfer $n=1$, the diffusion coefficient $D_R=7.60\times10^{-6}$ cm^{2}s^{-1}. Thus, from the slope of ip vs $\nu^{1/2}$ relation, the microscopic areas of TMOPPMn(III)Cl-modified GE was calculated to be 0.1968 cm^{-2}, which was about three times greater than the bare GE (0.08249 cm^{-2}).

Analytical Procedure

Proper amount of sodium nitrite solution was transferred into an electrochemical cell, which contained 10 ml of 0.1 M phosphate buffer solution, and then the three-electrode system was installed on the cell. The solution was de-aerated with nitrogen for 5 min. Differential pulse voltammograms were recorded between 0.0 and 1.0 V at a scan rate of 0.1 Vs^{-1}. Pulse amplitude of 50 mV, pulse period of 200 ms and pulse width of 50 ms were used. The potential step was 4 mV. The peak current for oxidation of nitrite at a potential of 0.690 V was measured.

Results and discussions

Electrocatalytic oxidation of nitrite on modified gold electrode

The electrochemical behaviour of nitrite at a TMOPPMn (III)Cl/GE has been investigated using DPV and square-wave voltammetry (SWV). Since the oxidation of nitrite occurs at a lower potential of 0.690 mV (0.043 mA) using DPV compared to SWV (0.740 mV, 0.039 mA) further studies are carried out using DPV technique. The voltammetric response of 1×10^{-3} M nitrite in 0.1 M phosphate buffer solution (pH 6) at (a) bare GE and (b) TMOPPMn (III)Cl/GE has been recorded (Fig.4). As can be seen from the figure, nitrite does not generate a voltammetric response on bare GE. On the other hand, TMOPPMn(III)Cl/GE exhibits a sharper oxidation peak at a potential of 0.690 mV. This result illustrates that the TMOPPMn(III) Cl could present a favourable activity towards the oxidation of nitrite, suggesting that TMOPPMn(III)Cl/GE will be an excellent sensor for nitrite determination.

Optimizing the experiment conditions

The conditions for sensor fabrication and determination of nitrite were optimized, which included amount of TMOPPMn(III)Cl, effect of buffer solution and influence of buffer pH.

Effect of the amount of TMOPPMn (III)Cl

The amount of TMOPPMn(III)Cl solution on gold electrode directly determines the thickness of TMOPPMn(III)Cl film. As the amount was increased from 1 to 3 µl of 2.5 mg ml^{-1} TMOPPMn(III)Cl, oxidation peak current greatly enhanced. The enhancement of current indicates that the number of catalytic sites increased with increase in

