Int. J. Electrochem. Sci., 10 **(2015)** 3668 - 3679

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

A label-free Electrochemical Aptasensor Based on Electrodeposited Gold Nanoparticles and Methylene Blue for Tetracycline Detection

Yemin Guo[1,2], *Xiangyou Wang*[1,2,*], *Xia Sun*[2,*]

[1] School of Engineering, Northeast Agricultural University, No. 59 Mucai Street Xiangfang Distrct Harbin, P. R. China
[2] School of Agriculture and Food Engineering, Shandong University of Technology, No. 12, Zhangzhou Road, Zibo 255049, Shandong Province, P. R. China
[*]E-mail: wxy@sdut.edu.cn

Received: 20 January 2015 / *Accepted:* 12 February 2015 / *Published:* 24 February 2015

A label-free electrochemical aptasensor modified with electrodeposited gold nanoparticles and methylene blue was fabricated for convenient and sensitive determination of tetracycline. Firstly, gold nanoparticles were modified onto a glassy carbon electrode (GCE) by one-step electrodeposition method. Then the anti-tetracycline aptamer was immobilized onto gold nanoparticles modified gold electrode surface by Au-S bond. Finally, methylene blue was dripped onto immobilized aptamer GE surface to fabricate a sensitive aptasensor for tetracycline detection in milk. During the above process, gold nanoparticles can facilitate electron transfer, and methylene blue can provide much more adsorption sites for tetracycline. The electrochemical properties of the modified processes were characterized by cyclic voltammetry (CV). The morphologies of the nanocomposites were characterized by scanning electron microscopy (SEM). The experimental conditions such as the concentration of the aptamer, the time, temperature and the pH were optimized. Based on the above optimum conditions, this aptasensor showed a favorable analytical performance for tetracycline detection with a detection limit of 0.42×10^{-11} M.

Keywords: Lable-free; electrochemical aptasensor; gold nanoparticles; methylene blue; tetracycline.

1. INTRODUCTION

Tetracyclines (TET) are a group of broad-spectrum antibiotics containing four condensed aromatic rings. They have been extensively used as a bacteriostatic and antibiotic drug in livestock production to control bacterial infections and to increase the growth rate of animals owing to its broad-spectrum antibacterial activity [1]. However, their widespread use has led to TET residues in animal foods, which becomes one of the most noticeable problems for food safety [2]. To safeguard human

health, many organizations in the world have officially established a maximum TET residues limit of 100 μg/kg in the food-producing species [3-5]. Therefore, it should be highly desirable to develop a sensitive method that can monitor the residues of TET in food samples. The traditional analytical methods for TET detection involving gas chromatography (GC), high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and mass spectrometry (MS) are sensitive, reliable and standardized techniques [6-10], but these methods require expensive instruments, skilled analysts and involve extensive time consumption and complicated pretreatment procedure.

As alternatives to antibodies, aptamers have recently attracted increasing attention due to their capability to bind a wide range of targets: nucleic acids, proteins, metal ions and other molecules with high affinity and sensitivity [11, 12]. Aptamers are peptides or oligonucleotides, which are synthesized by in vitro process with no need for animal or cell cultures [13-15]. They possess high recognition ability to specific targets ranging from small molecules to proteins and even cells [16]. Moreover, the chemically modified aptamers can be easily immobilized on the surface of electrode, therefore, they are used as recognition molecules in biosensing to replace antibodies.

Electrochemical aptasensors, with high sensitivity, fast analysis, good portability and low cost, had drawn extensive attention in the biosensor fields for various small molecular targets [17-19]. Nanometer materials, as the new modified sensing interface, can facilitate the immobilization of biomolecules and improve electrochemical properties of the transducer, such as owning low-background current, high signal to noise ratio and fast electron transfer [20]. For example, gold nanoparticles (AuNPs) can allow efficient adsorption of proteins as a result of their exceptional properties, such as large surface area, superior mechanical properties, excellent conductivity and large thermal conductivity. Methylene blue (MB), a cationic dye, has well-known electrochemical properties in the solution phase. The electrochemical behavior of its monomer in conductive substrate is good and it has been used as a redox indicator [21-23]. In this study, we aimed to improve the stability and maintain the activity of aptamer to develop a simple, stable and sensitive lable-free aptasensor for tetracycline detection. AuNPs possessing good electrochemical behavior was employed to immobilize aptamer on the sensor substrate surface without decreasing their binding affinities and binding capacities [24]. Furthermore, methylene blue can provide much more adsorption site for TET. Here, the GCE was modified with AuNPs and MB to promote the electron transfer, and anti-TET aptamer was immobilized between AuNPs and MB for specifically recognizing TET. To date, however, the application of sandwich method to construct aptasensor for TET detection has been still very less reported. The experimental conditions involving the concentration of the aptamer, the time, temperature and the pH were investigated in detail. As a result, the obtained electrochemical aptasensor for the direct determination of tetracycline in milk exhibited excellent characteristics including high sensitivity, low detection limit and long-term stability.

