

Short Communication

Fabrication of Aptasensors Modified by MWCNTs-CS / Fe₃O₄-CS Based on SPEs

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In this paper, A new electrochemical aptamer biosensor for detecting tetracycline (TET) has been developed based on the signal amplification of MWCNTs-CS and Fe₃O₄-CS. The multiwalled carbon nanotube (MWCNTs) and ferroferric oxide (Fe₃O₄) were dispersed in chitosan evenly. Then the MWCNTs-CS, Fe₃O₄-CS and the aptamer of anti-TET were modified on the surface of screen printing electrode respectively for preparation of the aptasensor. The electrochemistry properties of the surface modification were investigated by using cyclic voltammetry (CV). The proposed aptasensor showed a high sensitivity and a good stability. The proposed aptasensor exhibited a wide linear range from 10⁻² to 10⁻⁹ M and with the correlation coefficients of 0.9651. The detection limit for tetracycline was 10⁻⁹ M. The application of this method for samples of TET-spiked raw milk suggested satisfactory recoveries between 92% and 98%. The proposed method was proven to be a feasible quantitative method for tetracycline analysis.

Keywords: Aptasensor; Tetracycline; Multiwalled Carbon Nanotube; Ferroferric Oxide

1. INTRODUCTION

Tetracyclines (TCs) have been widely used as a antibacterial agent, because it has a broad spectrum antibacterial activity, so it can control the bacterial infection and increase the growth rate of the animal in livestock and poultry production [1]. But the abuse of TET and other TCs in aquaculture and animal husbandry may result in excessive amounts of their residues in daily food [2-5]. Tetracyclines residues in milk not only cause great harm to people's health but also bring heavy economic losses to dairy processing enterprises. So, it is necessary to control antibiotic residues in milk. Besides controlling the use of antibiotics from the source, the timely and accurate detection of

antibiotic residues in animal derived food is an effective way to protect the safety of livestock products.

Traditional analytical methods involving high performance liquid chromatography (HPLC) [6], capillary electrophoresis (CE) [7,8], mass spectrometric detection (MS) [9,10], the microbiological multi-residue system [11] and fluorescence (FL) [12,13] etc. These traditional analytical methods are highly sensitive, highly veracity, good stability and specific. But the price of their equipment is very expensive, and sample pretreatment has a complicated procedures, and they need professional people to operate them. Above these methods are not suitable for wide and rapid detection of antibiotics.

In recent years, aptasensor is emerged with a driving development vigor which can meet the requirements of simplicity, sensitivity and specificity for the detection of diverse substances at trace levels [14,15]. Aptamers are RNA or DNA molecules with specific three-dimensional structures. They have the ability to recognize and combine the targets ranging from small molecules to organisms. Compared to antibodies, aptamers have more advantages, such as in vitro synthesis, have a short cycle, target versatility, low cost, easy modification and preservation, high specificity, good stability and low molecular weight [16-21]. The electrochemical aptasensors for the detection of tetracycline is widely used in the detection of tetracycline in food. But generally aptasensors have low sensitivity, in order to improve the sensitivity of the aptasensors, nanomaterials and composite nanomaterials have been used to modified the aptasensors. Such as gold nanoparticles, multiwall carbon nanotubes, chitosan, graphene. They have high electrical conductivity or a large specific surface area or better biocompatibility and so on.

In this study, the nanocomposite of MWCNTs, Fe_3O_4 and CS were used. MWCNTs have high electrical conductivity, chemical stability and mechanical strength [22]. Fe_3O_4 have high electrical conductivity, can enhance the surface area, then it can increase the amount of aptamer [23]. Chitosan as membrane material contains large groups of $-\text{NH}_2$ and $-\text{OH}$ which can keep the biological activity of the biomolecules that immobilized on the chitosan membrane [24]. The MWCNTs and Fe_3O_4 were dispersed in chitosan evenly. Then the MWCNTs-CS and Fe_3O_4 -CS were modified on the surface of screen printing electrode to immobilize the anti-TET aptamer. To the best of our knowledge, such an aptasensor has not been reported. The proposed aptasensor has the advantages of simple operation, high sensitivity, short detection time and high specificity. This electrochemical aptasensor is simple, rapid, sensitive and highly specific, and it was developed for the sensitive detection of Tetracyclines in real milk samples.