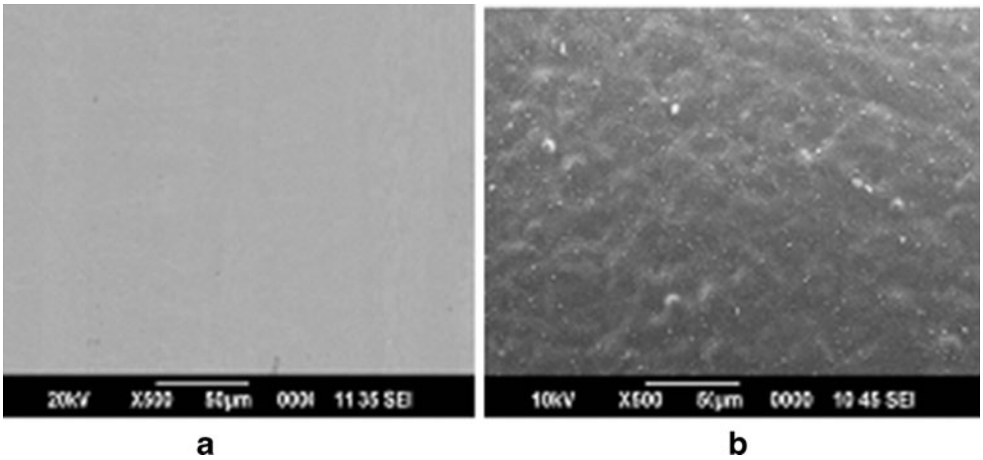

Fig. 2 SEM image of **a** bare GE and **b** TMOPPMn(III)Cl/GE

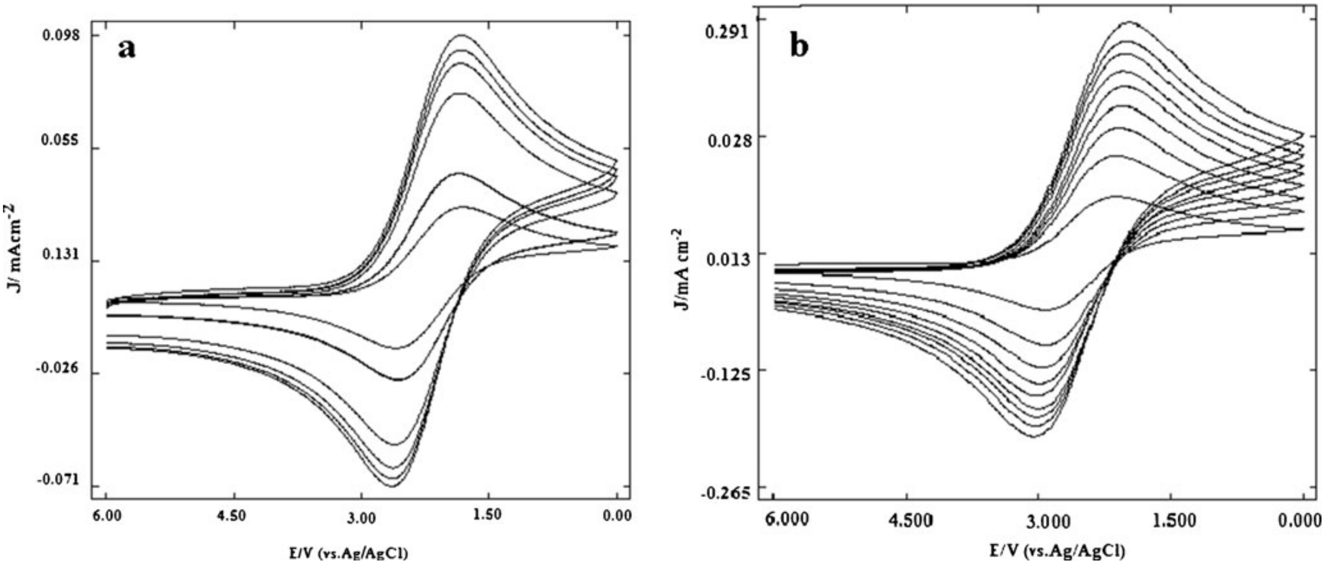

Fig. 3 Overlay of cyclic voltammograms of **a** bare GE and **b** TMOPPMn(III)Cl/GE in 1.0×10^{-3} M $K_3Fe(CN)_6$

the amount of TMOPPMn(III)Cl. Further increase in the volume of TMOPPMn(III)Cl, results in decrease of the peak current. This is because nafion is a kind of insulator (Yi et al. 2001), which blocks the electron transfer at higher concentrations. Hence, 3 μl of 2.5 mg ml^{-1} TMOPPMn(III) Cl was chosen for further analysis.

Effect of supporting electrolyte and pH

The influence of supporting electrolyte for nitrite determination was tested for different electrolytes of 0.1 M concentration (H_2SO_4, HCl, NaCl, KCl, NaOH, citrate and phosphate buffer). The best response was obtained with phosphate buffer solution and hence it was selected for further studies.

