

Manganese porphyrin sensor for the determination of bromate

Shanty Sheen¹ · Theresa Jos¹ · Leena Rajith¹ · Krishnapillai Girish Kumar¹

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Abstract The electro reductive behavior and determination of bromate on [5, 10, 15, 20-tetrakis (4-methoxyphenyl)porphyrinato] Manganese (III) chloride (TMOPPMn(III)Cl) modified Gold electrode(GE) was investigated by Square wave voltammetry (SWV). Bromate showed an irreversible reduction peak at -164 mV in 0.1 M pH 7 Na_2SO_4 solution. The cathodic peak of bromate showed a reduction in potential of 88 mV on modifying GE with a porphyrin film. The peak current varied linearly with concentration with a detection limit of 3.56×10^{-9} M. The influence of pH, scan rate, supporting electrolyte and interferents on the reduction peak current of bromate were studied. The developed sensor was proposed for the determination of bromate in bread samples and compared with the standard method.

Keywords Bromate · Porphyrin · Gold electrode · Square wave voltammetry · Sensor

Introduction

Bromate, a powerful oxidizing agent is a widely used food additive in bread making process for the maturation of flour and also used in the production of fish paste, fermented beverages and in cold wave hair lotion (AIRC 1986). Studies have shown that bromate can cause many adverse health effects such as peripheral neuropathy, anemia, renal diseases etc. (Crofton 2006; Ojeka et al. 2006; Fawell and Walker 2006);

if consumed more than the allowed level of $25 \mu\text{gL}^{-1}$ (WHO 1996). Therefore the detection of bromate in food is quiet important for controlling the food safety and guaranteeing the consumer's safety.

Until now several methods have been applied for the detection of bromate such as spectrophotometry (El harti et al. 2012), high-Performance Liquid Chromatography (Snyder et al. 2005), ion chromatography (Yashin and Yashin 2004) etc. Even though these methods are reliable, they require complicated and time consuming sample pretreatment.

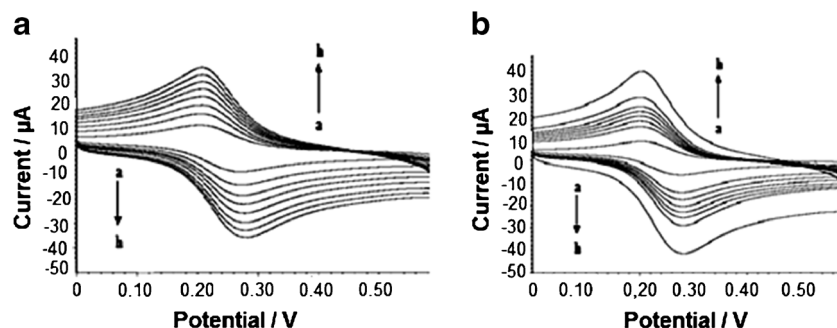
Electrochemical techniques, such as the voltammetric ones, are promising alternative to classical approaches due to their relatively low operational cost, good miniaturization potential, and rapid and sensitive detection procedures, which are suitable for faster analysis. Among the various electrochemical methods, Square wave voltammetric (SWV) technique is a very sensitive method widely used for trace analysis of analytes (Mirceski and Gulaboski 2014). The facility of electron transfer between the electro active species and the electrodes offer great promise for fabricating electrochemical sensors and biosensors (Lonappan et al. 2011; Jos et al. 2012). Still there are only few reports available in literature for the determination of food additives, colorants, antioxidants, emulsifiers etc. using electro analytical methods (Ohkita et al. 2002; Xu et al. 2003; Wang et al. 2000, 2001).

Metalloporphyrins are novel macrocyclic compounds which can act as electrode modifiers in catalyzing the electrochemical behaviour of various species probably due to the increased number of catalytic sites on the electrode surface. Among the various metalloporphyrins, Mn (III) macrocycle is particularly stable, (Rajith and Grish Kumar 2010; Joseph and Grish Kumar 2010) justifying our choice.

✉ Krishnapillai Girish Kumar
giri@cusat.ac.in

¹ Department of Applied Chemistry, Cochin University of Science and Technology, Kochi 682022, India

Fig. 1 Overlay of Surface area study at (a) bare GE and b TMOPPMn(III)Cl/GE in 1×10^{-3} M $\text{K}_3\text{Fe}(\text{CN})_6$, where a \rightarrow h indicates the direction of potential scan, a = 10 mV/s, b = 20 mV/s, c = 30 mV/s, d = 40 mV/s, e = 50 mV/s, f = 60 mV/s, g = 70 mV/s, h = 80 mV/s



As a continuation to our work on food analysis (Thomas et al. 2012; Vikraman et al. 2013) the present work is aimed to investigate the electrochemical behavior of the food additive, bromate at a Mn(III) porphyrin modified GE based on its reduction. The developed method has been applied for the determination of bromate in different bread samples, in which this additive may be present.