2. EXPERIMENTAL

2.1 Reagents

Tetracycline was provided by Sigma (America). The anti-TET oligonucleotides purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The sequences of aptamer is 5'-SH-(CH_2)₆-GTC TCT GTG TGC GCC AGA GAA CAC TGG GGC AGA TAT GGG CCA GCA CAG AAT GAG GCC C-3'. Chitosans (CS) were obtained from Sangon

Biotech Co., Ltd. (Shanghai, China). MWCNTs (purity>95%) was purchased from Shenzhen Nanotech Port Company (China). Other chemical reagents were of analytical grade. All solutions were prepared using double distilled water.

2.2 Apparatus

Cyclic voltammograms were performed with CHI660D electrochemical workstation (Shanghai Chenhua Co., China). The working electrode was screen printing electrode which purchased from Zensor R&D Co., LTD (Formosa).

2.3 Preparation of MWCNTs-CS

1% chitosan solution was prepared by dissolving 0.5 g chitosan flakes into 100 mL 1.0% acetic acid and stirring for 10 h. Then 2.5 mg MWCNTs was added into the 1% CS solution 10mL by ultrasonic treatment 2 h to get MWCNTs-CS solution.

2.4 Preparation of Fe₃O₄-CS

2.5mg Fe₃O₄ was weighted with the analytical balance. Then 2.5 mg Fe₃O₄ was added into the 1% CS solution 10mL by sonicating to get Fe₃O₄-CS solution. The Fe₃O₄-CS solution was uniform and steady.

2.5 Preparation of aptamer

The sequence of tetracycline was determined by consulting the literature [25]. In order to better fit the aptamer to the electrode surface, the 5'terminal of the aptamer was modified to be a thiol. and was synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. Shanghai, (China), with the sequence of 5'-SH-(CH₂)₆-GTC TCT GTG TGC GCC AGA GAA CAC TGG GGC AGA TAT GGG CCA GCA CAG AAT GAG GCC C-3'.

In order to avoid the loss of the aptamers, it was centrifuged before open the cover. According to the directions, 2 μM and 10 μM aptamer solution was prepared by added 700 μL and 140 μL PBS (pH 7.0 0.1 mol/L) to two tube of ptamers (1 OD) of tetracycline respectively, then vibrating for 5~10 min, stored at -20°C.

2.6 Activation of screen printed electrode

Firstly, immerse the screen printing electrode in 0.05 mol/L H₂SO₄ solution. After that, the electrode was cyclic scanned in the potential range from -0.5 to +1.1 V until the C-V curve was stable

by the cyclic voltammetry. The electrode was rinsed with ultrapure water, dried by nitrogen and stored at the environment of 4 ° C.

2.7 Fabrication of the aptasensor

MWCNTs-CS were added onto the surface of bare electrode and dried in the air at room temperature (noted as MWCNTs-CS/SPE). Then the electrode of MWCNTs-CS/SPE was modified with Fe₃O₄-CS and dried in the air at room temperature (noted as Fe₃O₄-CS/MWCNTs-CS/SPE). Finally 4 μL anti-TET aptamer was dropped onto the electrode of Fe₃O₄-CS/MWCNTs-CS/SPE and dried in the air at room temperature (noted as anti-TET /Fe₃O₄-CS/MWCNTs-CS/SPE), stored at 4 °C. The combination principle chart of aptasensor was shown in Fig. 1.