The effect of solution pH on the electrochemical response of nitrite was investigated in the pH range from 2.0 to 8.0 in 0.1 M phosphate buffer solution. Fig. 5 shows the effect of pH values on the oxidation peak current of 0.1 M nitrite. It can be seen that the maximal peak current appeared at pH 6.0. Therefore, pH 6.0 was chosen for further studies.

Effect of scan rate

The effect of scan rate on the oxidation peak current was studied by DPV. It was found that current density for

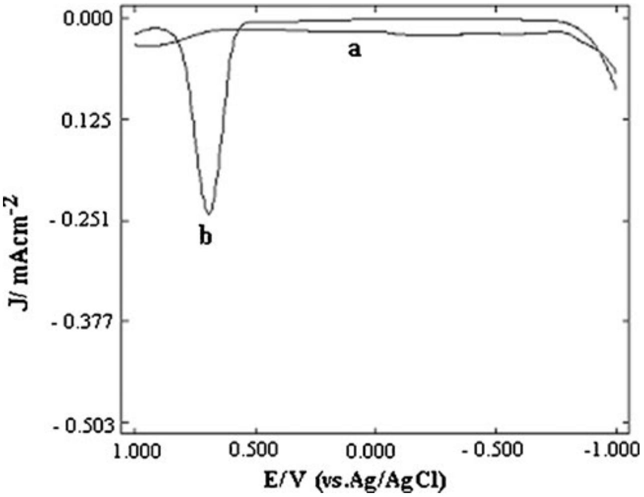

Fig. 4 Differential pulse voltammogram of nitrite at (*a*) bare GE (*b*) TMOPPMn(III)Cl/GE

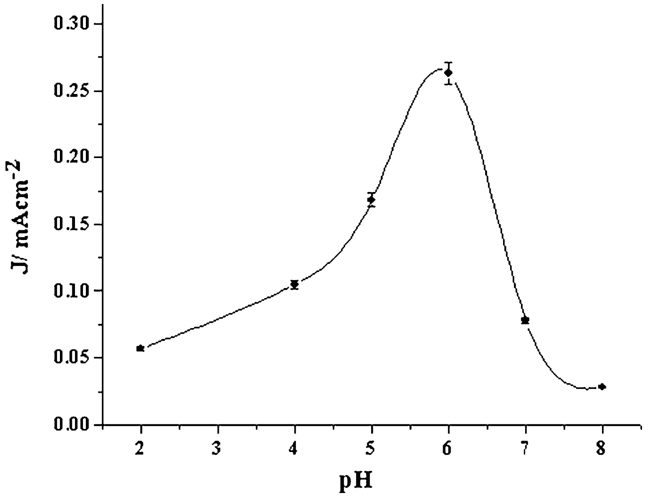

Fig. 5 Effect of pH

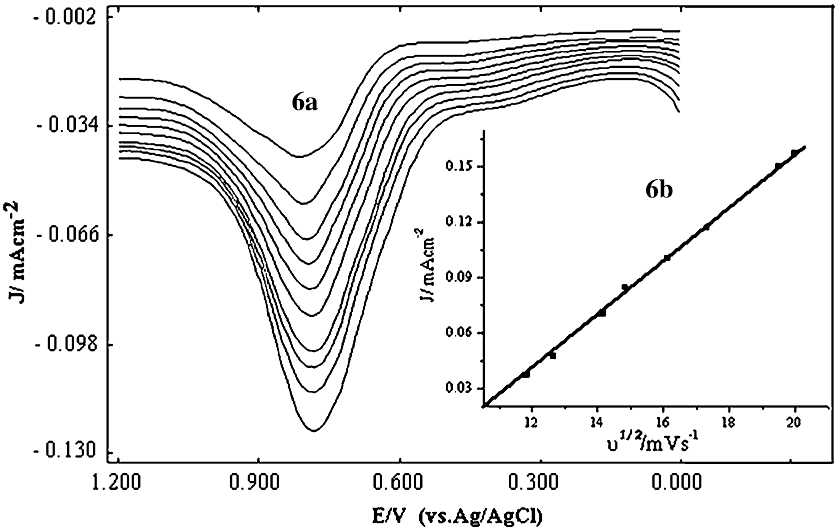

Fig. 6 (*a*) Overlay of differential pulse voltammograms for oxidation of nitrite at various scan rates. (*b*) Plot of square root of scan rate vs current density