Experimental

Apparatus and reagents

Voltammetric measurements were carried out with an Electrochemical Analyser (CH instrument, Austin, TX). A three electrode cell system consisting of TMOPPMn(III)Cl/GE as the working electrode, Pt wire as the auxiliary electrode and Ag/AgCl electrode as the reference electrode was used for all measurements. The pH measurements were carried out in a Metrohm pH meter (Eutech Instruments, India). Scanning electron microscopic (SEM) images were recorded using JOEL-6300LV USA.

All reagents used were of analytical grade and double distilled water was used throughout the studies. Potassium bromate (KBrO_3), sodium sulphate (Na_2SO_4), ascorbic acid, citric acid, sodium chloride, potassium chloride and sulphuric acid were purchased from s. d. fine chemicals. Nafion (5 %) and alumina powder were purchased from Sigma Aldrich. Porphyrin and metalloporphyrin (TMOPPMn(III)Cl) were prepared as previously reported (Maria da and Kevin 2014;

Rajith and Grish Kumar 2010). Pyrrole and anisaldehyde were obtained from Sisco Research Laboratories Ltd. (India) and purified prior to use.

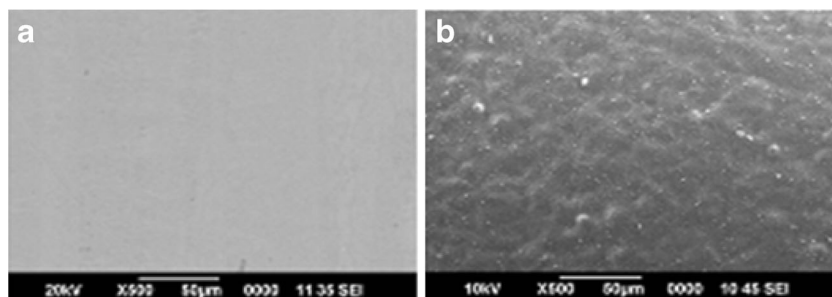
Fabrication of TMOPPMn (III)Cl/GE

Prior to modification, Au electrode was polished with alumina (<50 nm) slurries, rinsed with double distilled water and methanol, and then ultrasonicated for 5 min in a water bath. Following this mechanical process, an electrochemical cleaning process was carried out using cyclic voltammetry (CV) performed from 0 to 1500 mV in 0.5 M sulphuric acid solution at a scan rate of 100 mV/s until a stable cyclic voltammogram was obtained. 3 μL of this solution was dropped on to the clean gold surface.

Experimental procedure

A 10 mL Na_2SO_4 (0.1 M, pH = 7) solution with an appropriate amount of bromate of various concentration, was transferred into a cell, and then the three electrode system was immersed into the solution. A potential scan between 0 to 800 mV at a scan rate of 20 mV/s was triggered, and the cathodic peak was recorded using square wave voltammetry (SWV). A reduction peak around -164 mV was measured for 1×10^{-3} M bromate solution. All experiments were carried out at room temperature. No oxidation peak was observed for bromate in the reverse sweep of CV indicating an irreversible electrochemical process.

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



Determination of bromate in food sample

1.0 g of bread was transferred into a test tube and 10 mL of distilled water was added. The mixture was shaken and allowed to stand for 20 min and decanted 5 mL from the test tube (Emeje et al. 2010). From this solution was analyzed by SWV and the procedure was repeated for different concentration of bromate in bread samples.

Surface area studies

The microscopic area of the bare GE and TMOPPMn(III)Cl/GE were obtained by cyclic voltammetry (Fig. 1a, b) using 1.0×10^{-3} M $K_3Fe(CN)_6$ as a redox probe containing 0.1 M KCl at different scan rates. For a reversible process, according to Randles–Sevcik equation, the anodic peak current i_p is linear to square root of scan rate ($\nu^{1/2}$) (Jos et al. 2012).

$$i_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} C_0 \nu^{1/2}$$

where i_p refers to the peak current, A the surface area of the electrode, C_0 concentration of $K_3Fe(CN)_6$ and ν the scan rate. For 1×10^{-3} M $K_3Fe(CN)_6$, the electron transfer $n = 1$, the diffusion coefficient $D = 7.60 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ (Adams 1969). Thus from the slope of i_p vs $\nu^{1/2}$ relation, the microscopic area of TMOPPMn(III)Cl modified GE [$i_p = 0.452 + 3.75\sqrt{\nu}$ where i_p is the current in microamperes and ν is the scan rate with a correlation ($r^2 = 0.995$)] calculated to be 0.1859 cm^2 which was about two times greater than the bare GE [$i_p = 0.279 + 2.01\sqrt{\nu}$ where i_p is the current in microamperes and ν is the scan rate with a correlation ($r^2 = 0.996$)].