2. MATERIALS AND METHODS

2.1 Reagents

The anti-TET oligonucleotides purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China) with the following sequences were designed by

Niazi [24] and Zhou [25]: 5'-NH_2-(CH_2)-CGT ACG GAA TTC GCT AGC CCC CCG GCA GGC CAC GGC TTG GGT TGG TCC CAC TGC GCG TGG ATC CGA GCT CCA CGT G -3' and probe aptamer 5'-GCA TGC CTT AAG CGA TCG GGG GGC CGT CCG GTG CCG AAC CCA ACC AGG GTG ACG CGC ACC TAG GCT CGA GGT GCA C-6 FAM (FITC)-3'. Chloroauric acid ($HAuCl_4$) and ethanol were obtained from Sinopharm Chemical Reagent Co., Ltd. Methylene blue (MB) was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. Phosphate buffer solution (PBS, pH = 7.4) was prepared from 1.45 g $Na_2HPO_4 \cdot 12H_2O$, 0.1 g KH_2PO_4, 0.1 g KCl and 4 g NaCl in 500 mL ultrapure water with its pH adjusted by HCl and NaOH solutions. The other chemicals were analytical reagent grade and used without further purification. Ultrapure water (18.2 MΩ·cm) was produced by Millipore (PALL, Germany).

2.2 Apparatus

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) and measurements were performed with CHI660D electrochemical workstation (Shanghai Chenhua Co., China). A conventional three-electrode system was employed with a saturated calomel electrode (SCE) as the reference electrode, a platinum electrode as the auxiliary electrode, and a glassy carbon electrode (GCE) (d=3 mm) or modified GCE as the working electrode. The morphologies of GNPs and the fabrication process of the aptasensor were observed by a scanning electron microscope (SEM, SIRION, FEI, Netherlands.).

2.3 Preparation of the aptasensor

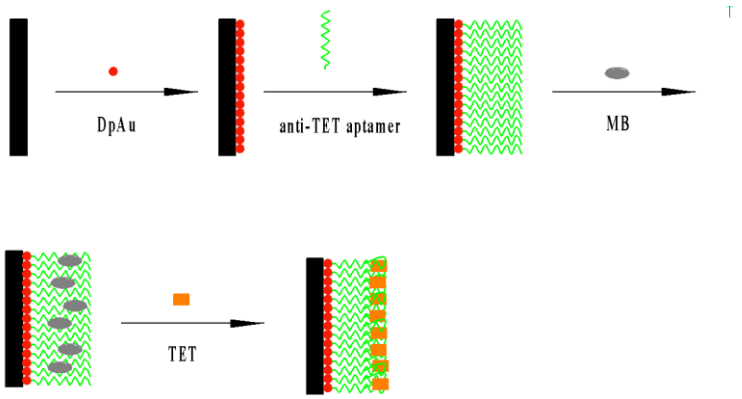
2.3.1 Electrode surface cleaning

GCE (with a diameter of 3 mm) were sonicated in a mixture solution of "piranha solution" (H_2SO_4:30% H_2O_2 = 3:1) and rinsed with distilled water. The bare GCE was polished carefully with 0.3 and 0.05 μm alumina powder, respectively. Then they were immerged in 6.0 M HNO_3, absolute ethanol and distilled water in sequence for an ultrasonic bath for 5 min. Before modification, the bare GCE was scanned in 0.5 M H_2SO_4 between -1 and 1 V with a scan rate of 0.1 V/s until a steady-state curve was obtained. Then the electrode was rinsed with distilled water, and dried in air.

