Microchimica Acta
https://doi.org/10.1007/s00604-017-2566-8

ORIGINAL PAPER

Aptamer based voltammetric determination of ampicillin using a single-stranded DNA binding protein and DNA functionalized gold nanoparticles

Jun Wang[1] · Kui Ma[2] · Huanshun Yin[2] · Yunlei Zhou[2] · Shiyun Ai[2]

Received: 17 August 2017 / Accepted: 9 November 2017

Abstract
An aptamer based method is described for the electrochemical determination of ampicillin. It is based on the use of DNA aptamer, DNA functionalized gold nanoparticles (DNA-AuNPs), and single-stranded DNA binding protein (ssDNA-BP). When the aptamer hybridizes with the target DNA on the AuNPs, the ssDNA-BP is captured on the electrode surface via its specific interaction with ss-DNA. This results in a decreased electrochemical signal of the redox probe $Fe(CN)_6^{3-}$ which is measured best at a voltage of 0.188 mV (vs. reference electrode). In the presence of ampicillin, the formation of aptamer-ampicillin conjugate blocks the further immobilization of DNA-AuNPs and ssDNA-BP, and this leads to an increased response. The method has a linear reposne that convers the 1 pM to 5 nM ampicillin concentration range, with a 0.38 pM detection limit (at an S/N ratio of 3). The assay is selective, stable and reproducible. It was applied to the determination of ampicillin in spiked milk samples where it gave recoveries ranging from 95.5 to 105.5%.

Keywords Electrochemical bioassay · Glassy carbon electrode · Antibiotic detection · Differential pulse voltammetry · Milk sample

Introduction

Antibiotics are in widespread use but sometimes overdosed, and this leads to bacterial resistance and to enlarged concentrations of antibiotics in food such as meat and milk, in wastewater and in other samples. Hence, sensitive and specific methods are needed for the determination of antibiotics in complex matrices. Various analytical techniques have been applied to detect antibiotics, such as high performance liquid chromatography (HPLC) [1], liquid chromatography − mass spectrometry (LC-MS) [2], liquid chromatography − tandem mass spectrometer (LC-MS/MS) [3], high performance liquid chromatography–mass spectrometry/mass spectrometry (HPLC-MS/MS) [4], fluorescence [5, 6], surface plasmon resonance (SPR) [7], capillary electrophoresis (CE) [8] and colorimetric determination [9, 10]. Though these methods are sensitive, they still require expensive and bulky instruments, skill operators, complicated pretreatment process, which might limit their application.

Electrochemical analysis techniques are widely applied in the filed of analytical chemistry due to the advantages of operation simplicity, instruments miniaturization, low cost, fast detection, etc. [11–14]. One of the factors affecting the detection performance of electrochemical method is target molecule recognition element. DNA aptamers are synthetic and screened single-stranded deoxyribonucleic acid (DNA) sequences, which can tightly bind to their target molecules with high specificity [15]. Though similar to the interaction between antibody and antigen, DNA aptamers have the advantages of low cost, easy synthesis and labeling and good stability [16]. Therefore, DNA aptamers have been successfully applied to fabricate methods for detecting various targets, such as proteins [17], pesticides [18], antibiotics [13], bacteria [19], virus [20], metal ions [21], small organic molecules [22], etc.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00604-017-2566-8) contains supplementary material, which is available to authorized users.

✉ Yunlei Zhou
zhyl@sdau.edu.cn

[1] College of Resources and Environment, Key Laboratory of Agricultural Environment in Universities of Shandong, Shandong Agricultural University, Taian 271018, People's Republic of China

[2] College of Chemistry and Material Science, Shandong Agricultural University, 271018 Taian, Shandong, People's Republic of China

