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Short Communication

Study on Aptasensors Modified by Ionic Liquid–Fe_3O_4 Based on Microarray Electrodes for Tetracycline Detection

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Aptasensors modified by ionic liquid (IL)-ferroferric oxide(Fe_3O_4) based on the microarray electrodes were developed for the detection of tetracycline. The aptasensor was prepared by dropping ionic liquid-ferroferric oxide mixture on a microarray electrode surface. The mixture exhibited a significant synergistic effect towards the proposed aptasensor. Ionic liquid and ferroferric oxide as the nanocomposites mediator possess the advantage of good electrical conductivity and fast response speed.The electrochemical properties of the modified processes were characterized by electrochemical impedance spectroscopy (EIS). Different concentration of tetracyclines were detected with the fabricated aptasensor. Under the optimized conditions, the proposed aptasensor provided high sensitivity and a low detection limit (1×10^{-9}M). In addition, it was successfully used to detect the tetracyclines in a real sample.

Keywords: Antibiotic residues; Rapid detection; Aptasensor; Microarray electrodes

1. INTRODUCTION

Tetracycline, a kind of broad spectrum antibiotics, is not only used for the prevention and treatment of animal diseases in livestock and poultry industry, but also has effect on promoting growth [1]. When it is abused in humans, it will be seriously harmful to health for human beings[2]. Therefore, the timely and accurate detection of antibiotic residues is an effective way to protect the safety of livestock products. More recently, many methods have been used for the detection of tetracycline,, such as the microbiological method[3], high performance liquid chromatography[4-6], gas chromatography[7-9], immunoassays[10], have been reported for the detection of tetracycline. However, most of the above-mentioned methods are often tedious, time-consuming and require

professional personnel to operate relevant apparatus. Thus, a simple, highly sensitive and specific technique for tetracycline detection is very fulfilling.

Aptamers are RNA or DNA molecules with specific 3-D structures[11]. They can be selected through an in vitro selection process called systematic evolution of ligands by exponential enrichment (SELEX)[12]. Aptamers have been used as antibody mainly because of their distinct characteristic of excellent chemical recognition, high accuracy, ease of modification, high purity and chemically stability[13-19] in the analysis application. Currently, the detection and diagnosis technology used antibody is almost can be replaced by aptamers.

To enhance the sensitivity of the aptasensors, mixture of nanomaterials and conducting polymers have been attracted much attention. As a kind of conductor, ferroferric oxide has good electrical conductivity mainly because Fe^{2+} and Fe^{3+} is basically disordered in octahedral position of magnetite, electrons can quickly shift between two kinds of oxidation state of iron[20]. In addition, ferroferric oxide can form a film on the electrode surface and increase the electrode area, at the same time, the redox active substances can be transported to the electrode surface. So using ferroferric oxide modified electrodes can greatly improve the ability of combining with biological molecules, which will be very good for fixing biomolecules to the electrode surface and improving the sensitivity of sensors. Moreover, a kind of biocompatible materials, ionic liquid, has also been introduced into the modified layers to improve the stability of aptasensors. Ionic liquid possesses good thermal stability, electrical conductivity, low manufacturing cost, and, for most of the inorganic, organic and polymer materials, ionic liquid is a kind of good solvent[21-27].

In this work, we selected interdigitated array microelectrodes for the operation platform. While the major drawback of microelectrodes was that impedance changes due to biorecognition are very small, the IDAMscan maximize the impedance change at the surface, lower the detection time and minimize interfering effects of non-target analytes in the solution. Particularly, IDAMs possess higher sensitivity than conventional electrodes and it has demonstrated particular advantages for applications in the fields of chemical and biochemical processing and environmental monitoring。

Electrochemical impedance spectroscopy (EIS) is a relatively sensitive technique depending on the frequency of the alternating current employed (typically, from 1Hz to 100 kHz). EIS is superior to other analysis methods not only for their good interfacial characterization, but also for the discussion of experimental data can be represented using a variety of forms, such as Nyquist Plot, Bode Phase,Bode Impedance, which makes the experimental data can be analyzed from different perspectives and the results could be more accurate.