2.3.2 The fabrication of aptasensor

The cleaned GCE was treated by electrodeposition method performed with current-time curve scanning at potential of -0.2V for 200s in 3 mM $HAuCl_4$ (denoted as DpAu/GCE). A 20μL anti-TET aptamer with 5 mM concentration was dropped onto the DpAu/GCE surface. Then a certain concentration MB solution was dropped onto the electrode surface (denoted as MB/anti-TET aptamer/DpAu/GCE). 4 h latter, the obtained MB/anti-TET aptamer/DpAu/GCE was rinsed with 0.1 M Tris- HCl buffer to remove unbound aptamer, and then dried in the air atmosphere. Finally, the

resulted aptasensor was stored above the 0.1 M PBS at 4°C when not in use. The stepwise fabrication procedure of aptasensor was shown in Scheme 1.



Scheme 1. Schematic illustration of the stepwise immunosensor fabrication process.

2.4 Electrochemical measurements

The scanning electron micrograph of DpAu on the GCE surface was observed with SEM. All electrochemical measurements were performed in 10 mL of 0.1 M PBS (pH 7.5) containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1 mixture as a redox probe) and 0.1 M KCl at room temperature. Cyclic voltammetric (CV) measurements were performed over a potential range from -0.2 to 0.6 V at a scan rate of 50 mV/s (vs. SCE) in the above mentioned detection solution (pH 7.5). The impedance spectra was measured at a potential of 0.2 V in the frequency range from 0.1 to 10^5 Hz with a voltage amplitude of 5 mV. The TET detection was based on relative change in current response ($\%\Delta I = (I_0 - I_1)/I_0 \times 100\%$), where I_0 was the cathodic peak current of MB/anti-TET aptamer/DpAu/GCE and I_1 was the cathodic peak current of the CV after exposure to the TET.

2.5 Preparation and determination of real milk samples

The milk samples used in this study were all purchased from a super- market in China. The milk samples were prepared as follows: the milk was firstly diluted at the ratio of 1:10 and ultracentrifugated at 30 000 rpm for 90 min. In this way, the intermediate layer milk serum was obtained without fat and casein.

3. RESULTS AND DISCUSSION

3.1 SEM characterization of the different modified GCE interfaces

SEM was employed to observe the morphologies and microstructures after modified with electrodeposition gold nanoparticles. As shown in SEM profiles (Fig. 1A), the uniform dispersion

DpAu without obvious aggregation could be found. The SEM image revealed that the DpAu were well distributed on the surface of the GCE with the formation of small nano-particles. Therefore it could be concluded that DpAu had been successfully dispersed on the surface of the GCE.