Further evidence for the modification of the gold electrode surface by TMOPPMn(III)Cl was obtained from the SEM images. Comparison of SEM images of the bare GE and TMOPPMn(III)Cl/GE points to the effective modification of bare GE (Fig. 2a, b).

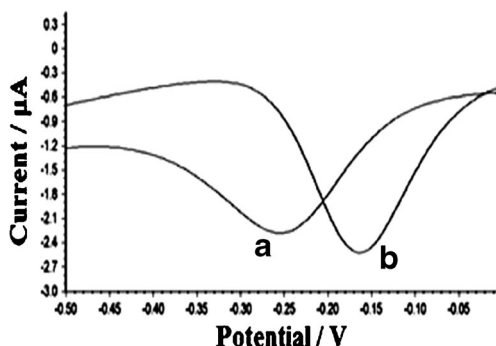


Fig. 3 Comparison of the response of 1×10^{-3} M bromate at (a) bare and b TMOPPMn(III)Cl modified gold electrode by SWV

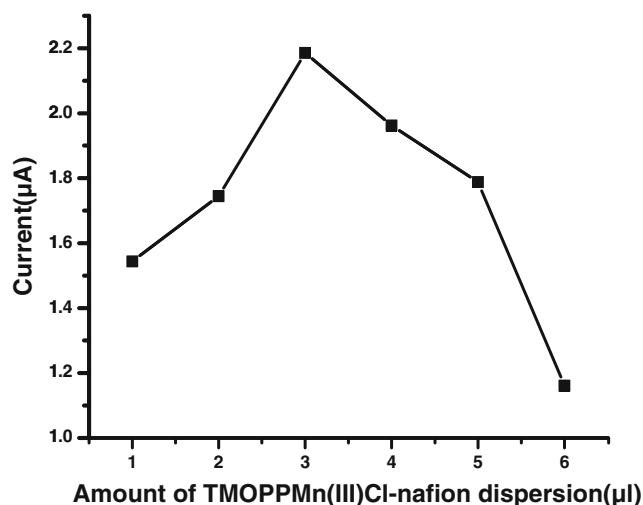


Fig. 4 Effect of volume of TMOPPMn(III)Cl-nafion solution on the peak current of 1×10^{-3} M bromate

Results and discussion

Electrochemical behavior of bromate at bare GE and TMOPPMn(III)Cl/GE

The electrochemical behavior of 1×10^{-3} M bromate at bare GE and TMOPPMn(III)Cl/GE was studied by SWV (Fig. 3). At TMOPPMn(III)Cl/GE bromate exhibited a well-defined and sensitive reduction peak at -164 mV , which was 88 mV less than for a bare GE. The reduction of 1×10^{-3} M bromate occurs at a lower potential for SWV (Recorded with amplitude 0.025 V and potential increment 0.004) compared to CV and DPV (Recorded from 0 to 800 mV at a scan rate of 20 mV/s , with pulse amplitude of 50 mV , pulse period of 0.5 s , pulse width of 0.2 s and potential step of 4 mV). Hence further studies were carried out using square wave technique. The decrease of peak potential and enhancement of peak current proved the electrocatalytic activity of TMOPPMn(III)Cl modified GE towards the reduction of bromate.

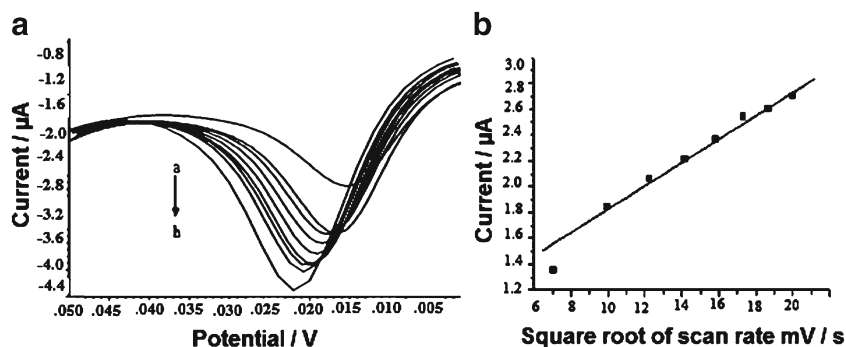
Effect of amount of TMOPPMn(III)Cl on peak current

