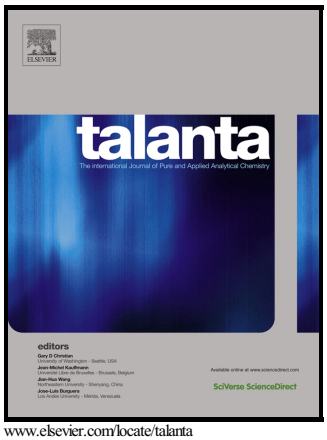
A facile and practical biosensor for choline based on manganese dioxide nanoparticles synthesized in–situ at the surface of electrode by one–step electrodeposition

Guangxia Yu, Qiang Zhao, Weixiang Wu, Xiaoyun Wei, Qing Lu



www.elsevier.com/locate/talanta

PII: S0039-9140(15)30068-0
DOI: http://dx.doi.org/10.1016/j.talanta.2015.06.037
Reference: TAL15714

To appear in: *Talanta*

Received date: 28 February 2015
Revised date: 9 May 2015
Accepted date: 13 June 2015

Cite this article as: Guangxia Yu, Qiang Zhao, Weixiang Wu, Xiaoyun Wei and Qing Lu, A facile and practical biosensor for choline based on manganese dioxide nanoparticles synthesized in–situ at the surface of electrode by one–step electrodeposition, *Talanta,* http://dx.doi.org/10.1016/j.talanta.2015.06.037

A facile and practical biosensor for choline based on manganese dioxide nanoparticles synthesized in–situ at the surface of electrode by one–step electrodeposition

Guangxia Yu, Qiang Zhao, Weixiang Wu, Xiaoyun Wei, Qing Lu*

Key Laboratory of Environment and Health, Ministry of Education & Ministry of Environmental Protection, and State Key Laboratory of Environmental Health (Incubating), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, #13 Hangkong Road, Wuhan, Hubei, 430030, China

* Corresponding author:

Qing Lu, E–mail: qi_weiliao@126.com

Address: School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, #13 Hangkong Road, Wuhan, Hubei, 430030, China, Phone: +86–27–83625912 , Fax: +86–27–83657765

ABSTRACT

In this paper, a facile and sensitive biocompatible biosensor based on Nafion/choline oxidase/manganese dioxide composite film was developed for the determination of choline chloride. Manganese dioxide (MnO_2) nanoparticles, possessing the advantages of large specific surface areas, good hydrophilicity, great permeability as well as excellent biocompatibility, were synthesized in–situ at the surface of the glassy carbon electrode (GCE) by one–step electrodeposition. And then, choline oxidase (ChOx) was immobilized on the MnO_2 modified GCE with coating a Nafion film to hold the ChOx/MnO_2 film on the electrode surface firmly. Upon optimized conditions, a linear range of 8.0 μM–1.0 mM was obtained for the sensor in a cyclic voltammetry method, with a detection limit as low as 5.0 μM. Besides, the biosensor was successfully employed to detect choline in milk, milk powder

and feedstuff samples, providing a promising alternative for the practical application.

KEYWORDS: Choline biosensors; Manganese dioxide; Electrochemical; Electrodeposition.

1. Introduction

Choline, generally classified into the B–complex vitamins, is an essential nutrient necessary for several biological functions in human body. The major fate of choline is to synthesize neurotransmitter acetylcholine, which is intricately connected with the cholinergic neural networks associated with memory [1]. Choline can be also oxidized to betaine in kidney and liver, where it functions as an important methyl donor in the methionine cycle [2]. In addition, in human body, choline also takes important roles in the processes of phosphatidylcholine synthesis, membrane construction and signaling [3], lipoprotein transport and metabolism [4]. In view of its essentiality, in 1998, choline was classified as an essential nutrient by the Food and Nutrition Board of the Institute of Medicine (the USA). And the institute has estimated an adequate intake (AI) of choline of 550 mg/day for men and 425 mg/day for women. Consequently, the quantitative determination of choline is crucial in clinical analysis, food industry, feed additives and many other fields [5].

The common methods of choline detection include liquid chromatography–tandem mass spectrometry (LC–MS/MS) [6], high performance liquid chromatography (HPLC) [7], colorimet [8], fluorescence spectrometry [9] and chemiluminescence analysis [10]. Although these methods have reliable sensitivity, high cost and time consuming restrict their practicability. On the other hand, amperometric enzyme–based biosensors have received considerable attention due to their rapid response, high sensitivity, portability, low cost (disposability) and minimal power requirements in the past few years [11]. Thus, the choline oxidase–based biosensor is a promising alternative for detecting choline. As shown in Eq. 1, in the presence of oxygen, choline oxidase (ChOx) can catalyze the oxidation of choline to produce betaine and hydrogen peroxide (H_2O_2)

$$\text{Choline} + O_2 + H_2O \xrightarrow{\text{ChOx}} \text{betaine} + 2H_2O_2 \quad (1)$$

Based on the determination of H_2O_2, the indirect detection of choline can be achieved by a

ChOx–based biosensor.

As is well–known, nanostructured metal oxides not only possess large surface area, good biocompatibility and high chemical stability, but also show fast electron communication features. All these virtues make the nano–materials be able to function as ideal catalysts, and good biomimetic membranes. Many nanostructured metal oxides, such as SnO_2, Fe_3O_4, TiO_2, ZnO and ZrO_2 nanoparticles and so on, have been utilized for the immobilization of enzymes to develop sensitive bioelectronic devices [12, 13]. Particularly, MnO_2 as a kind of attractive and potential material in catalyst, adsorbent, capacitor and battery [14–16], has been applied successfully to construct biosensors. For example, Emir Turkusic et al. fabricated a screen–printed amperometric glucose biosensor based on MnO_2 and glucose oxidase [17]. In this system, MnO_2 reacted chemically with H_2O_2 to generate manganese species at lower oxidation states, which could be electrochemically reoxidized to MnO_2. Then, an oxidative current was obtained, which had direct relationship with the glucose concentration and could be applied to detect glucose. Nanostructured MnO_2 can be synthesized by sol–gel technique [18], coprecipitation [19], hydrothermal reaction [20] and so on. However, during the process of synthesis, complex experimental steps and rigorous experimental conditions are usually required in those methods. Moreover, it is still a challenge to produce nano–MnO_2 with highly pure crystallographic phases and uniform morphology [21].