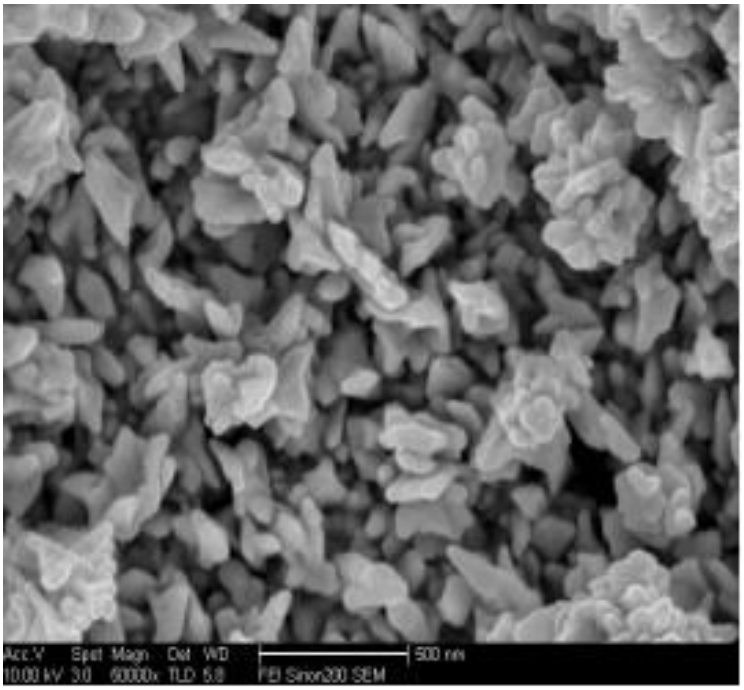


Figure 1. SEM images of DpAu/GCE

3.2 Electrochemical characterization of the modifying process

Fig. 2 showed the CVs of the modifying process in the presence of 0.1 M PBS (pH 7.5) and 5.0 mM $[Fe(CN)_6]^{3-/4-}$ at a scan rate of 50 mV/s. It can be seen that a couple of well-defined redox peaks (Fig. 2a) appeared when the bare GCE was immersed in the detection solution. The peak current increased to 90 μA after DpAu was electrodeposited on the surface of GCE (Fig. 2b), which increased significantly compared with gold colloidal nanoparticles [26] indicated that the DpAu film functioned as an electron-conducting tunnel could promote the electron transfer between electrode surface and $[Fe(CN)_6]^{3-/4-}$. When anti-TET aptamer was immobilized onto DpAu /GCE, the peak current decreased obviously (Fig. 2c), attributed to their non-electrochemical activity which partially blocked the electron transfer between $[Fe(CN)_6]^{3-/4-}$ solution and the electrode. This phenomenon showed that the anti-TET aptamer have been immobilized onto the modified electrode surface. After MB was modified onto anti-TET aptamer/DpAu/GCE, the peak current increased again. Because MB served as electron conducting materials can accelerate the electron transfer between the electrode and the detection solution. At the same time, it can provide a favorable microenvironment to obtain much more adsorption site for TET (Fig. 2d). Here, a sandwich method was used for constructing aptasensor. Anti-TET aptamer was immobilized between AuNPs and MB for specifically recognizing TET. AuNPs and MB can promote the electron transfer. Therefore, the current signal was 4 folds that of other

nanomaterials modified method [27]. After TET molecules were combined with anti-TET aptamer, a decrease of the redox peaks was observed (Fig. 2e).

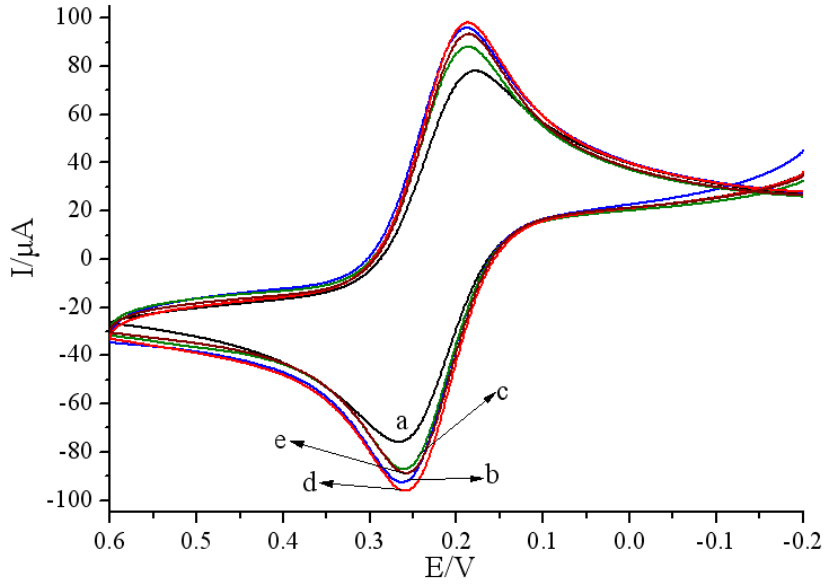


Figure 2. CVs of modified GCE recorded in 0.1 M PBS (pH 7.5) containing 5.0mM $[Fe(CN)_6]^{3-/4-}$ and 0.1M KCl: (a) bare GCE; (b) DpAu /GCE; (c) anti-TET aptamer/DpAu/GCE; (d) MB/anti-TET aptamer/DpAu/GCE; (e) TET/MB/anti-TET aptamer/DpAu/GCE.

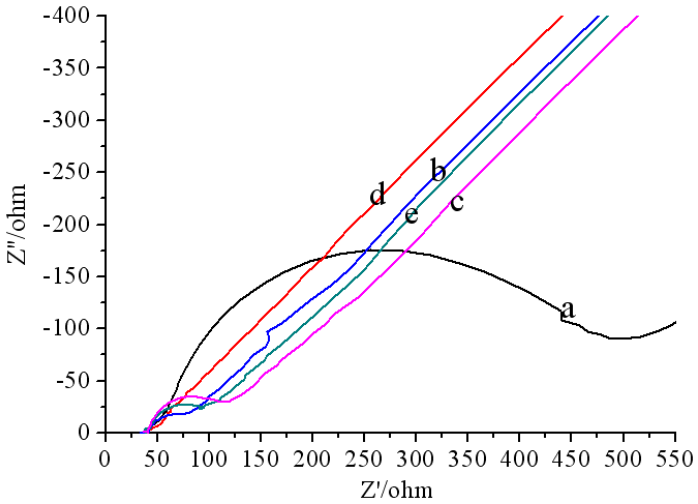


Figure 3. EIS of modified GCE recorded in 0.1 M PBS (pH 7.5) containing 5.0mM $[Fe(CN)_6]^{3-/4-}$ and 0.1M KCl: (a) bare GCE; (b) DpAu /GCE; (c) anti-TET aptamer/DpAu/GCE; (d) MB/anti-TET aptamer/DpAu/GCE; (e) TET/MB/anti-TET aptamer/DpAu/GCE.