Published online: 20 December 2017

Inspired by the above work, a sensitive analytical strategy was designed using electrochemical technique for antibiotic detection, where glassy carbon electrode was used as substrate electrode, gold nanoparticles (AuNPs) were engaged as DNA aptamer immobilization matrix and electron transfer improvement reagent, single-strand DNA binding protein (ssDNA-BPSSDNA-BP) was employed as electrochemical signal inhibition reagent [23], ampicillin was adopted as model antibiotic (Scheme 1). After DNA aptamer was assembled on electrode surface, capture DNA functionalized AuNPs (DNA-AuNPs) was then further modified on electrode surface via hybridization with DNA aptamer. Afterwards, through the specific interaction between ssDNA-BP and unhybridized capture DNA, more ssDNA-BP was loaded on electrode surface, which significantly increased the steric-hinerance effect, blocked the transfer of redox probe to electrode surface. Therefore, a weak electrochemical signal was achieved. However, in the presence of ampicillin, it can bind with its DNA aptamer to form a stable composite, which can block the further immobilization of capture DNA functionalized AuNPs and ssDNA-BP, and lead to a strong electrochemical signal. Based on the relationship between electrochemical signal and antibiotic concentration, the latter can be sensitively detected. More importantly, this method can be applied to detect other antibiotics just changing aptamer.

Experimental section

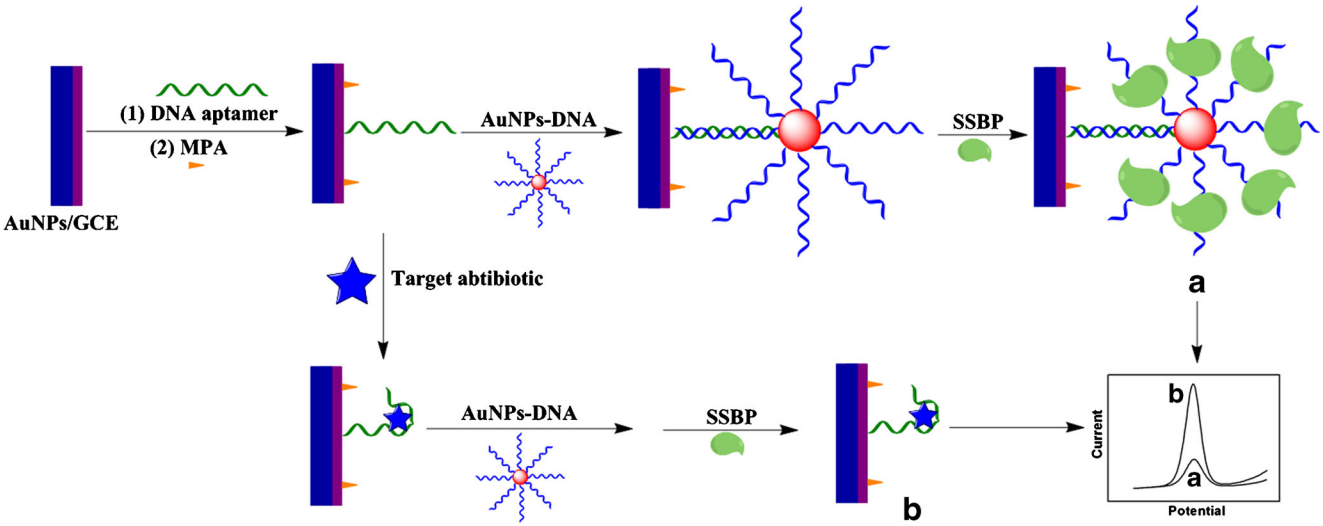
Reagents and instruments

Single-stranded DNA binding protein (ssDNA-BP) was purchased from Affymetrix (ThermoFisher, USA, https://www.thermofisher.com). Sodium cholate, chloroauric acid ($HAuCl_4$) and mercaptopropionic acid (MPA) was supplied by Sigma-Aldrich (USA, http://www.sigmaaldrich.com). Sodium citrate, NaCl, $MgCl_2$, Al_2O_3, EDTA, KNO_3, glycerine, ampicillin sulfate, tetracycline hydrochloride, oxytetracycline hydrochloride, ampicillin trihydrate, penicillin, chloramphenicol, streptomycin, ciprofloxacin, ascorbic acid (AA), tris(hydroxymethyl)aminomethane (Tris) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was offered by Aladdin (Shanghai, China, http://www.aladdin-e.com/). AuNPs and DNA-AuNPs were prepared according to previous work, respectively [24, 25]. DNA sequences were synthesized by Sangon (Shanghai, China, http://www.sangon.com) with HPLC purification. DNA aptamer, 5′-SH-TGG GGG TTG AGG CTA AGC CGA C-3′. Capture DNA, 5′-SH-GTC TTA GCC TCA ACC CCC A-3′.