In this work, nano–MnO_2, was synthesized in–situ at the surface of the glassy carbon electrode by one–step electrodeposition. The as–prepared nano–MnO_2 with high specific surface areas, uniform morphology, favorable hydrophilic property and super catalytic ability was successfully synthesized with a simple electrodeposition method, and was further used to construct a choline biosensor.

2. Experimental

2.1. Reagents

Choline oxidase (13 Units/mg) and Nafion (5%) were obtained from Sigma. Choline chloride, $KMnO_4$, H_2SO_4, HCl and H_2O_2 (30%) were purchased from Sinopharm Chemical Reagent Co. Ltd (China). Phosphate buffer solution (PBS 0.1 M, pH 8.0) was prepared by

mixing the stock solutions of Na_2HPO_4 and NaH_2PO_4 obtained from Tianjin Chemical Reagent Co. Ltd (China). The deposition bath was 10 mM $KMnO_4$ solution mixed with 45 mM H_2SO_4. ChOx was dispersed in 0.1M pH 8.0 PBS, and the gained solution was stored in a refrigerator at 4 °C for further use. The practical samples were purchased from the local market. All the other reagents were of analytical grade. Double–distilled water was applied for all the aqueous solutions.

2.2. Apparatus

Electrochemical measurements were carried out by employing a conventional three–electrode system. A bare or modified GCE was served as a working electrode, a saturated calomel electrode (SCE) as a reference electrode and a platinum wire as a counter electrode. Current–time parameters (i–t) and cyclic voltammetry (CV) measurements were performed on a CHI440A electrochemical workstation (Shanghai Chenhua Apparatus, China). Field emission scanning electron microscopy (FESEM) and X–ray energy dispersive spectrometer (EDS) were operated on a Sirion 200 field–emission scanning electron microanalyzer with EDAX energy disperse spectroscopy (FEI, Netherlands). Transmission electron microscopy (TEM) was taken at a Tecnai G2 F20 S–TWIN microscope (200 kV, Netherlands). Electrochemical impedance spectroscopy (EIS) was carried out with an Autolab potentiostat/galvanostat (PGSTAT302, Echo Chemie B.V., Netherlands) equipped with a frequency response analyzer module.

2.3. The assembly process for the choline biosensor

2.3.1. Preparation of electrode modified with MnO_2

First of all, a disk glassy carbon working electrode was polished with 0.05 μm alumina slurry, and then sonicated successively in nitric acid (1:1), ethanol and twice–distilled water. Thin film of MnO_2 was deposited on GCE in the aqueous solution of 10 mM $KMnO_4$ and 45 mM H_2SO_4 at –0.2 V for 200 s. After the deposition, the film was bathed with double–distilled water and then dried for approximately 10 min at room temperature.

2.3.2. Immobilization of choline oxidase on the modified electrodes

Choline oxidase was dissolved in the PBS (0.1 M, pH 8.0). Then, 5 μL of the mixed solution was pipetted onto the MnO_2 modified electrodes. The gained electrodes should be dried for about 2 h at room temperature. Before using, 2 μL 0.5% Nafion (diluted with double–distilled water) was utilized to cover the ChOx/MnO_2 film as a binder to hold the film on the electrode surface stably. The Nafion/ChOx/MnO_2/GC electrode was stored in a refrigerator at 4 °C for further use.

2.4 Sample preparation

Milk, milk powder and feedstuff were prepared for the practical detection. The preparation of milk sample was carried out by the procedure described by Woollard and Indyk [22] in a collaborative study for the AOAC official method. In short, a 20 mL milk sample was firstly mixed with 10 mL of 3 M HCl in a flat bottomed flask with glass stopper. Secondly, the flask was connected to a condenser, heated for 3 h in a covered water bath (70 °C) and agitated periodically. The obtained mixture was cooled and centrifuged for 15–20 min. A 10 mL aliquot of the supernatant was next transferred to a 20 mL volumetric flask. Then the pH of the gained solution was adjusted to 7.0–7.4 with 50% (w/w) NaOH solution and the volume was made to 20 mL with the carrier solution. The pretreatment of milk powder was according to the National Standard of China (GBT 5413.20–1997). In a word, 5 g milk powder was mixed in a flat bottomed flask with 30 mL of 1 M HCl, the rest of the steps were similar to the milk preparation. As for the preparation of the third sample, feedstuff sample was pretreated on the basis of the National Standard of China (GBT 17481–2008). Briefly, 1 g feedstuff was firstly mixed with 100 mL methanol–trichloromethane mixture (10:1) in an Erlenmeyer flask and oscillated for 30 min prior to the centrifugation. Secondly, the gained supernatant of 10 mL was heated and dried out in a covered water bath (50 °C). The residue was then dissolved with the carrier solution. All the sample solutions were filtered through a 0.45 μm cellulose acetate membranes. If not immediately analyzed, all the samples were stored frozen at –18 °C.

3. Results and discussion

3.1 Characterization of modified electrode

3.1.1. FESEM, TEM, Contact angle and EDS image of MnO_2

The morphology of the as–synthesized MnO_2 was characterized by FESEM and TEM. Fig. 1a showed the typical FESEM image of a MnO_2 sample, which revealed that the sample composed many uniform spherical nanoparticles approximately 200 nm in diameter. With a closer examination of the sample by FESEM and TEM, as was shown in Fig. 1b and c, it was found that these nanoparticles had highly sponge–like nanostructures, which were good for transporting electrons and mass when used as electrocatalysts [23] The entire structure of these nanoparticles was built from numerous nanosheets, which could provide more active sites for electrochemical reactions due to their large specific surface areas. Furthermore, this distinctive structure might also offer excellent permeability, which might facilitated the hydrogen peroxide (the enzymatic product of choline) to transfer into the surface of electrode. Consequently, the reaction time could be reduced.