Fig. 3 illustrated the Nyquist diagram of EIS corresponding to the stepwise modification procedure using $[Fe(CN)_6]^{3-/4-}$ as the redox probe, and the results were in agreement with the conclusions obtained from the CV data. It can be seen a well defined semicircle at high frequencies and a linear part at low frequencies in the EIS of the bare GCE (Fig. 3a). Compared to the bare GCE,

when DpAu (Fig. 3b) was assembled onto the electrode surface, a significant lower resistance was obtained, implied that DpAu served as electron conducting materials and accelerated the electron transfer between the electrode and the detection solution. When anti-TET aptamer was immobilized onto the modified electrode surface (Fig. 3c), the Ret increased obviously, attributed to that aptamer acted as inert electron layers and hindered the electron transfer. After MB was modified onto anti-TET aptamer/DpAu/GCE, the Ret decreased obviously (Fig. 3e). At last, the Ret increased again after the modified electrode was incubated in TET (Fig. 3f), because of the nonconductive properties of biomacromolecule.

3.3 Optimization parameters of the experimental conditions

3.3.1 Influence of the anti-TET aptamer concentration

The specific aptamer is an important role on the establishment of the aptasensor. In order to study the effect of the concentration of anti-TET aptamer on the performance of the electrode, we investigated the concentration of aptamer varying from 2μM to 6μM. It is clear that the concentration of anti-TET aptamer immobilized onto the electrode should play a key role in the analytical characteristics. As shown in Fig. 4A, the inhibition ratio, that is the relative change in current response (ΔI), was found to increase with the increasing of the anti-TET aptamer concentration. The inhibition ratio reached the maximum at a concentration of 5μM. Whereas, the inhibition ratio (ΔI) had no obviously increase with the further increase in the concentration of anti-TET aptamer. The reason might be that the immunoreation of anti-TET aptamer and TET reaches equilibrium, so with subsequent increases of the aptamer concentration, the inhibition ratio did not increase Therefore, 5μM anti-TET aptamer was chosen in further experiments.

3.3.3 Influence of the pH of the detection solution

The pH value of the detection solution is an important factor of the aptasensor performance. The anti-TET aptasensor was incubated in PBS solutions containing 5.0mM $[Fe(CN)_6]^{3-/4-}$ and 0.1M KCl with different pH of 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5, and the relative change in current response (ΔI) were measured. The experimental results were shown that the inhibition ratio increased with pH value from 6.0 to 7.5, and then decreased when pH value increase again from 7.5 to 8.5 (shown in Fig. 4B). That is to say the the maximum inhibition ratio appeared at pH 7.5. The reasons might that were the biological activity of the antibody as protein declined in acid and alkaline solutions and the antigen-antibody complex could easily be dissociated in the unsuitable pH of working solution. Thus, the detection solution of pH 7.5 was chosen in subsequent experiments.

3.3.4 Influence of incubation time

Incubation time is another factor that could influence the response of the immnunosensor, for that a short incubation time would lead to the insufficiency of the reaction and a long incubation time

would cause the dissociation of the TET-aptamer complex. As displayed in Fig. 4C, the inhibition ratio reached the maximum in 40 min and were stable when the time was extended, indicated that the interaction between aptamer and TET had reached saturation. Thus, 40 min was chosen as the optimal incubation time in subsequent experiments.