Buffers: TE buffer: 10 mM Tris-HCl (pH 8.0) containing 1 mM EDTA. DNA immobilization buffer: 10 mM Tris-HCl (pH 7.4), 1.0 mM EDTA, 1.0 M NaCl, and 1.0 mM TCEP. DNA hybridization buffer: 10 mM Tris–HCl (pH 7.4), 50 mM NaCl, 1 mM $MgCl_2$, 5 mM KCl. ssDNA-BP storage buffer: 50 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.1 mM EDTA, 1.0 mM DTT, 50% glycerol. ssDNA-BP reaction buffer: 10 mM Tris-HCl (pH 7.4), 200 mM NaCl, 20 mM $MgCl_2$, 1 mM EDTA. Washing buffer: 10 mM Tris-HCl (pH 7.4) and 50 mM KCl. Detection buffer: 10 mM phosphate buffer (pH 7.5) containing 2 mM $Fe(CN)_6^{3-}$ and 0.1 M KCl.

Assembly of DNA aptamer on electrode surface

Prior to electrode modification, a glassy carbon electrode (GCE) was firstly polished to a mirror-like surface with 30 nm Al_2O_3 slurry, then washed with double distilled ionized water and ethalnol under ultrasonication for 5 min, respectively. After rinsed thoroughly with double distilled deionized



Scheme 1 Schematic illustration of antibiotic assay based on aptamer-antibiotic binding induced electrochemical signal improvement

water and dried under N_2 blowing, the GCE was immersed into 10 mL of 3 mM $HAuCl_4$ and 0.1 M KNO_3 solution and the electrochemical deposition of gold nanoparticles were performed at −0.2 V for 200 s under magnetic stirring. The electrode was rinsed with double distilled ionized water and noted as AuNP/GCE. Then, the electrode was incubated with 10 μL of DNA immobilization buffer containing 1.5 μM DNA aptamer for 12 h at room temperature in a humid cell. After rinsed with washing buffer for three times, the electrode was dried under N_2 blowing and noted as Aptamer/AuNP/GCE. Subsequently, the electrode was incubated with 10 μL of 5 mM MPA for 1 h and rinsed with washing buffer.

Immobilization of DNA-AuNPs and ssDNA-BP

10 μL of a solution of DNA-AuNPs was dropped on Aptamer/AuNP/GCE surface and the hybridization event was performed at 37 °C for 150 min under humid conditions. The electrode was then rinsed with washing buffer and noted as DNA-AuNP/Aptamer/AuNP/GCE. Then, the electrode was incubated with 10 μL of ssDNA-BP reaction buffer containing 30 $\mu g \cdot mL^{-1}$ ssDNA-BP at room temperature for 90 min. The electrode was named as ssDNA-BP/DNA-AuNP/Aptamer/AuNP/GCE. After rinsed with washing buffer, the electrode was storage at 4 °C before use.

Ampicillin capture and its inhibition on the immobilization of DNA-AuNPs and ssDNA-BP

For ampicillin detection, Aptamer/AuNPs/GCE was used as working electrode. Firstly, 10 μL of 10 mM Tris-HCl (pH 7.4) and 50 mM KCl containing different concentrations of ampicillin was dripped on electrode surface and incubated for 60 min at room temperature under humid conditions. Then, the electrode was rinsed with washing buffer and noted as Ampicillin-Aptamer/AuNP/GCE. Then, the electrode was further incubated with DNA-AuNPs and ssDNA-BP as described process in section 2.3.

Electrochemical detection and EIS characterization

Differential pulse voltammetry (DPV) was performed at CHI830D electrochemical workstation (Austin, USA) in 10 mM phosphate buffer (pH 7.5) containing 2 mM $Fe(CN)_6^{3-}$ and 0.1 M KCl. The potential scan range is from 0.6 to −0.2 V with other parameters as initial potential, 0.5 V, final potential, −0.2 V, step potential, 0.004 V, amplitude, 0.05 V, pulse width, 0.05 s, pulse period, 0.2 s, quiet time, 2 s. Electrochemical impedance spectroscopy (EIS) was carried out in 10 mL of 10 mM phosphate buffer containing 5 mM $Fe(CN)_6^{3-/4-}$ and 0.1 M KCl (pH 7.4) with frequency ranging from 10^{-1} to 10^5 Hz.