In this work, the proposed MnO_2 was synthesized in the aqueous phase, which could provide the nanoparticles with excellent hydrophilicity. To confirm it, the contact angles of water dropped on bare and MnO_2 film modified glassy carbon slices were measured (Fig. 2A). When MnO_2 was deposited on the glassy carbon slice, the contact angle decreased significantly, indicating that this new synthesized nanopaticles had good hydrophilicity. In Rahimi's study [24], the effect of hydrophilicity of the nano–compsites on the electrochemical and electrocatalytic behaviours of choline oxidase had been discussed. The results indicated that more hydrophilic nano–materials not only could provide a suitable biocompatible environment for the enzyme, but also preserve its bioactivity and prepare a suitable micro–environment for enzyme–substrate interaction. Thereby, the proposed hydrophilic nano–MnO_2 could also be benefit for the immobilization of choline oxidase.

Chemical composition analysis of the manganese oxide was also performed by EDS, and the Mn/O atomic ratio of 33/67 was found (Fig. 1d). The experimental data implied that Mn (VII) could transform to Mn (IV) after the reduction process. The deposition mechanism could be attributed to the diffusion and cathodic reduction of anionic MnO_4^- species. In acidic aqueous solutions, the MnO_4^- species could be reduced as Eq. 2, which was consistent with the previous studies [25].

$$MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O \quad (2)$$

3.1.2. EIS and characterizations of the assembly process

The information on the impedance changes of the electrode surface in the modification process was obtained from the study of electrochemical impedance spectroscopy (EIS) [26]. In EIS, the semicircle diameter of EIS equals the electron–transfer resistance (R_{et}), and this resistance controls the electron–transfer kinetics of the redox probe at the electrode interface. Fig. 2B demonstrated the results of Faradic impedance spectroscopy on a bare GCE (curve a), MnO_2/GCE (curve b), Nafion/MnO_2/GCE (curve c) and Nafion/ChOx/MnO_2/GCE (curve d) respectively. It could be found that the bare GCE exhibited a very small semicircle domain, and the R_{et} value was merely 200 Ω. When MnO_2 was immobilized on the electrode surface, the R_{et} value increased to about 1200 Ω, due to the restriction of the electron transfer kinetics by the assembly layer. Two semicircles were exhibited on the Nafion/MnO_2 modified electrode, which could be because two interfaces, a hydrophilic MnO_2 film and a hydrophobic Nafion film were formed on the surface of GCE. Then the semicircle reverted to one during further assembly of the enzyme layer. The phenomenon suggested that MnO_2 and Nafion could be linked together closely by the choline oxidase due to its favorable hydrophily and abundant amino acid residues. Besides, the R_{et} value of the ChOx modified electrode increased to about 4400 Ω, which was attributed to protein molecules obstructing the electron transfer process of the electrochemical probe. All of the above indicated the facts that various layers had been successfully assembled onto the GCE and the choline biosensor was fabricated successfully.

3.2. CV behavior of choline chloride at Nafion/ChOx/MnO_2 modified electrode

The cyclic voltammetry behavior of choline at non-modified GCE, Nafion/ChOx/GCE and Nafion/ChOx/MnO_2/GCE had been tested. As shown in Fig. 3, no significant redox responses were observed at bare GCE and Nafion/ChOx/GCE, neither in the absence (Fig. 3a and c) nor in the presence(Fig. 3b and d) of 0.1 mM choline chloride. However, at the Nafion/ChOx/MnO_2/GCE, in the absence of choline (Fig. 3e), there was a pair of broad but barely observable peaks between 0.2 V to 0.8 V. It might be assigned to the reduction of

MnO_2 to Mn (II, III) and the reoxidation of Mn (II, III) back to MnO_2 [17]. In the presence of choline, the oxidation peak current had significantly increased at potential between 0.5 V and 0.8 V (Fig. 3f), which could be attributed to the parallel catalytic reaction. When choline chloride was added to the cell, it could be oxidized to H_2O_2 (process as Eq. 1). As soon as MnO_2 was reduced to lower states by H_2O_2, it would be electro–oxidized back to MnO_2 at the electrode surface just as follows Eq. 3 and 4:

$$MnO_2 + H_2O_2 \rightarrow MnO \text{ (or } Mn_2O_3) + O_2 + H_2O \quad (3)$$

$$MnO \text{ (or } Mn_2O_3) \rightarrow MnO_2 + 2e^- \quad (4)$$

Within the above reaction, MnO_2 was used as a catalyst which could catalyze the hydrogen peroxide and produce H_2O and O_2. The oxygen produced could be recycled for choline oxidation reaction. When the concentration of choline increased, more H_2O_2 would be produced, initiating more parallel catalytic reactions as well as increasing the oxidation current finally. As shown in Fig. 4A, the oxidation currents at Nafion/ChOx/MnO_2/GCE increased proportionally along with the addition of choline chloride.