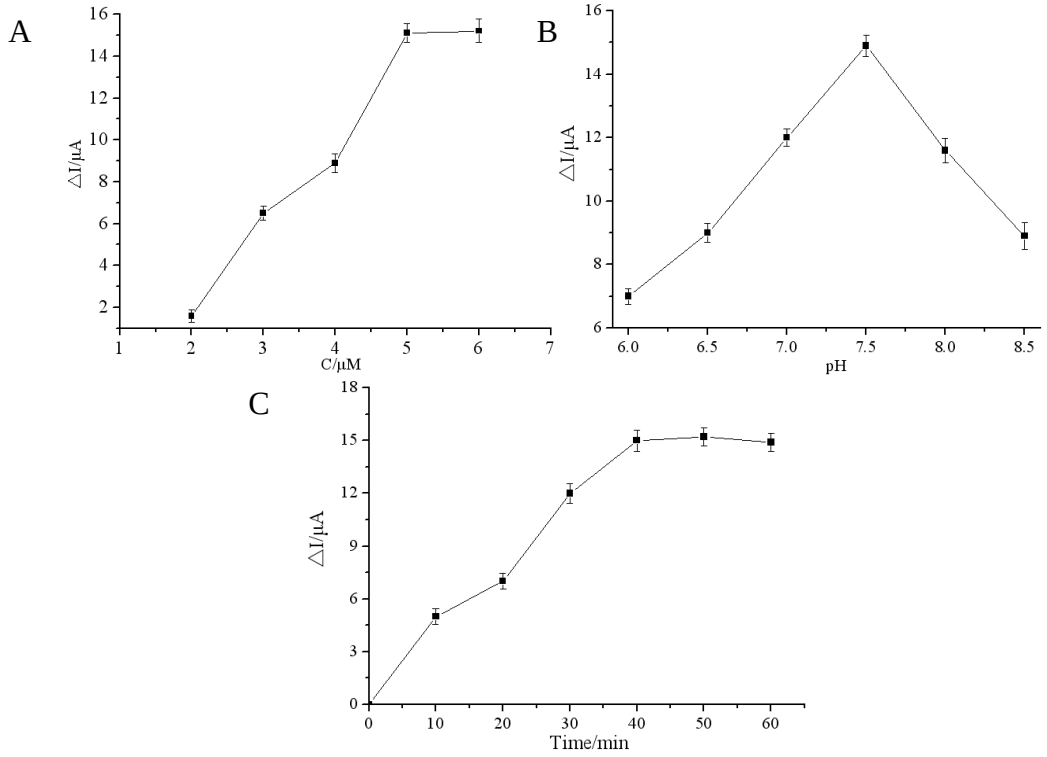


Figure 4. Optimization of experimental parameters: (A) Influence of anti-TET concentration; (B) Influence of detection solution pH; (C) Influence of incubation temperature; (D) Influence of incubation time.

3.4 Calibration curve for TET detection

Under optimal experimental conditions, the calibration plot for detecting TET with the proposed aptasensor is shown in Fig. 5. A good linear relationship between the relative current change and logarithm of TET concentration in the range from 10^{-3} to 10^{-10} M was obtained. The linear regression equation is ΔI=23.305+2.124lgC (M) with the correlation coefficients of 0.9878. The detection limit was estimated to be 0.42×10^{-11} M at a signal/noise of 3 (S/N=3) between the detection signal of low concentration samples and the noise of blank samples, lower than that of TET detection in other ways showed in Table. 1. The low detection limit may be attributed to three factors: (1) The DpAu immobilized on the electrode formed a thin film with relatively high electroactivity; (2) MB served as electron conducting materials can accelerate the electron transfer between the electrode and the detection solution. At the same time, it can provide a favorable microenvironment to obtain much more adsorption site for TET.

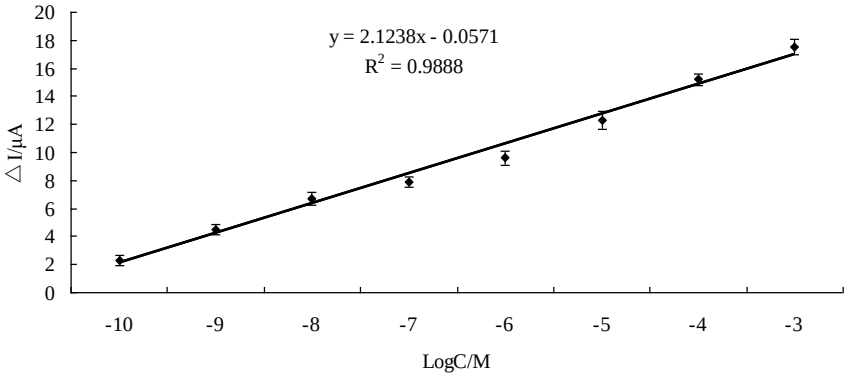


Figure 5. The calibration curve of the relative current changes (ΔI) of the proposed aptasensor versus the logarithm of TET concentration.

Table 1. Comparison with other electrochemical aptasensors for the determination of TET.