Optimization of the assay

DNA aptamer and ampicillin binding time

Aptamer/AuNP/GCE was used as working electrode. 10 μL of 10 mM Tris-HCl (pH 7.4) and 50 mM KCl containing 100 nM ampicillin was dripped on electrode surface and incubated for different time (15, 30, 45, 60, 75, 90, 105, 120 min) at room temperature. After rinsing with washing buffer, the electrode was further incubated with 10 μL DNA-AuNPs and 10 μL of 30 $\mu g \cdot mL^{-1}$ ssDNA-BP successively. The incubation time was 120 and 90 min, respectively. Then, differential pulse voltammetry (DPV) detection was performed in detection buffer.

DNA hybridization time

Aptamer/AuNP/GCE was used as working electrode. 10 μL DNA-AuNPs was dripped on electrode surface, and then the electrode was incubated for different time (15, 30, 60, 90, 120, 150, 180 min) at 37 °C in a humid cell. After rinsed with washing buffer, the electrode was further incubated with 10 μL of 30 $\mu g \cdot mL^{-1}$ ssDNA-BP for 90 min. After dried under N_2 blowing, DPV detection was carried out in detection buffer.

ssDNA-BP concentration and incubation time

DNA-AuNP/Aptamer/AuNP/GCE was employed as working electrode. The electrode was incubated with different time (0, 15, 30, 45, 60, 75, 90, 105, 120 min) with 30 $\mu g \cdot mL^{-1}$ ssDNA-BP. Then, electrochemical detection was performed. For ssDNA-BP concentration optimization, 10 μL of different concentration (0.1, 0.5, 1, 10, 30, 50 $\mu g \cdot mL^{-1}$) of ssDNA-BP was dripped on electrode surface and incubate for 90 min. After that, the DPV response was compared.

Determination of ampicillin in milk samples

Milk samples were purchased from a local supermarket. 2 mL milk was added into 8 mL of 10 mM Tris-HCl (pH 7.5) and completely mixed for 10 min under magnetic stirring. After that, 10 μL of different concentrations of ampicillin was spiked into the diluted milk samples. After centrifugation, the supernate was collected and the content was detected.

Results and discussion

Choice of materials

The choice of materials for electrochemical bioassay is crucial, the material should have the merits of good conductivity, high specific surface area and excellent biocompatibility. Therefore, in this work we have selected AuNPs nanomaterial

because they not only meet the above requirements, but also act as a kind of good immobilization matrix material for thiol group modified DNA aptamer.

Electrode characterization by EIS

The electrode preparation process was monitored by EIS. The results are illustrated in Fig. S1 (see Electrical Supplementary Material), which demonstrate the successful preparation of the electrodes.