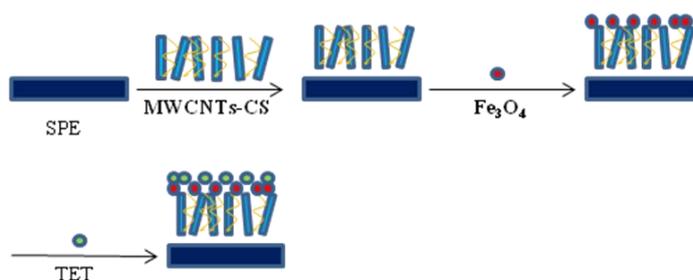


Figure 1. Combination principle chart of aptasensor

2.8 Electrochemical measurements

The Fe₃O₄-CS/MWCNTs-CS/SPE biosensor was employed for the determination of tetracycline using cyclic voltammetry (CV) method. The performance of the biosensor was tested by its CV response in 5 mM [Fe(CN)₆]^{3-/4-}. Then the electrode was rinsed with distilled water and incubated in the desired concentration of tetracycline for a period of time. Finally, the electrode was characterized by CV for the detection of TET. The CV measurements were carried out under the following conditions: The voltage was scanned from -0.2 V to +0.6 V, the sweep rate was 0.05 V/s.

3. RESULTS AND DISCUSSION

3.1 Current characterization of the assembly process of the aptasensor

In this paper, cyclic voltammetry (CV) was used to compare the electrochemical behavior of the nano-composites. The cyclic voltammograms of bare screen-printed carbon electrode and differently modified electrodes are presented in Fig. 2. The cyclic voltammogram of bare electrode identified an apparent peak current (curve a), and the peak current was about 8 μA. Because the solution of [Fe (CN)₆]³⁻ has Fe³⁺ that can produce the charge transfer with the electrode. The peak currents of the MWCNTs-CS/SPE (curve d) increase since the large specific surface area and good conductivity of MWCNTs, and the peak current was about 20 μA. The highest redox peaks was appear

at the $\text{Fe}_3\text{O}_4\text{-CS/MWCNTs-CS/SPE}$ (curve e) due to the good electrical conductivity of Fe_3O_4 , and the peak current was about $26 \mu\text{A}$. While the $5 \mu\text{L}$ 5 mM anti-TET aptamer was immobilized successfully on the modified electrode, the peak currents decreased (curve c). Because the adapter hindered the transfer of charge between the solution and the electrode. Finally, the anti-TET/ $\text{Fe}_3\text{O}_4\text{-CS/MWCNTs-CS/SPE}$ was placed in the sample solution for a period of time, and then the CV measurement of the aptasensor was performed. The peak currents of the TET/anti-TET/ $\text{Fe}_3\text{O}_4\text{-CS/MWCNTs-CS/SPE}$ (curve b) was smaller than the peak currents of the anti-TET/ $\text{Fe}_3\text{O}_4\text{-CS/MWCNTs-CS/SPE}$. This indicates that the specific binding of the adapter and tetracycline form a complex of immune complex, which can further hinders the charge transfer between the solution and the electrode.