Method of detection	Detection limit (M)	Liner range (M)	References
Label-free aptasensor	0.5265×10^{-9}	1×10^{-8}-5×10^{-5}	[25]
Indirect competitive enzyme-linked aptamer assay	2.2×10^{-10}	2.25×10^{-10}-2.25×10^{-6}	[28]
Label-free aptasensor	2.25×10^{-9}	1.125×10^{-8}-1.125×10^{5}	[29]
Label-free aptasensor	0.42×10^{-11}	1×10^{-10}-1×10^{-3}	this work

3.5 Performance of the aptasensor

3.5.1 Reproducibility and stability of the aptasensor

To evaluate the reproducibility of the aptasensor, a series of five electrodes were prepared for the detection of 10^{-5}M TET. The relative standard deviation (RSD) of the parallel measurements for the five electrodes was 6.4%, confirming that the proposed aptasensor possessed good reproducibility and precision.

The stability of the aptasensor was studied by comparing the ΔI after a storage period of 7, 14 and 21 days. The electrodes were stored in refrigerator at 4°C and ΔI was periodically measured. The response current retained above 95%, 91% and 86% of its initial response, respectively, which indicated the storage stability of the aptasensor was quite good.

3.5.2 Specificity of the aptasensor

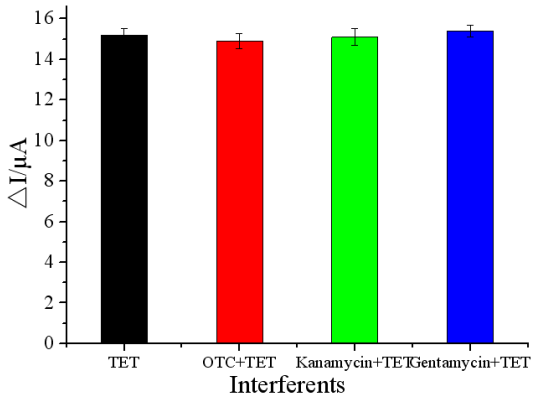


Figure 6. The specificity of the aptasensor in the presence of 5 μM TET, 5 μM OTC, 5 μM Kanamycin Monosulfate, 5μM Gentamycin Sulfate.

To further investigate the specificity of the proposed aptasensor for TET, the selectivity test was carried out by measuring and comparing the response of TET (5 μM) to some possible interferences including a structurally similar tetracycline derivative Oxytetracycline Hydrochloride (OTC) (5 μM) and two structurally distinct molecules of Kanamycin Monosulfate (5 μM) and Gentamycin Sulfate (5 μM). It could be observed in Fig. 6, the TET sample had a significant peak current change, while the same concentrations of the other three chemicals had slight emissions. These tests indicated that the developed strategy could be used to identify TET with high specificity.

3.6 Determination of TET in milk samples

In order to evaluate the feasibility of the aptasensor system for possible applications, the standard addition method was used. The milk samples were prepared as follows: the milk was firstly diluted at the ratio of 1:10 and ultracentrifugated at 30 000 rpm for 90 min. In this way, the intermediate layer milk serum was obtained without fat and casein. Then we added TET to the collected TET-free milk serum to the final concentration of 5×10^{-9} M, 5×10^{-7}M and 5×10^{-5} M. As shown in Table 2, the recovery tests were performed using 3 replicates with the aptasensor that prepared in the same way. The recovery of 96~108% indicated that the aptasensor was available for the analysis of TET in milk samples.

Table 2. Determination of TET in milk samples

Sample	Added (M)	Total found (M)	RSD(%, n=3)	Recovery (%)
1	5×10^{-9}	4.8×10^{-9}	4.8	96
2	5×10^{-7}	5.1×10^{-7}	6.3	108
3	5×10^{-5}	4.7×10^{-5}	5.6	94

4. CONCLUSIONS

This work developed a novel label-free electrochemical aptasensor for the rapid and effective determination of TET residues in milk. Gold nanoparticles were firstly modified onto the glassy carbon electrode (GCE) by one-step electrodeposition method. Then the anti-tetracycline aptamer was immobilized onto gold nanoparticles modified gold electrode surface by Au-S bond. Finally, methylene blue was dripped onto immobilized aptamer GE surface to fabricate a sensitive aptasensor for TET detection. Here, DpAu served as electron conducting materials and accelerated the electron transfer between the electrode and the detection solution. MB acted as an efficient biointerface film with good biocompatibility can not only enhance the conductivity, but also enlarge the specific surface area of the aptasensor interface, which helps to immobilize aptamer more efficiently. This strategy contributed to improve the sensitivity and stability of aptasensor, thus, it provided a novel promising platform of aptasensor for TET detection.