In this work, a novel aptasensor was prepared for the detection of tetracycline based on ionic liquid-ferroferric oxide. Ionic liquid-ferroferric oxide nanocomposites constructed an effective immobilization matrix for electron transfer. The results showed that the fabricated aptasensor has the advantages of high sensitivity and it solves the problem of fixing aptamers inconveniently to microelectrode and weak signal. In addition, it can be applied to the sensitive detection of tetracycline in real milk samples.

2. EXPERIMENTAL

2.1. Reagents and chemicals

$NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$ were purchased from Beijing Chemical Technology Co., Ltd. (Beijing, China). The 0.1 M pH 7.5 phosphate buffer solutions (PBS) were prepared by mixing the stock solutions of $NaH_2PO_4 \cdot 2H_2O$ and $Na_2HPO_4 \cdot 12H_2O$. Ferroferric oxide was obtained from Shanghai crystal pure reagent co., Ltd.(Shanghai, China). $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ were purchased from Yong da Chemical Reagent Co., Ltd.(Tianjin, China). The aptamer sequences specific for tetracycline , were as identified by Aniela Wochner et al.[28]， DNA oligonucleotides modified with mercapto groups (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') were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The 5 mM tetracycline was obtained from the Sigma company (USA). All other chemicals were of analytical reagent grade. All the solutions were prepared with ultrapure water which was purified with a Milli-Q purification system (Branstead, USA).

2.2. Apparatus

Electrochemical measurements were carried out using a CHI660D workstation (China). The solution pH values were measured using an FE 20K Mettler-Toledo pH meter (Switzerland). Ultrasonication was performed using a SK3300H ultrasonic cleaner (Shanghai, China). The solution was blended using a PTR – 35 SPC vortex mixer (Britain). The interdigitated microelectrod(IDAM) was selected for the operation platform of this work. In addition, all steps of the electrochemical measurements were carried out at room temperature (RT).

2.3. Preparation of ionic liquid–Fe_3O_4 nanocomposites

Firstly, all the glassware used in the preparation was cleaned with aquaregia(3 parts HCl, 1part HNO_3), rinsed in ultrapure water and oven-dried prior to use. The ionic liquid-Fe_3O_4 nanocomposites were prepared as follows: . 0.1 g of chitosan was added into 50mL of 1.0% acetic acid solution, after stirring until it became translucent with no visible particulate matter. 1 mg ferroferric oxide powder was dispersed into the prepared 4 mL 0.2% chitosan solution. After this the dispersed solution was sonicated over 6 h until it became stable dispersion. At the same time, 2 mg ionic liquid was added into 4 mL of ethanol, after ultrasonic dispersing over 6 h until it became stable dispersion.The resulting mixture was used for all the characterizations of the proposed aptasensor.

2.4. Preparation of the aptamer

According to the illustrations of the tetracycline aptamer, 14 μL buffer was added into an OD primer and formated into 100 μM storage liquid, thus, 140 μL of 0.1 M PBS(pH 7.0) was respectively added into each OD tube of tetracycline aptamer and configured to 10μM concentration of tetracycline aptamer in the test. After dissolving, cover the tube lid and shake it fully for 5-10 minutes. Various

concentrations of tetracycline aptamer was obtained by the diluted the 10μM of tetracycline aptamer for the follow-up experiments using, The resulting aptamer was preserved in - 20 ℃when not in use.