oxidation of 1×10^{-3} M nitrite shows a linear relationship with scan rate in the range 100–400 mV s^{-1} (at 95% confidence interval, $j_p=0.0253+0.0027\sqrt{\nu}$); where j_p is the current density in milliamperes per square centimetre and ν is the scan rate with a good correlation ($r^2=0.9998$; Fig. 6a and b). The result demonstrates that the oxidation process of nitrite at TMOPPMn(III)Cl-modified gold electrode is controlled by the mass transport of nitrite ion from the bulk solution to the electrode surface.

Interferences

The selectivity and anti-interference ability of TMOPPMn (III) Cl/GE was tested by studying the effects of common interfering ions in the determination of nitrite. No obvious interference was seen in nitrite determination, on addition of 100-fold excess of Ca^{2+}, Cu^{2+}, K^{+}, PO_4^{2-}, CO_3^{2-} and NO_3^{-}. However, a 50-fold excess of ascorbic acid showed a noticeable interference in nitrite determination using TMOPPMn (III) Cl/GE sensor.

Analytical characteristics of the sensor

Under optimized conditions (0.1 M phosphate buffer solution of pH 6), differential pulse voltammetric studies for oxidation of nitrite was carried out at different concentrations (Fig.7a). The current densities calculated were plotted against various nitrite concentrations (Fig.7b), which gave a straight line [at 95% confidence interval, j_p (mA)=36.73+2458.06 C]; where j_p is the current density in

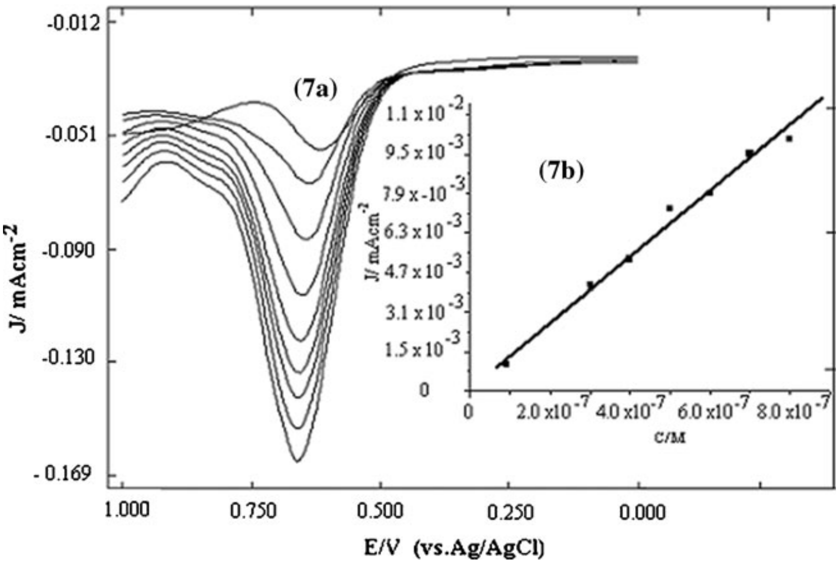

Fig. 7 (*a*) Overlay of differential pulse voltammograms for oxidation of nitrite at various concentrations. (*b*) Plot of various nitrite concentrations vs current density

Table 1 Comparison of different nitrite sensors for DPV determination in phosphate buffer solution