ACKNOWLEDGEMENTS
This work was supported by the National Natural Science Foundation of China (No.30972055, 31101286, 31471641), Agricultural Science and Technology Achievements Transformation Fund Projects of the Ministry of Science and Technology of China (No.2011GB2C60020), Special project of independent innovation of Shandong Province (2014CGZH0703) and Shandong Provincial Natural Science Foundation, China (No.Q2008D03, ZR2014CM009).

References

1. K. Kishida, *Food Chem*. 126 (2011) 687.
2. D. Chen, D. Yao, C. Xie , D. Liu, *Food Control*, 42 (2014), 109.
3. Commission Regulation 508/1999/EC, 1999.
4. Japanese Ministry of Health Welfare and Labor, 2008.
5. US Food and Drug Administration, 1975.
6. J. W. Fritz,Y. Zuo, *Food Chem*. 105(2007) 1297.
7. K. Ng, S. W. Linder, *J. Chromatogr Sci*. 41 (2003) 460.
8. A. C. Martel, S. Zeggane, P. Drajnudel, J. P. Faucon, M. Aubert, *Food Addit. Contam*. 23 (2006) 265
9. P. Kowalski, *J. Pharm. Biomed*. 47 (2008) 487.
10. B. Y. Deng, Q. X. Xu, H. Lu, L. Ye, Y. Z. Wang, *Food Chem*. 134 (2012) 2350.
11. P. Chandra, H. B. Noh, M. S. Won, Y. B. Shim, *Biosens. Bioelectron*., 26 (2011) 4442.
12. S. Song, L. Wang, J. Li, C. Fan, J. Zhao, *Trac-Trend Anal Chem*, 27 (2008), 108.
13. W. Winkler, A. Nahvi, R. R. Breaker, *Nature*, 419 (2002) 952.
14. E. González-Fernández, N. de-los-Santos-Álvarez, M. J. Lobo-Castañón, A. J. Miranda-Ordieres, P. Tuñón-Blanco, *Biosens. Bioelectron.*, 26 (2011) 2354.
15. Edward N. Brody, Michael C. Willis, Jonathan D. Smith, Sumedha Jayasena, Dominic Zichi, Larry Gold， Molecular Diagnosis,4(1999) 381
16. R. Pauliukaite, M.E. Ghica, O. Fatibello-Filho and C.M.A. Brett, *Electrochim. Acta,* 55 (2010) 6239
17. Y.C. Zhang and C. Ji, *Anal. Chem.*, 82 (2010) 5275
18. K.S. Novoselov, A.K. Geim, S.V. Morozov, D. Jiang, Y. Zhang, S.V. Dubonos, I.V.Grigorieva and A.A. Firsov, *Science*, 306 (2004) 666

19. C.Q. Ruan, W. Shi, H.R. Jiang, Y.N. Sun, X. Liu, X.Y. Zhang, Z. Sun, L.F. Dai and D.T. Ge, *Sens. Actuat. B Chem*, 177
20. H. Yao, N. Li, S. Xu, J.Z. Xu, J.J. Zhu and H.Y. Chen, *Biosens. Bioelectron.*, 21 (2005) 372
21. X.H. Lin, P. Wu, W. Chen, Y.F. Zhang and X.H. Xia, *Talanta*, 72 (2007) 468
22. J.L. Wang, F. Wang and S.J. Dong, *J. Electroanal. Chem.*, 626 (2009) 1
23. K.X. Mao, D. Wu, Y. Li, H.M. Ma, Z.Z. Ni, H.Q. Yu, C.N. Luo, Q. Wei and B. Du, *Anal. Biochem.*, 422 (2012) 22
24. J. H. Niazi, S. J. Lee and M. B. Gu, Bioorg. Med. Chem. 16(2008) 7245.
25. L. Zhou, D. J. Li, L. Gai, J. P. Wang and Y.B. Li, Sens. Actuators, B: Chem. 162(2012)201-208.
26. S. Du, X. Wang, X. Sun, Analytical Letters, 45 (2013)1230
27. G.Shen, Y. Guo, X. Sun X. Wang, *Nano-Micro Letters,6(2014):143*
28. S. Wang, J. Liu, W. Yong, Q. Chen, L. Zhang, Y. Dong, H. Su, T. Tan, Talanta, 31(2015) 562
29. D.Chen, D. Yao, C. Xie, D. Liu, Food Control, 42(2014)109