2.5. The fabrication of the aptasensor

Prior to modification, the microelectrode were sequentially soaked in 0.1 M NaOH solution and HCl solution for 10 min, then the electrode surface was wiped respectively with lens wiping paper dipping absolute ethyl alcohol and finally washed with ultrapure water and blowed under nitrogen. After being dried at RT, 15μL of the ionic liquid-Fe_3O_4 nanocomposites was dropped onto the surface of the microelectrode. Then 10μL of the aptamer was assembled on the above-modified microelectrode surface. Finally, 8μL of the prepared tetracycline was added onto the modified electrode surface. After adsorption steps being finished, the modified electrode was thoroughly rinsed with ultrapure water and dried with nitrogen. The process of preparation for the aptasensor was shown n in Fig.1.

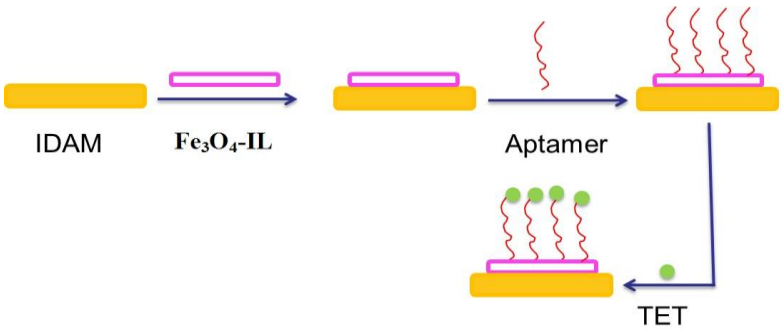

Figure 1. Combination process of aptasensor

2.6. Electrochemical measurements

Electrochemical measurements were carried out in a conventional electrochemical cell at 37℃. Electrochemical impedance spectroscopy（EIS）measurements of the modified microelectrodes were carried out in PBS (pH 7.0) containing 5mM $K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$ (1:1) and 0.1 M KCl. The frequency was measured from1 Hz to100 KHz and the ac voltage amplitude was 55 mV. Record the Nyquist impedance spectrum in the process of reaction (imaginary part of impedance vs real part of impedance), the impedance-frequency curve of Bode diagram and phase frequency curve. The sensitivity and the specificity of the proposed aptasensor were investigated by EIS.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of the modified electrodes

The stepwise fabrication of the aptasensor was characterized by EIS, and the results as shown in Fig.2. Curve a presented a small semicircle domain implying a low electron transfer resistance (R_{et}) about 500 Ω on the bare gold electrode. After ionic liquid-Fe_3O_4 composites was dropped onto the

electrode surface, the R_{et} decreased remarkably to about 250 Ω owning to the excellent conductivity of ionic liquid-Fe3O4 composites which have larger effective surface area than the bare electrode surface(as shown in curve b).. After the tetracycline aptamer was modified onto ionic liquid-Fe_3O_4s/ IDAM, the EIS presented an apparent increase and the R_{et} was about 1250 Ω (curve c). The reasons for this phenomenon is that tetracycline aptamers are macromolecules which has the inhibition effect for electron transfer. Finally, when the target molecule tetracycline was covered onto the TET-aptamer / ionic liquid-Fe3O4s /IDAM, the R_{et} further increased to about 1650 Ω which mainly because that the non-conductive properties of tetracycline(curve d).

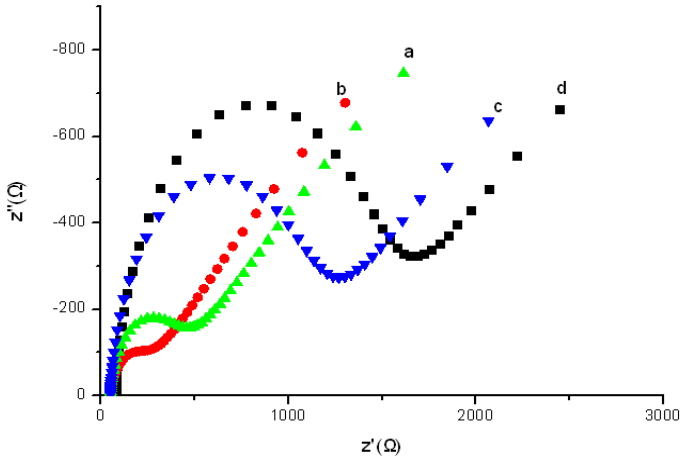

Figure 2. EIS of aptasensor (a) bare electrode (b) ionic liquid-Fe_3O_4 (c) TET aptamer (d)TET; under testing solution of 5 mM $[Fe(CN)6]^{3-/4-}$ and 0.1 mol/L KCl(pH 7.0 PBS)