3.3. Other techniques for the detection of choline

The amperometric current–time response was described in our study. Suitable potential for the i–t method had been evaluated varying from 0.6 V up to 0.8 V, and 0.7 V was selected in this work due to its largest current response. Fig. 4B had showed the amperometric current–time record in a stirred 0.1 M PBS (pH 8.0) with successive addition of choline chloride at 0.7 V. The upper–right inset indicated that Nafion/ChOx/MnO_2/GC electrode had a linear amperometric response to choline concentration from 0.05 mM to 0.45 mM with a sensitivity of 7.86 μA/mM, which was higher than 3.56 μA/mM reported in the previous research [27]. The average time to reach 90% of the current at steady state for each concentration was approximately 40 seconds for this biosensor, with few fluctuation of the response in steady state. It was more rapid compared with the results reported so far as 2 minutes [28]. The phenomenon could be due to the permeability of these nano–materials, which could improve the electron transfer process. Moreover, differential pulse voltammetry

(DPV) performance of the biosensor had also been discussed. The results were shown in Fig. S1 in the supporting information, which also displayed a linear relationship between the oxidation current and the concentration of choline chloride in the range between 10.0 μM and 0.3 mM, with a correlation coefficient of 0.995. The linear ranges achieved from the i–t and DPV method were narrower than those obtained from the CV method. Thus, cyclic voltammetry was chosen as the final technique to detect choline in this study.

3.4. Influences of scan rate and pH

Under the mode of CV, the effect of scanning rate was examined. As shown in Fig. 5A, it was obvious that the redox peak currents enlarged gradually with the increase of scan rate. Besides, the oxidation peak currents of the composite film were linearly proportional to the square root of scan rate with the related coefficient 0.998 (Fig. 5A inset). The results suggested that the electrochemical kinetics was a quasi reversible and diffusion–confined electrochemical process [29].

The effect of the pH value of the supporting electrolyte on the response behavior of the biosensor was also studied between pH 5.5 and 9.0 in Fig. 4B. It was displayed that the pH value of external solution had a strong influence on the redox potentials of the Nafion/ChOx/MnO_2 film. In additionally, with the increase of the solution pH value, both the oxidation (E_{pa}) and reduction (E_{pc}) peak potential would shift to a negative direction. And the oxidation peak potentials of the composite film were linear to the pH value with a related coefficient as 0.992 (Fig. 5B inset (the red line)). It suggested a proton–transfer was involved within the redox reaction [30].

The pH value was also a key factor to influence the current of the oxidation peak. Fig. 5B inset (the blue line) indicated that the peak current increased along with the enhancement of pH value from 5.5 to 8.0, and declined following the decrease of the pH value from 8.0 to 9.0. The highest responses were obtained at pH 8.0, which was consistent with the optimal pH of ChOx [10]. Thus subsequent studies were done at pH 8.0.

3.5. Linear response range and detection limit

As shown in Fig. 4A, under the optimized condition, the oxidation peak current was

increased proportionally when series concentrations of choline were added in the phosphate buffer solution. A calibration curve shown in the inset of Fig. 4A was applied for the determination of choline. The current intensity was linear to the concentration of choline in the range of 8.0 μM–1.0 mM with a detection limit of 5.0 μM. The regression equation could be shown as below Eq. 5:

$$i = -0.07951 - 3.22919C \ (r = 0.996,\ C \text{ in mM},\ i \text{ in } \mu\text{A}) \quad (5)$$

Comparative studies between the proposed biosensor and other published choline biosensors had been conducted, and the results had been summarized in Table 1. It was worth noting that Bai *et al* had also fabricated choline biosensors with nano–MnO_2, and had deeply evaluated the performances of different manganese dioxides (amorphous MnO_2 nanoparticles, α–MnO_2 nanoparticles and β–MnO_2 nanowires) modified electrode for the detection of choline. The results displayed that β–MnO_2 modified biosensors possessed the best amperometric response for the determination of choline with a wide linear range from 1.0 μM to 0.79 mM and a low detection limit of 0.3 μM [33]. However, the method of their work was complex and required complicated procedures for the synthesis of nano–MnO_2. In the proposed study, the nano–MnO_2 was synthesized in–situ at the surface of glassy carbon electrode by one–step electrodeposition, which was more convenient than most of the previous report. Besides, compared with Bai's work, the herein biosensor had comparative performances in detection limit and linear range, and had even better performance than some other biosensors reported previously. The good performance of the biosensor might be attributed to the fine electrocatalytic property and outstanding biocompatibility of the synthesized nano–MnO_2. On one hand, these nanoparticles had highly sponge–like nanostructures, which could increase the electrocatalytic active area and promote mass and electrons transfer. On the other hand, the nano–MnO_2 had good hydrophilicity, which could provide a suitable biocompatible environment for the enzyme and prepare a suitable micro–environment for enzyme–substrate interaction.

3.6. Reproducibility and stability of the choline biosensor

The reproducibility of the biosensor was investigated. The RSD estimated using the CV

response for five measurements of 0.1 mM choline at Nafion/ChOx/MnO_2/GCE was about 2.7%, which was less than the previously reported values of 4.3% and 4.6% [33, 36]. Moreover, the RSD value for the slopes of different calibration sets had also been evaluated and the results had been displayed in the supporting information. The RSD value was calculated to be 5.6% for the slopes of three different calibration curves (Fig. S2). All of the results above indicated that a good reproducibility of this choline biosensor was obtained. The reason could be that the preparation method of this biosensor was simple, which might reduce the measurement deviations. The stability of the developed choline biosensor was also studied. After being stored at 4 °C for over one month, the biosensor still remained 85% of its initial CV response for 0.1 mM choline. Moreover, a new calibration curve with this biosensor was still obtained after being stored for one month without the slope being changed significantly (Fig. S3). The result demonstrated this biosensor possessed of acceptable stability.

3.7. Interference studies

The interference of sampling "contaminating" compounds, which could be easily oxidized at positive potentials, was an important factor for evaluating the analytical performance of the choline biosensor applications. The interference tests were performed in PBS containing 0.1 mM choline chloride in the presence of six potential interference substrates. Effect of interferences on the biosensor response was listed in Table 2. For this biosensor, all of the listed compounds did not cause severe interference, and the signal change was below 4.5%. The results had confirmed high selectivity of the choline biosensor.