Feasibility of the assay

To investigate the detection feasibility of this electrochemical method for ampicillin assay, several experiments were performed in 10 mM phosphate buffer (pH 7.5) containing 2 mM $Fe(CN)_6^{3-}$ and 0.1 M KCl. As shown in Fig. 1a, the bare GCE presents a weak electrochemical signal of the redox probe of $Fe(CN)_6^{3-}$ in detection buffer (curve a). After AuNPs are electrochemical deposited on GCE surface, the fabricated AuNP/GCE shows strong electrochemical reduction signal (curve b), which can be ascribed to the increased electrochemical effective surface area and the improved conductibility of the GCE by AuNPs. However, the electrochemical signal decreases obviously when aptamer DNA is assembled on AuNP/GCE surface (curve c). This phenomenon can be explained as the fact that the negative charged phosphate group of ssDNA increased the electronegativity of the electrode surface, which blocked the transfer of redox probe to electrode surface, causing a decreased electrochemical response. When the electrode is seal with MPA, the electrochemical signal further decreases (curve d). The reason is same to that for ssDNA. After ssDNA-Au (curve e) and ssDNA-BP (curve f) are modified on the electrode surface, the electrochemical reduction signals decrease successively. It can be attributed to the negatively charged phosphate group in ssDNA and the bulk protein, which further hindered the transfer of $Fe(CN)_6^{3-}$ towards electrode surface. According to the above results, it can be confirmed that ssDNA-BP/ssDNA-Au/Aptamer/AuNP/GCE was successfully constructed. Fig. 1b illustrates the influence of ampicillin on the electrode fabrication and the corresponding electrochemical response. Curve a is DPV response of Aptamer/AuNP/GCE incubated with MPA. After this electrode is further incubated with 5 nM ampicillin, the electrochemical reduction signal decreases (curve b). When Aptamer/AuNP/GCE is incubated with ampicillin (5 nM), the aptamer ssDNA can interacted with ampicillin to form a folded structure, which would increase the steric-hinerance effect of the electrode and decrease the transfer rate of redox probe. As a result, the electrochemical signal decreases compared with Aptamer/AuNP/GCE. However, when the electrode is further incubated with ssDNA-Au (curve c) and ssDNA-BP (curve d) successively, the electrochemical reduction signal only decrease a little. When aptamer ssDNA and its target molecule of ampicillin formed stable conjugate, the hybridization event between aptamer ssDNA and ssDNA-Au is inhibited, which decreases the immobilization amount of ssDNA-Au on electrode surface. It further causes the reduced immobilization of ssDNA-BP. Therefore, the electrochemical signal only decreases a little. In addition, the electrochemical signal for curve d is much higher than that for ssDNA-BP/ssDNA-Au/Aptamer/AuNP/GCE (curve e), which further confirms the small immobilization amount of ssDNA-Au and ssDNA-BP causing by the formation aptamer-ampicillin conjugate. In summary, in the presence of ampicillin, the electrochemical signal still increases though the electrode was incubated with ssDNA-Au amd ssDNA-BP. According to the results, this method can be applied to detect ampicillin.