Electrode	Ep (V)	Analytical range (M)	LOD (M)	pH	References
MC/GCE[a]	0.810 vs SCE	5.0×10^{-7}–1.0×10^{-4}	1.0×10^{-7}	3.5	Karyakin et al. 1999
Thionine/ACNTs/GCE[b]	0.800 vs SCE	3.0×10^{-6}–1.0×10^{-2}	9.0×10^{-7}	2.0	Lubert et al. 1999
FeT4MPyP/CuTSPc/GCE[c]	0.710 vs SCE	5.0×10^{-7}–7.5×10^{-6}	1.4×10^{-7}	7.0	Santos et al. 2009
TMOPPMn (III)Cl/GE	0.690 vs Ag/AgCl	5.0×10^{-2} –9.0×10^{-8}	2.9×10^{-9}	6.0	Proposed sensor

LOD limit of detection

[a] MC/GCE chitosan-carboxylated multiwall carbon nanotube modified glassy carbon electrode.

[b] Thionine/ACNTs/GCE thionine modified aligned carbon nanotube electrode

[c] FeT4MPyP/CuTSPc/GCE alternated layers of iron(III) tetra-(*N*-methyl-4-pyridyl)-porphyrin (FeT4MPyP) and copper tetrasulfonated phthalocyanine (CuTSPc) modified glassy carbon electrode

milliamperes per square centimetre and *C* is the concentration in molar with good correlation (r^2=0.9954). The proposed sensor showed a linear response range from 5×10^{-2} to 9×110^{-8} M nitrite with a limit of detection of 2.9×10^{-9} M. This indicates that the presence of nitrite in food samples with concentration as low as 2.9×10^{-9} M can be determined using the proposed sensor.

The analytical characteristics of TMOPPMn(III)Cl/GE sensor in comparison with those reported in the literature is summarized in Table. 1.

The relative standard deviation of the peak current, corresponding to the oxidation of 1×10^{-3} M nitrite, for five determinations was found to be 1.46%. The TMOPPMn (III)Cl-modified gold electrodes were found to have reserved 97% of their initial activity for more than 2 weeks. These results demonstrated the good reproducibility and stability of the proposed sensor.

Determination of nitrite in food samples

To confirm the validity and accuracy of the developed sensor, nitrite content in chicken sausage, chicken ham and pickled vegetables was determined. The concentration of nitrite in food samples was determined by standard-addition method. Each test was repeated five times. The obtained results are in good agreement with those of standard spectrophotometric method (Table. 2). The results indicate that the proposed electrochemical sensor is reliable for the determination of nitrite in real samples.

Conclusion

The electrocatalytic oxidation of nitrite on TMOPPMn(III) Cl-modified gold electrode sensor can be used as an effective method for the determination of nitrite. Optimization of the experimental conditions yield a detection limit and linear range for NO_2^- much better than those reported in the literature. The developed sensor has high sensitivity, good stability, reproducibility and anti-interference ability. The sensor is also promising for nitrite determination in food samples and the results are consistent with those obtained with standard spectrophotometric method. The use of less toxic reagents in the proposed method makes it more

Table 2 Determination of nitrite in food samples (*n*=5)

Samples	Added (mg/kg^{-1})	Griess assay		Proposed method	
		Found (mg/kg^{-1}±RSD[a])	Recovery (%)	Found (mg/kg^{-1}±RSD[a])	Recovery (%)
Sample 1[b]	5.00	5.01±0.11	100.2	5.10±0.14	99.6
	10.00	10.10±0.21	101.0	10.08±0.22	100.8
Sample 2[c]	5.00	4.97±0.19	99.4	5.09±0.27	101.8
	10.00	9.9±0.09	99.0	9.9±0.11	99.0
Sample 3[d]	5.00	5.03±0.13	100.6	4.97±0.25	99.4
	10.00	10.18±0.11	101.8	9.91±0.27	99.1

[a] Average of five determination±relative standard deviation

[b] Sausage

[c] Chicken ham

[d] Pickled vegetable

advantageous over the standard method for nitrite determination based on carcinogenic reagents.

Acknowledgement One of the authors (D. Thomas) is grateful to the Council of Scientific and Industrial Research (CSIR), India for fellowship.

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