3.8. Determination of choline in practical samples

The applicability of the biosensor was assessed by the determination of choline in the prepared samples. To verify the reliability of the choline biosensor, the samples were analyzed by the choline biosensor comparing with a Richard salt colorimetry method. The concentrations of choline in each sample were displayed in Table 3. As is shown, the results of each sample detected by the choline biosenor were in good accordance with those obtained by the Richard salt colorimetry method, demonstrating high accuracy and excellent reliability.

The recoveries values were in the range of 96.3±2.0–107.8±2.4%. The above results had adequately demonstrated that the proposed biosensor could be applied for the choline detection in real samples with acceptable accuracy and reliability.

4. Conclusions

A facile and practical biosensor for choline was fabricated with the utilization of Nafion/ChOx/MnO_2 composite film. MnO_2 nanoparticles, possessing the advantages of large specific surface areas, good hydrophilicity, great permeability, extraordinary catalytic ability for hydrogen peroxide (H_2O_2) as well as excellent biocompatibility for choline oxidase, were successfully electrodeposited on the glassy carbon electrode within one step. The fabricated choline biosensor exhibited high sensitivity and good stability. Furthermore, satisfactory results were obtained when employing this biosensor in analyzing the practical samples. In a word, the constructed choline biosensor had been proven to be a promising alternative for determining of choline in food industry, feed additives and many other fields.

Acknowledgements

The financial supports from National Basic Research Program of China (973 Program, No. 2015CB352100) and Program for New Century Excellent Talents in University (No. NCET–13–0232) are greatly acknowledged. The Center of Analysis and Testing of Huazhong University of Science and Technology is thanked for its help in FESEM, EDS and TEM.

Reference

[1] E. Nurk, H. Refsum, I. Bjelland, C.A. Drevon, G.S. Tell, P.M. Ueland, S.E. Vollset, K. Engedal, H.A. Nygaard, D.A. Smith, Br. J. Nutr. 109 (2013) 511–519.

[2] P.H. Pritchard, D.E. Vance, Biochem. J. 196 (1981) 261–267.

[3] P. Tso, K. Fujimoto, Brain Res. Bull. 27 (1991) 477–482.

[4] S.H. Zeisel, J.K. Blusztajn, Annu. Rev. Nutr. 14 (1994) 269–296.

[5] R.D. Allowances, Aufl. Washington, DC (1968).

[6] Y. Xiong, Y.–Y. Zhao, S. Goruk, K. Oilund, C.J. Field, R.L. Jacobs, J.M. Curtis, J. Chromatogr. B 911 (2012) 170–179.

[7] X. Shao, B.G. Hockley, R. Hoareau, P.L. Schnau, P.J. Scott, Appl. Radiat. Isot. 69 (2011) 403–409.

[8] M. Takayama, S. Itoh, T. Nagasaki, I. Tanimizu, Clin. Chim. Acta 79 (1977) 93–98.

[9] X. Qin, H. Wang, X. Wang, Z. Miao, L. Chen, W. Zhao, M. Shan, Q. Chen, Sens. Actuat. B–Chem. 147 (2010) 593–598.

[10] H. Dai, Y. Chi, X. Wu, Y. Wang, M. Wei, G. Chen, Biosens. Bioelectron. 25 (2010) 1414–1419.

[11] A.A. Ansari, A. Aldwayyan, M. Alhoshan, M. Alsalhi, Nanostructured metal oxides based enzymatic electrochemical biosensors, INTECH Open Access Publisher 2010.

[12] H.–P. Peng, R.–P. Liang, L. Zhang, J.–D. Qiu, Biosens. Bioelectron. 42 (2013) 293–299.

[13] Y.–C. Shen, C.–H. Yang, S.–W. Chen, S.–H. Wu, T.–L. Yang, J.–J. Huang, Biosens. Bioelectron. 54 (2014) 306–310.

[14] K. Chen, Y. Dong Noh, K. Li, S. Komarneni, D. Xue, J. Phys. Chem. C, 117 (2013) 10770–10779.

[15] X. Tao, J. Du, Y. Sun, S. Zhou, Y. Xia, H. Huang, Y. Gan, W. Zhang, X. Li, Adv. Funct. Mater. 23 (2013) 4745–4751.

[16] K.J. Babu, A. Zahoor, K.S. Nahm, R. Ramachandran, M.J. Rajan, J. Nanopart. Res. 16 (2014) 1–10.

[17] E. Turkusic, J. Kalcher, E. Kahrovic, N.W. Beyene, H. Moderegger, E. Sofic, S. Begic, K. Kalcher, Talanta 65 (2005) 559–564.

[18] X. Wang, A. Yuan, Y. Wang, J. Power Sources 172 (2007) 1007–1011.

[19] T. Brousse, M. Toupin, R. Dugas, L. Athouël, O. Crosnier, D. Bélanger, J. Electrochem. Soc. 153 (2006) A2171–A2180.

[20] W.N. Li, J. Yuan, X.F. Shen, S. Gomez–Mower, L.P. Xu, S. Sithambaram, M. Aindow, S.L. Suib, Adv. Funct. Mater. 16 (2006) 1247–1253.

[21] T.T. Truong, Y. Liu, Y. Ren, L. Trahey, Y. Sun, Acs Nano 6 (2012) 8067–8077.

[22] D.C. Woollard, H.E. Indyk, J. AOAC Int. 83 (2000) 131–138.

[23] C. Li, H. Wang, Y. Yamauchi, Chem. Eur. J. 19 (2013) 2242–2246.

[24] P. Rahimi, H. Ghourchian, S. Sajjadi, Analyst 137 (2012) 471–475.

[25] Y.J. Yang, S. Hu, Electrochimi. Acta 55 (2010) 3471–3476.

[26] H. Liang, D. Song, J. Gong, Biosens. Bioelectron. 53 (2014) 363–369.

[27] B. Wu, Z. Ou, X. Ju, S. Hou, J. Nanomater., 2011 (2012) 1–6.