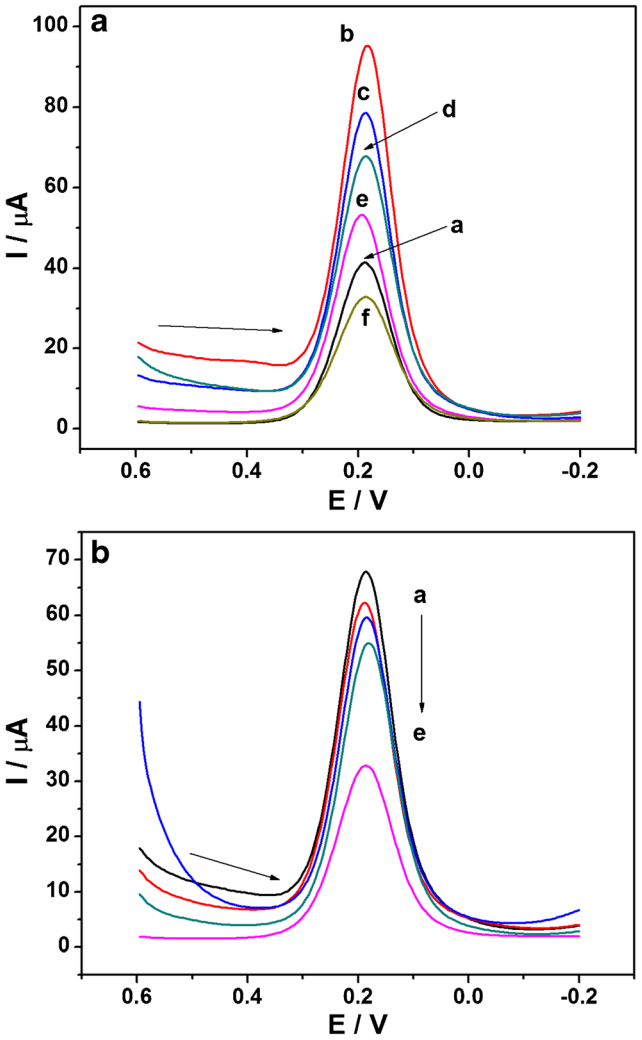


Fig. 1 Differential pulse voltammograms of different electrodes in 10 mM phosphate buffer (pH 7.5) containing 2 mM $Fe(CN)_6^{3-}$ and 0.1 M KCl. (A) **a** GCE, **b** AuNP/GCE, **c** Aptamer/AuNP/GCE, **d** Aptamer/AuNP/GCE after incubating with MPA. **e** ssDNA-Au/Aptamer/AuNP/GCE, **f** ssDNA-BP/ssDNA-Au/Aptamer/AuNP/GCE. (B) **a**-**d** Aptamer/AuNP/GCE incubated with MPA **a**, 5 nM ampicillin **b**, ssDNA-Au **c** and ssDNA-BP **d** sucessively. **e** ssDNA-BP/ssDNA-Au/Aptamer/AuNP/GCE. Ampicillin concentration, 5 nM

Optimization of method

The following parameters were optimized: (a) DNA aptamer and ampicillin binding time; (b) DNA hybridization time; (c) ssDNA-BP concentration and (d) ssDNA-BP incubation time. Respective data and Figures (Fig. S2) are given in the Electronic Supporting Material. The following experimental conditions were found to give best results: (a) DNA aptamer binding time:60 min; (b) DNA hybridization time: 150 min (c) ssDNA-BP concentration: 30 μg· mL^{-1}, and (d) ssDNA-BP incubation time: 90 min.

Detection performances

The detection performances of this bioassay for ampicillin were investigated. As shown in Fig. 2a, under optimal experiment conditions, the electrochemical response enhances with the increase of ampicillin concentration. The photocurrent shows a linear relationship with the logarithm value of ampicillin and the logarithm of electrochemical signal in the range of 1 pM to 5 nM (Fig. 2b). The linear regression can be expressed as $\log I\ (\mu A) = 1.49 \log c\ (pM) + 0.06$ ($R = 0.9964$) and the electrochemical sensitivity was 5.17 μA·μM^{-1}·cm^{-2}. The limit of detection (LOD) is estimated to be 0.38 pM ($S/N = 3$). This result demonstrates that this method possesses great potential for ampicillin detection. Moreover, the comparison of detection performances with different methods is shown in Table 1. Compared with previous work, this electrochemical method presents wide linear range and low detection

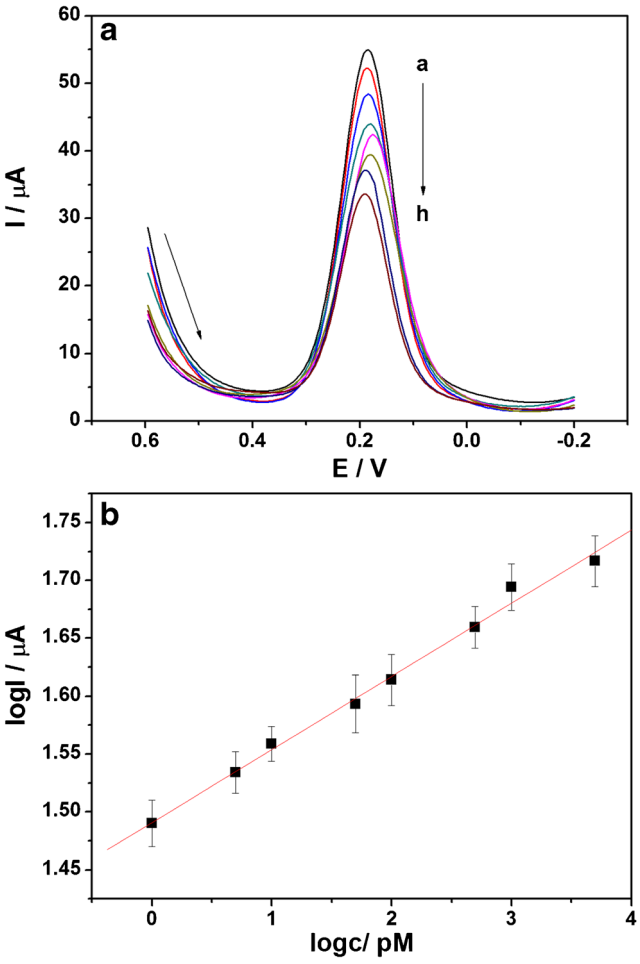


Fig. 2 **a** Differential pulse voltammograms of the electrodes with different concentrations of ampicillin10 mM phosphate buffer (pH 7.5) containing 2 mM $Fe(CN)_6^{3-}$ and 0.1 M KCl. a-h, 10,000、5000、1000、500、100、50、10、1 pM. **b** The linear relationship between the logarithm value of the electrochemical signal and the logarithm value of ampicillin concentration. Working potential, 0.188 V