3.2. Calibration curve

The calibration curve for tetracycline detection with the prepared aptasensor. As we can see from Fig.3A, different concentrations of tetracycline were detected ,which presented the change of impedance value. As tetracycline concentration increasing, the R_{et} was also increased gradually. Moreover, the corresponding calibration curve exhibited good linearity. As shown in Fig.3B, the changes of the impendence contrast response of the aptasensor were proved to be proportional to the tetracycline concentration, with a detection limit of 10^{-9}M (S/N=3). The equation was ΔR (Ω)= 22.600LogC(M)+217.26 (R=0.9745).

A variety of adptasensors have been reported to detect the tetracycline in milk. For example, the establishment of enzyme - linked aptamer assay with the detection limit of 2.5ng/mL[29]; another biosensor was fabricated using the identify molecular which was made by isothermal heat drops of quantitative method of screening to detect tetracycline, the detection limit is 1ng/mL[30]; a label-free aptamer-based sensor system for the detection of tetracycline in aqueous solution was designed with the lowest detection limits of 17.6 nM in colorimetric and 24.2 nM in resonance light scattering[31]. Except for these examples, the proposed aptasensor displayed a higher sensitivity,a wider liner range

and a lower detection limit in which was compared to other reported aptasensor of detecting tetracycline (Table 1).