[28] T. Shimomura, T. Itoh, T. Sumiya, F. Mizukami, M. Ono, Talanta 78 (2009) 217–220.

[29] L. Li, Z. Du, S. Liu, Q. Hao, Y. Wang, Q. Li, T. Wang, Talanta 82 (2010) 1637–1641.

[30] I. Yamazaki, T. Araiso, Y. Hayashi, H. Yamada, R. Makino, Adv. Biophys. 11 (1977) 249–281.

[31] Y.–H. Bai, Y. Du, J.–J. Xu, H.–Y. Chen, Electrochem. Commun., 9 (2007) 2611–2616.

[32] H. Zhang, Y. Yin, P. Wu, C. Cai, Biosens. Bioelectron., 31 (2012) 244–250.

[33] Y.–H. Bai, H. Zhang, J.–J. Xu, H.–Y. Chen, J. Phys. Chem. C, 112 (2008) 18984–18990.

[34] J. Wang, G. Liu, Y. Lin, Analyst, 131 (2006) 477–483.

[35] X. Ren, F. Tang, R. Liao, L. Zhang, Electrochim. Acta, 54 (2009) 7248–7253.

[36] Z. Zhang, J. Wang, X. Wang, Y. Wang, X. Yang, Talanta, 82 (2010) 483-487.

Captions

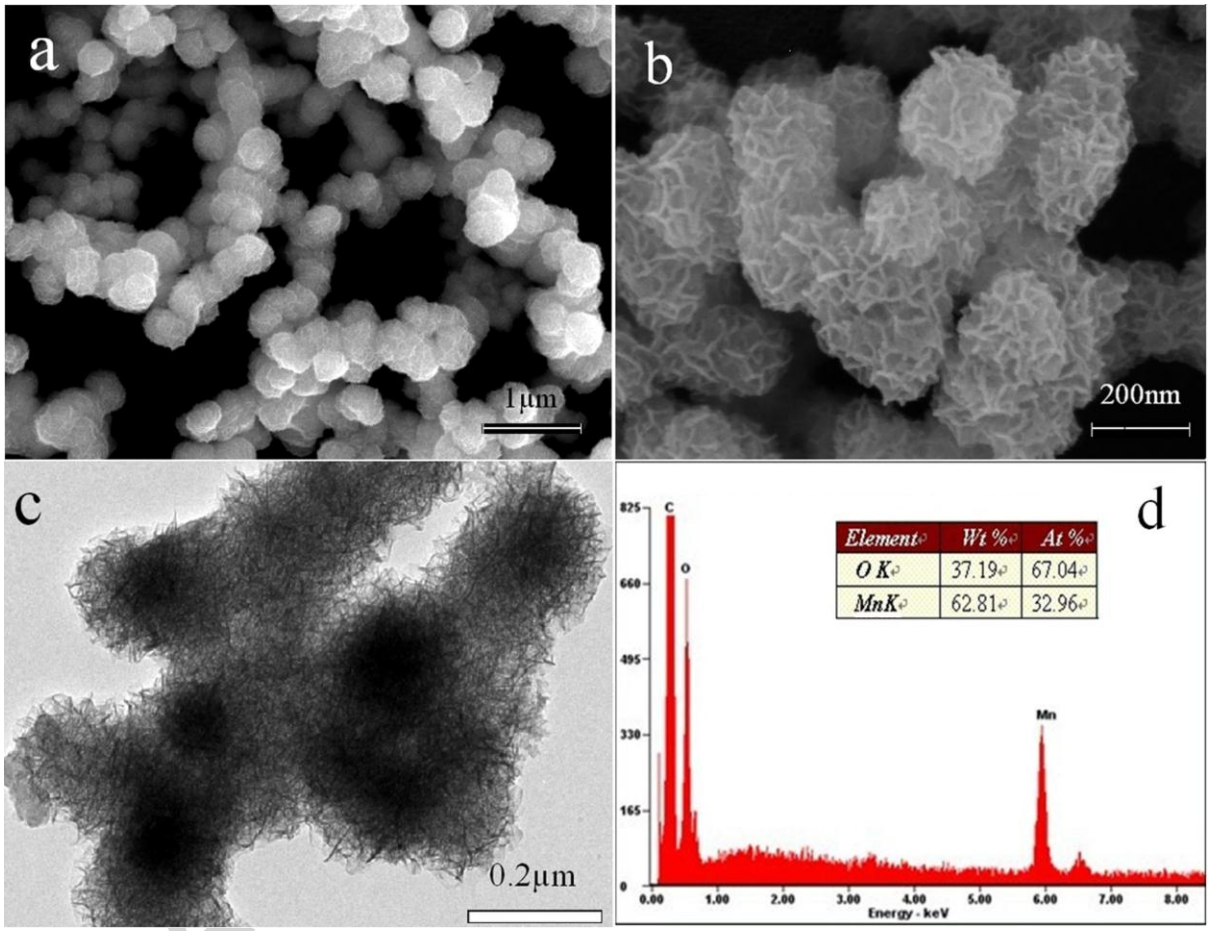


Fig. 1 a) and b) FESEM images, c) TEM image and d) EDS image for the electrodeposited manganese dioxide films.