The relationship between the amount of TMOPPMn(III)Cl and the reduced current of bromate was studied. The current

Table 1 Effect of pH on the peak current of 1×10^{-3} M bromate at TMOPPMn(III)Cl modified gold electrode

pH	Ep(V)	Ip(μA)
3	−0.196	1.075
4	−0.188	1.161
5	−0.172	1.451
6	−0.168	1.742
7	−0.164	2.07
8	No peak	No peak

Fig. 5 **a** Overlay of SWV for reduction of 1×10^{-3} M bromate at different scan rates on TMOPPMn(III)Cl modified gold electrode. Where, a = 50 mV/s, b = 100 mV/s, c = 150 mV/s, d = 200 mV/s, e = 250 mV/s, f = 300 mV/s, g = 350 mV/s, h = 400 mV/s. **b** Plot of square root of scan rate vs current



increased as the amount of the TMOPPMn(III)Cl solution increased from 1 μ L to 3 μ L. The enhancement of current indicates that the number of catalytic sites increased with the amount of TMOPPMn(III)Cl. Further increase in the volume of TMOPPMn(III)Cl, result in decrease of the peak current (Fig. 4). This may be because nafion is an insulator, which blocks the electron transfer at higher concentration. Hence, 3 μ L of 2.5 mg/500 μ L TMOPPMn(III)Cl was used to modify the gold electrode for further studies.

Effect of supporting electrolyte

The electro chemical response of 1×10^{-3} M bromate at a TMOPPMn(III)Cl modified gold electrode in 0.1 M concentration of various supporting electrolytes like H_2SO_4 , HCl, NaCl, KNO_3 , NaOH, acetate buffer, phosphate buffer, citrate buffer, Na_2SO_4 were studied. When H_2SO_4 , NaOH, citrate buffer were used as the supporting electrolyte, no peak was obtained for TMOPPMn(III)Cl modified and bare gold electrode. With KNO_3 , TMOPPMn(III)Cl/GE gave reduction peak at -182 mV with peak current 1.1619×10^{-6} A. When acetate buffer was used as the supporting electrolyte, TMOPPMn(III)Cl/GE gave reduction peak at -224 mV with peak current 1.429×10^{-6} A and with phosphate buffer reduction peak at -192 mV with peak current 1.313×10^{-6} A. However in Na_2SO_4 solution, TMOPPMn(III)Cl/GE gave a peak at -164 mV with

peak current 2.07×10^{-6} A. As potassium bromate gave the best reduction peak in Na_2SO_4 solution, it was used as the supporting electrolyte throughout the experiment.

Effect of pH

The influence of solution pH on the peak current for bromate determination was investigated from pH 2–8, pH of the sodium sulphate solution was controlled by using sulphuric acid and sodium hydroxide. Table 1 depicts the effect of pH on the peak current of 1×10^{-3} M bromate. The best response was obtained at pH 7. Hence, 0.1 M Na_2SO_4 with pH 7 was used as the optimum pH for the effective determination of bromate.

Mechanism

The reduction peak current of 1×10^{-3} M bromate at different scan rates ranging from 50 to 400 mV/s was measured by SWV. It was found that the cathodic peak current increases with increase in the scan rate (Fig. 5a). The reduction peak current varies linearly with square root of scan rate [At 95 % confidence interval, $i_p = 0.104 + 0.716\sqrt{\nu}$] where i_p is the current in microamperes and ν is the scan rate with a good correlation ($r^2 = 0.975$) indicating that the reduction of bromate at the TMOPPMn(III)Cl/GE diffusion controlled (Fig. 5b).

Fig. 6 **a** Overlay of SWV of bromate at different concentrations on TMOPPMn(III)Cl modified gold electrode. Where a = 1×10^{-7} M, b = 2×10^{-7} M, c = 4×10^{-7} M, d = 6×10^{-7} M, e = 8×10^{-7} M, f = 1×10^{-6} M, g = 4×10^{-5} M, h = 2×10^{-5} M. **b** Plot of various bromate concentration vs current