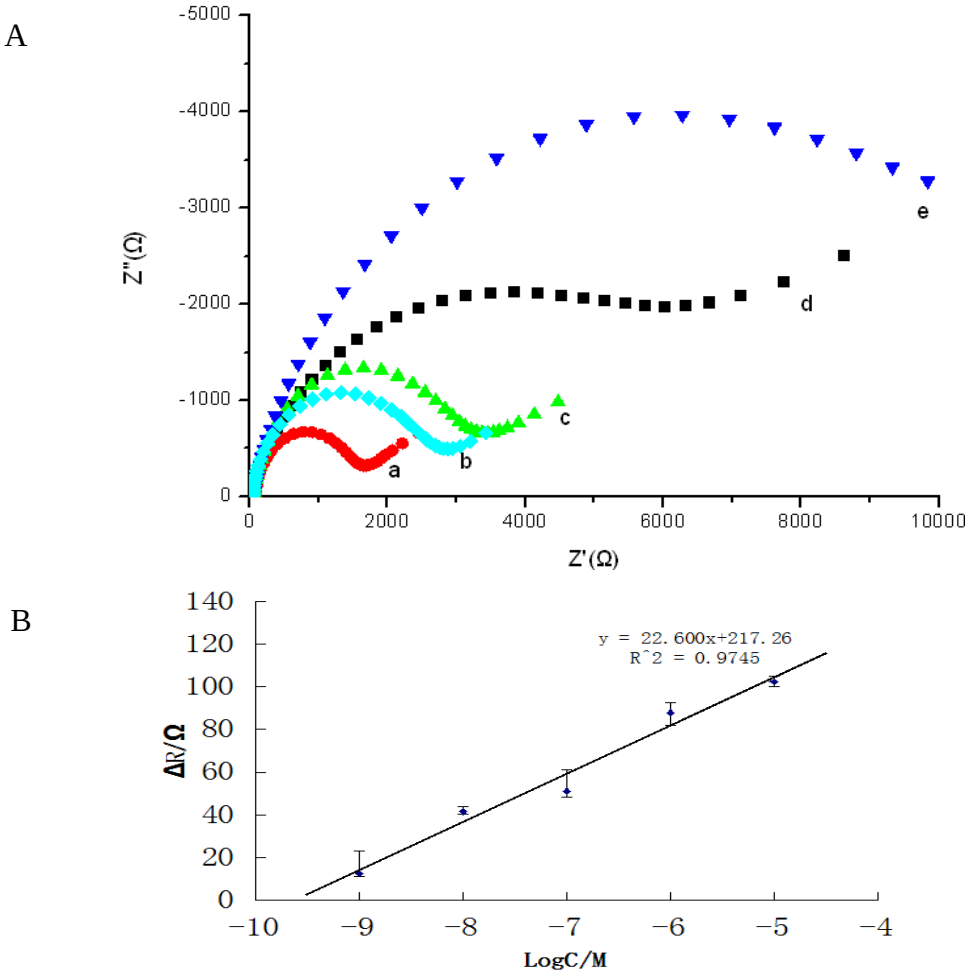

Figure 3. (A) EIS of different concentrations of TET: impedance change with 10^{-9} to10^{-5}g/mL TET(a)1ng/mL； (b)10ng/mL； (c)100ng/mL； (d)1μg/mL； (e)10μg/mL (B) Detection standard curve of aptasensor to tetracycline

Table 1. Comparison with other methods of detecting tetracycline

Method of detection	Limit of detection/M	Linear range/M	Ref
ELISA	3×10^{-7}	3×10^{-7}-3×10^{-5}	[32]
RP-HPLC	2×10^{-8}	5×10^{-8}-1×10^{-6}	[33]
LC-MS/MS	5×10^{-5}	0 -5×10^{-4}	[34]
Fluorescence detection	2.06×10^{-6}	5×10^{-5}-2.5×10^{-3}	[35]
Aptasensor detection	1.0×10^{-9}	1×10^{-9}-1×10^{-5}	this work

3.3. Determination of tetracycline *in real samples*

Although the proposed aptasensor showed good selectivity towards tetracycline in the electrochemical measurements, it is worth probing the analytical ability for practical application. The milk samples used in this study were all purchased from a supermarket in China. Preprocessing: the milk sample was diluted according to dilution ratio of 1:10, and then centrifuged in 20000 rpm for 90 min, milk is finally divided into three layers.

Table 2. Testing results of TET in milk samples

Milk found(M)	Added (M)	Total found (M)	Recovery (%)
Not detected	1×10^{-9}	0.95×10^{-9}	95%
Not detected	1×10^{-7}	0.97×10^{-7}	97%
Not detected	1×10^{-5}	0.99×10^{-5}	99%
Not detected	1×10^{-9}	0.98×10^{-9}	98%
Not detected	1×10^{-7}	0.96×10^{-7}	96%
Not detected	1×10^{-5}	1.05×10^{-5}	105%

Remove the upper and lower layer that is the macromolecular material such as fat and casein. Further, the prepared tetracycline standard solution was spiked into the diluted milk to prepare the tetracycline concentrations of 10^{-9}, 10^{-7},10^{-5}M, and then experiments were carried out with the fabricated aptasensor. The results provided that the tetracycline concentration recoveries were between 95 and 105% (Table 2), which powerfully proved that the aptasensor was appropriate for the detection of tetracycline in real milk samples.

4. CONCLUSIONS

In this paper, we have developed an aptasensor for tetracycline detection based on the ionic liquid-Fe_3O_4/ aptamer / target configuration, which possess high sensitivity and specificity. A detection limit down to 1.0×10^{-9} M for tetracycline has been achieved, which mainly because that nanocomposites enhanced the conductivity, fast charge transfer and unique electrochemical properties The proposed aptasensor was more sensitive and specific. In addition, the method used in this paper was successfully applied for tetracycline detection in milk samples. Therefore, the presented aptasensor provides the potential applications for tetracycline detection in food analysis and clinical diagnosis.

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