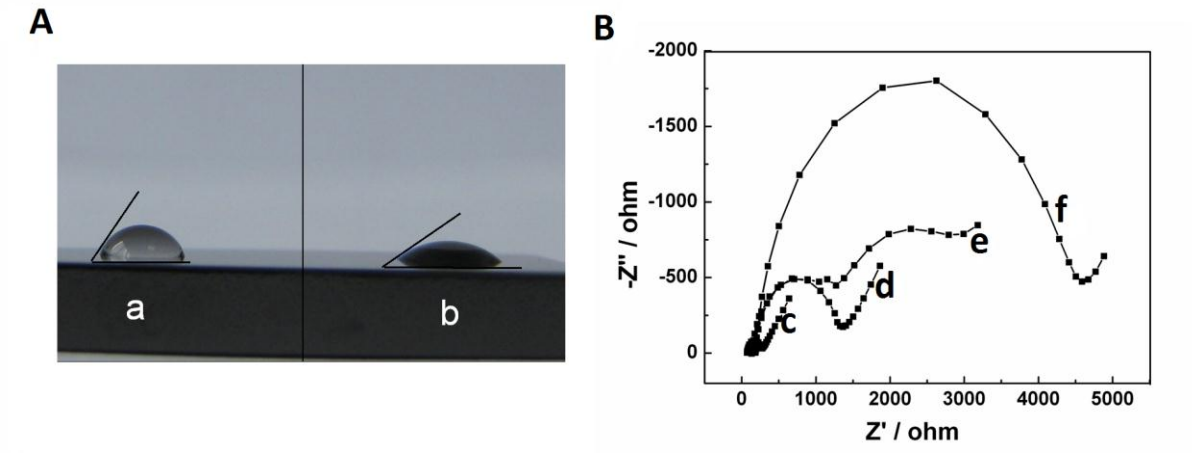


Fig. 2 (A) Contact angle photographs of 5 µL water dropped on a) bare glassy carbon slice, b) MnO_2 modified glassy carbon slice. (B) The EIS of the assembly process in the presence of 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) mixture containing 0.1 M KCl as a redox probe at the formal potential of the system 0.223V. c) GCE, d) MnO_2/GCE, e) Nafion/MnO_2/GCE, f) Nafion/ChOx/MnO_2/GCE.

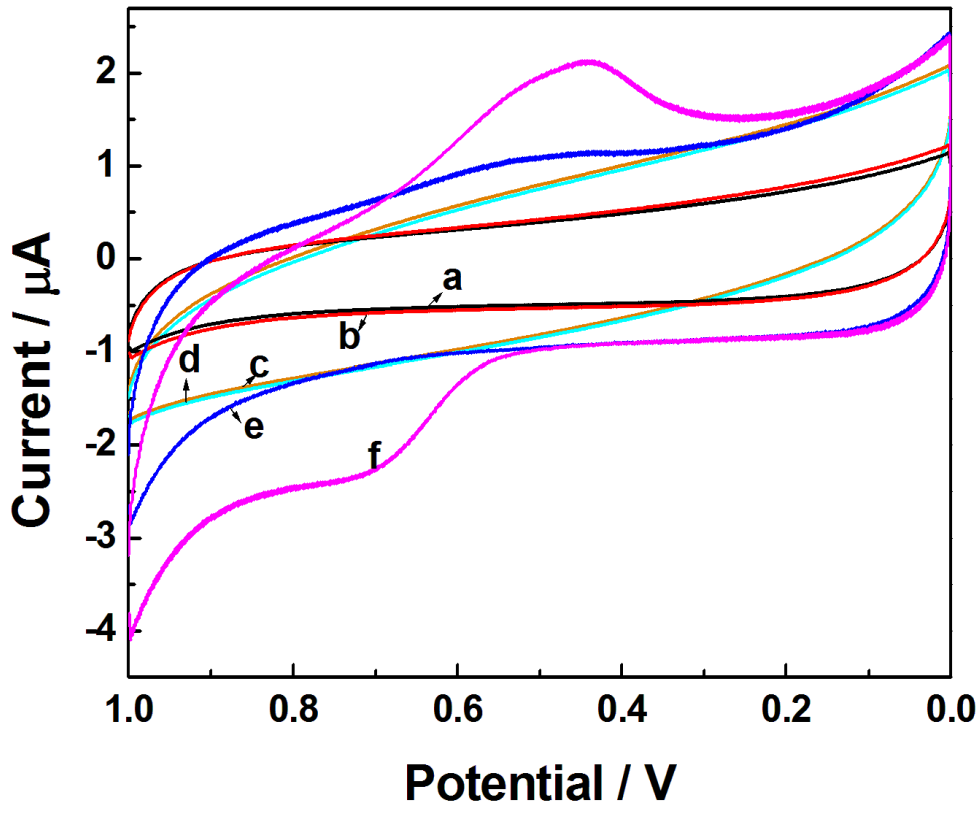


Fig. 3 Cyclic voltammograms of different electrodes a): bare GCE, c): Nafion/ChOx/GCE and f): Nafion/ChOx/MnO_2/GCE in the absence of 0.1 mM choline chloride; b): bare GCE d): Nafion/ChOx/GCE

and f): Nafion/ChOx/MnO_2/GCE in the presence of 0.1 mM choline chloride.