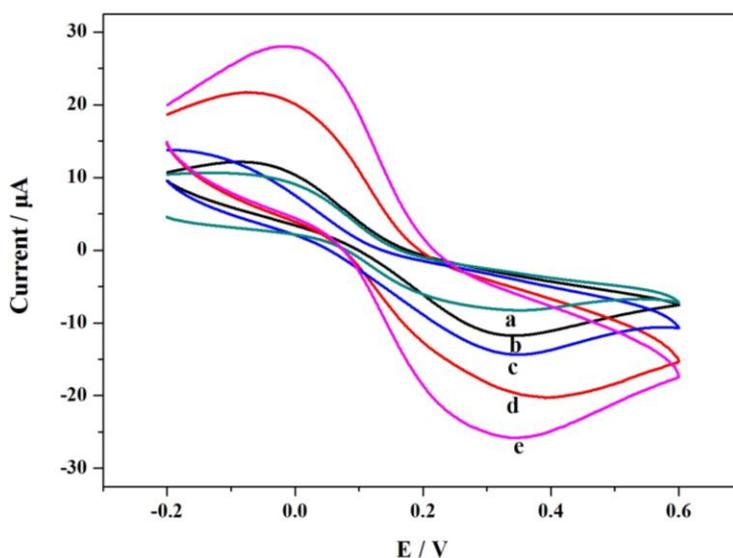


Figure 2. CV characteristics of aptasensors (a) bare SPE, (b) TET/ anti-TET / $\text{Fe}_3\text{O}_4\text{-CS/MWCNTs-CS/SPE}$, (c) anti-TET / $\text{Fe}_3\text{O}_4\text{-CS/MWCNTs-CS/SPE}$, (d) MWCNTs-CS/SPE, (e) $\text{Fe}_3\text{O}_4\text{-CS/MWCNTs-CS/SPE}$

3.2 Detection of TET

We studied the relationship between the anti-TET / $\text{Fe}_3\text{O}_4\text{-CS/MWCNTs-CS/SPE}$ sensor and the different concentrations of pesticides. The CV responses were examined before and after exposure to different concentration tetracyclines. As shown in Fig.3A, as the concentration of tetracycline increased gradually, the value of the current was decreased gradually (curve a-h). As shown in Fig.3B, curve indicates the current variables of CV showing a good linear relationship with the logarithmic values of the TET concentrations in ranges of $1 \times 10^{-9} \text{ M} \sim 1 \times 10^{-2} \text{ M}$. The linear equation was $\Delta I (\mu\text{A}) = 1.0643 \text{ Log} C (\text{M}) + 11.229$, with a correlation coefficient of 0.9651, the detection limit was $1 \times 10^{-9} \text{ M}$. Compared with other previously reported methods of detecting tetracycline, the proposed aptasensor exhibited a higher sensitivity and a lower detection limit (As shown in Table 1 and Table 2).