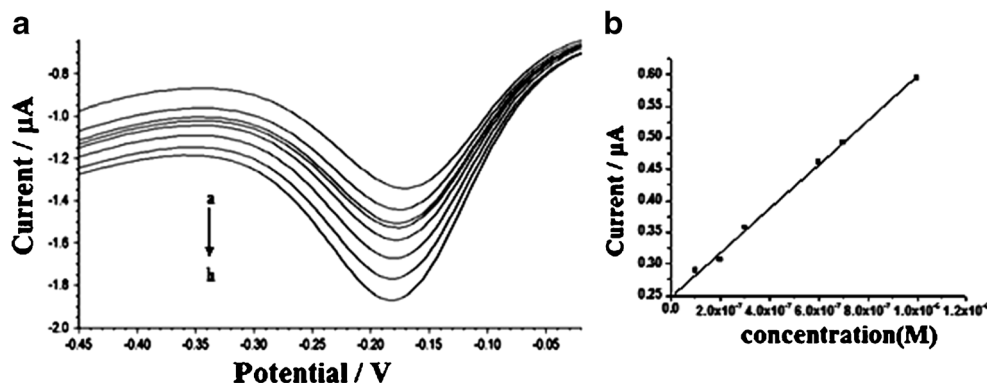
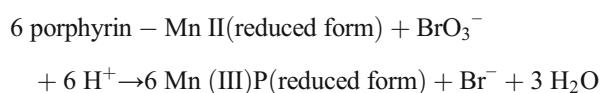
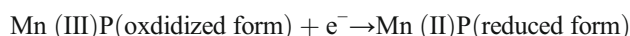


Table 2 Analytical application

Samples	Added (M)	Iodometric method		Proposed method	
		Found (M)	Recovery (%) (S.D)	Found(M)	Recovery (%) (S.D)
Sample1	1×10^{-6}	0.992×10^{-6}	99.20 ± 0.15	0.998×10^{-6}	99.8 ± 0.20
	1×10^{-7}	1.002×10^{-7}	100.2 ± 0.12	0.993×10^{-7}	99.3 ± 0.13
Sample2	1×10^{-6}	0.99×10^{-6}	99.0 ± 0.13	0.992×10^{-6}	99.2 ± 0.11
	1×10^{-7}	1.002×10^{-7}	100.2 ± 0.11	0.991×10^{-7}	99.1 ± 0.13

The number of electrons involved in the reaction (n_a) can be calculated from the scan rate study using Laviron's equation (Lonappan and Grish kumar 2011, Wei Guo et al. 2013) It was found that E varies linearly with $\ln v$. The slope of this plot is equal to, $RT/\alpha n_a F$, where R is the universal gas constant, T is the temperature, α is a constant (for a totally irreversible electrode process, the value of α is assumed to be 0.5) and F is 96,500 C. The obtained value for n_a was 5.54 (around 6). This confirms that 6 electrons are involved in the reduction of bromate (Salimi et al. 2007).



Interference study

In order to evaluate the selectivity and anti-interference ability of the proposed sensor, the effect of various foreign species on the electrochemical reduction of 1×10^{-3} M bromate was studied. Even a 100 fold excess of glucose, sodium carbonate, sodium chloride, K^+ and Ca^{2+} , has no influence on the determination of bromate (signal change below 5 %).

Calibration curve

The relationship between the cathodic peak current of bromate and its concentration was investigated by SWV (Fig. 6a). The analyte concentration was varied from 1×10^{-2} to 1×10^{-7} M. Linear increase in peak current was obtained in the range 1×10^{-6} M - 1×10^{-7} M under optimized conditions (Fig. 6b), [At 95 % confidence interval, $i_p = 0.249 + 34,667 \text{ C}$ with correlation $r^2 = 0.996$ where i_p is the current in microamperes and C is the concentration in molar]. The lower detection limit obtained from the graph was found to be 3.56×10^{-9} M. Comparison of the developed sensor with the previously reported methods demonstrates that the proposed method is a better method for the determination of bromate.

The reproducibility of peak potential was estimated in a standard solution of 1×10^{-3} M bromate under the selected optimum conditions. The relative standard deviation of the peak potential was ± 0.87 ($n = 6$). The high reproducibility indicates that this method is highly stable.

Analytical applications

To confirm the validity and accuracy of the developed sensor, bromate content in different bread samples were determined. Each test was repeated five times. The obtained results were in good agreement with those of standard iodometric method (Furman 1991) (Table 2). The results indicated that the proposed sensor reliable for the determination of bromate in real samples.

Conclusion

The electrochemical behaviour of bromate at a (TMOPPMn(III)Cl)/GE was investigated by SWV and an irreversible reduction peak that involved six electrons was observed. Owing to the large surface area and numerous active catalytic sites, TMOPPMn(III)Cl film remarkably increased the reduction signal of bromate on GE. Finally it was successfully used for the determination of bromate in various bread samples and the accuracy was testified using the standard iodometric method.

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