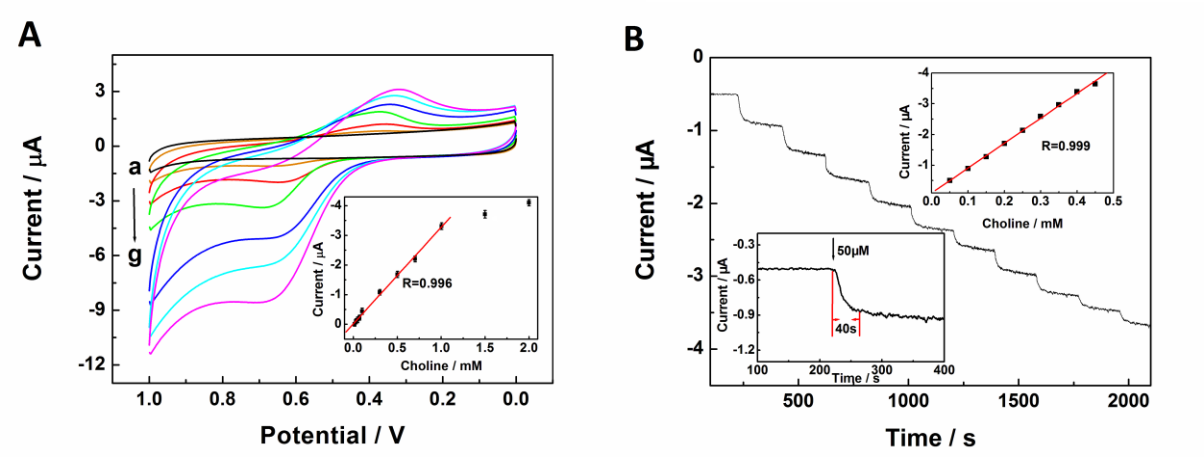


Fig. 4 (A) Cyclic voltammetry for Nafion/ChOx/MnO_2/GC electrode in 0.1 M PBS of pH 8.0 at different concentration of choline chloride a) 0, b) 0.05 mM, c) 0.1 mM, d) 0.3 mM, e) 0.5 mM, f) 0.7 mM and g) 1 mM. The inset shows the relationship between the oxidation peak current and the choline concentration. Error bars indicated the standard deviations ranging from 1.2% to 2.7% (n=3). (B) Amperometric responses obtained upon the successive addition of choline chloride at the applied potential 0.7 V. Each current step corresponds to an increase of 0.05 mM in choline concentration. The upper–right corner inset shows the relationship between the current and the choline concentration from 0.05 mM to 0.45 mM. And the lower–left corner inset shows the response time.

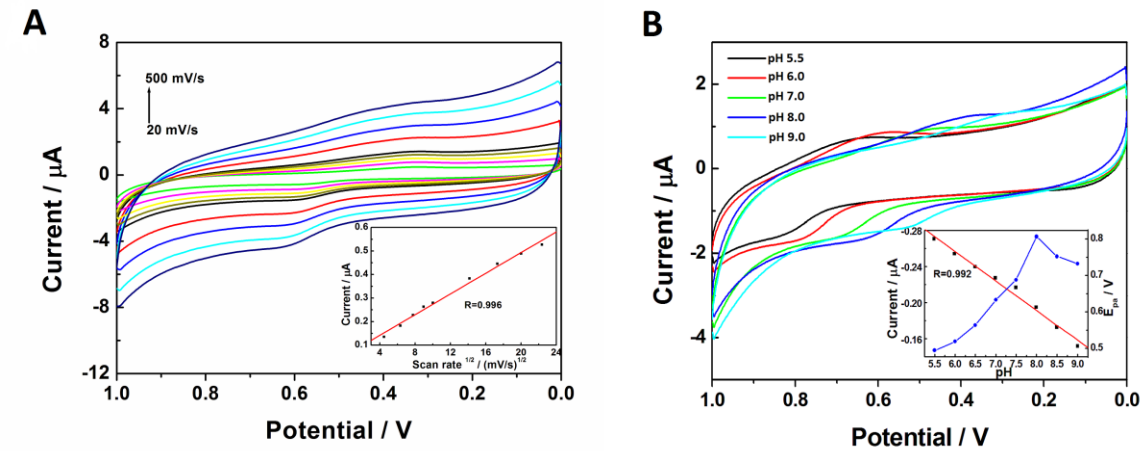


Fig. 5 (A) Cyclic voltammograms of the Nafion/ChOx/MnO_2/GCE in present of 0.1 mM choline chloride at different scan rates of 20 mV/s, 40 mV/s, 60 mV/s, 80 mV/s, 100 mV/s, 200 mV/s, 300 mV/s, 400 mV/s and 500 mV/s. The inset is a plot of the oxidation peak current against the square root of scan rate. (B) Cyclic voltammograms of the Nafion/ChOx/MnO_2/GCE in 0.1 M PBS at pH of 5.5, 6.0, 7.0, 8.0, and 9.0

in present of 0.1 mM choline chloride. The inset shows the relationship between oxidation peak current (the blue line), E_{pa} (the red line) of the Nafion/ChOx/MnO_2/GCE and the solution pH value.

Table 1 Comparisons of the proposed biosensor for detection of choline with other biosensors.

Table 2 The effect of interfering compounds on analysis of choline.

Cyclic voltammetry response was obtained with Nafion/ChOx/MnO_2/GC electrode in 0.1 M PBS of pH 8.0. Choline concentration in the electrochemical cell was 0.1 mM.

Table 3 Results of determination of choline in practical samples

The values were averages of four measurements by the choline biosensor and Richard salt colorimetry.

TABLES

Table 1

Sensors	Linear range (mM)	Detection limit (μM)	References
ChOx/MnO_2/Chit/GCE	0.01–2.1		[31]
ChOx–PDDA–PB–$FePO_4$/GCE	0.002–3.2	0.4	[32]
ChOx/β MnO_2/Chit/GCE	0.001–0.79	0.3	[33]
PDDA/ChO/PDDA/HRP/PDDA/CNT/GCE	0.05–5	10	[34]
PVA/Au nanorods/ChOx/Pt electrode	0.02–0.4	10	[35]
Nafion/ChOx/MnO_2/GCE	0.008–1.0	5	This work

Table 2

Possible interferents	Concentration (mM)	Signal change (%)
Glucose	1.0	0.3
Acetaminophen	1.0	2.3
Serine	1.0	–0.3
Dopamine	1.0	0.7
Ascorbic acid	0.1	4.5
Uric acid	0.5	3.7

Table 3

Sample	Richard salt colorimetry (g/kg)	The proposed biosensor (g/kg)	Recovery (%)
Milk	0.116±0.002	0.125±0.005	107.8±2.4

Milk powder	0.761±0.011	0.733±0.004	96.3±2.0
Feedstuff	1.995±0.043	1.961±0.057	98.3±5.1

Highlights

1. A facile and practical choline biosensor was fabricated by MnO_2 nanoparticles.
2. The MnO_2 was synthesized in-situ at GCE surface by one–step electrodeposition.
3. The synthesized MnO_2 had good hydrophilicity and excellent permeability.
4. The biosensor was successfully employed to detect choline in real samples.

Graphical abstract