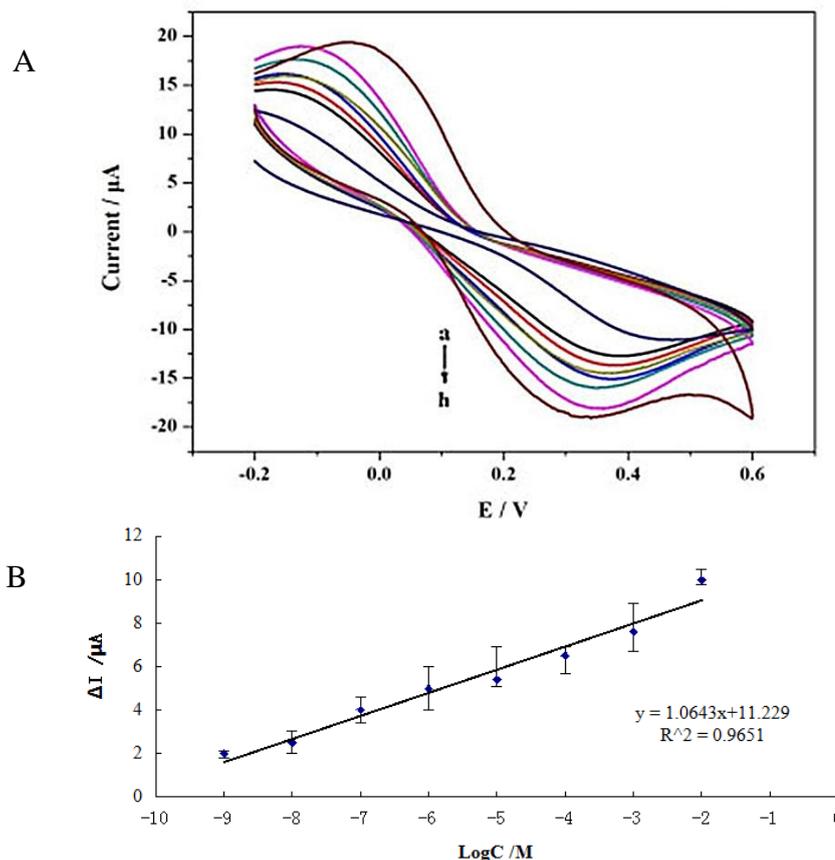


Figure 3. (A) CV chart of aptasensor with different concentration of TET(a-h 1×10^{-2} M, 1×10^{-3} M, 1×10^{-4} M, 1×10^{-5} M, 1×10^{-6} M, 1×10^{-7} M, 1×10^{-8} M, 1×10^{-9} M). (B) Standard curve of aptasensor to TET.

Table 1. Comparison with other electrochemistry methods of detecting tetracycline

Electrode	Liner range (M)	Detection limit (M)	References
aptamer/poly-DPB(AuNP)/SPE	5×10^{-8} to 9×10^{-6}	$9.4 \pm 0.4 \times 10^{-9}$	[26]
Apt/MWCNTs-CoPc/GR-AuNPs/CS-AuNPs/GE	1×10^{-8} to 1.5×10^{-6}	5.8×10^{-9}	[27]
Apt/MWCNTs/GCE	1×10^{-8} to 5×10^{-5}	5×10^{-9}	[28]
Apt/ Fe ₃ O ₄ -CS/MWCNTs-CS/SPE	1×10^{-9} to 1×10^{-2}	1×10^{-9}	This work

Table 2. Comparison with other methods of detecting tetracycline

Method of detection	Liner range (M)	Detection limit (M)	References
HPLC	—	5.58×10^{-8}	[29]
ELISA	—	2.1×10^{-8}	[30]
Colorimetric detection	—	2.5×10^{-8}	[31]
Aptasensor detection	1×10^{-9} to 1×10^{-2}	1×10^{-9}	This work

3.3 Determination of TET in milk samples

In order to further evaluate the performance of the aptasensor system for the detection of tetracycline in real samples, the standard addition method was used. Milk (Yi li was manufactured by the Inner Mongolia Yili Industrial Group Co., Ltd., De yi was manufactured by the Shandong De yi Dairy Co., Ltd., China) were bought from a local supermarket, and they were prepared as follows: the milk was firstly diluted at the ratio of 1:10 with the double distilled water, then, it was centrifugated at 20000 rpm for 90 min. We take the intermediate layer milk serum without fat and casein [32]. 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. Finally experiments were carried out according to the aforementioned method for TET detection with the developed aptamer sensor. The results as shown in table 3, the tetracycline concentration recoveries were between 92% and 98%. This suggests that the aptasensor can be used as a detection of tetracycline in milk.

Table 3. Testing results of tetracycline in milk samples

Sample	Blank sample	Added (M)	Total detected (M)	Recovery (%)
De yi 1	Not detected	5×10^{-5}	4.9×10^{-5}	98
De yi 2	—	5×10^{-7}	4.6×10^{-7}	92
De yi 3	—	5×10^{-9}	4.7×10^{-9}	94
Yi li 1	Not detected	5×10^{-5}	4.8×10^{-5}	96
Yi li 2	—	5×10^{-7}	4.8×10^{-7}	96
Yi li 3	—	5×10^{-9}	4.7×10^{-9}	94

4. CONCLUSION

In this work, the sensor of anti-TET/Fe₃O₄-CS/MWCNTs-CS/SPE had been successfully fabricated for the detection of tetracycline. Fe₃O₄ and MWCNTs and CS have good conductive ability, film-forming ability and excellent biocompatibility that can increase the surface area to capture a large amount of aptamer, thus increased detection sensitivity. Because of the synergistic effects of the Fe₃O₄, MWCNTs and CS, the aptasensor exhibited higher sensitivity, better stability, wider linear response range and short response time. And the low detection limit was 10^{-9} M. Thus, it is more suitable for trace detection of tetracycline residue compared with the other biosensor.

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