Table 1 Comparison of the detection performances of different methods for ampicillin detection

Methods	Linear range	LOD	Refs.
Colorimetry	25–150 nM	25 nM	[26]
SPR	20 nM - 8 mM	2 nM	[7]
HPLC	0.52–4.13 mM	78.4 μM	[27]
LC-MS	21–210 μM	2.1 μM	[28]
LC-MS/MS	0.413–20.6 μM	0.413 μM	[3]
Electrochemistry	0.05–9 μM	9.4 nM	[29]
Electrochemistry	0.1–60 nM	0.06 nM	[30]
Fluorescence	0.01–3 nM	9 pM	[31]
CE	8 μM - 1 mM	1.3 μM	[32]
CE	50 nM - 10 μM	8.9 nM	[33]
Luminescence	0.2–150 μM	0.143 μM	[34]
Electrochemistry	1 pM – 5 nM	0.28 pM	This work

PEC: Photoelectrochemical

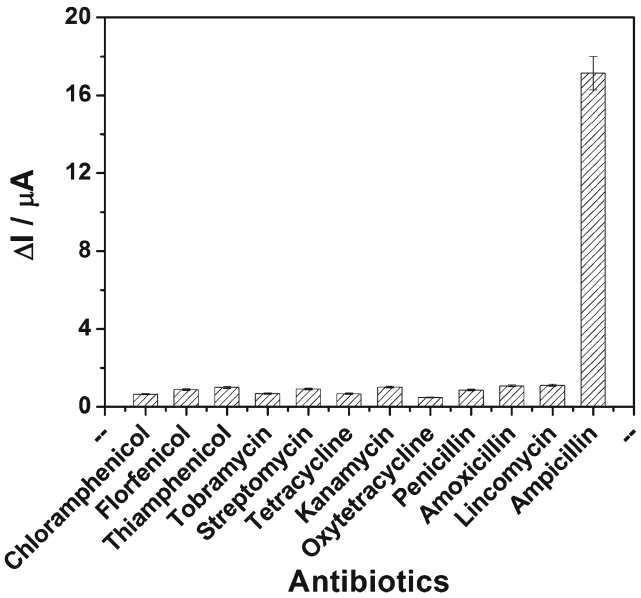


Fig. 3 The histogram for the change of electrochemical response of the electrode with 1 nM of different antibiotics. Working potential, 0.188 V

limit. In addition, the instrument used in this work is inexpensive and easily operation. Thus this electrochemical method presents comparable detection sensitivity and LOD with some of previous work.

The detection selectivity of this electrochemical analysis was investigated, seven other antibiotics were applied, including chloramphenicol, florfenicol, thiamphenicol, tobramycin, streptomycin, tetracycline,kanamycin,oxytetracycline, amoxicillin, penicillin, lincomycin. As illustrated in Fig. 3, in 10 mM phosphate buffer (pH 7.5) containing 2 mM $Fe(CN)_6^{3-}$ and 0.1 M KCl, the electrochemical signal change of the electrode with ampicillin (1 nM) is higher than that at with other antibiotics (1 nM), indicating excellent detection selectivity.

The reproducibility of this method was also evaluated by detecting 1.0 nM ampicillin with nine electrodes in 10 mM phosphate buffer (pH 7.5) containing 2 mM $Fe(CN)_6^{3-}$ and 0.1 M KCl, which were fabricated independently with same procedure. As seen in Fig. S3, the electrochemical signal changes a little and the RSD is 5.09%, indicating decent detection reproducibility.

Analytical application

To validate the applicability of this method in real sample analysis, The content of ampicillin in milk samples was detected. Before spiked ampicillin samples into milk, the content of ampicillin in milk samples were detected by this method and HPLC method. The results indicates that no ampicillin is detected in milk samples. Thus, the quantitative detection of ampicillin in spiked milk samples was performed. As shown in Table S1, the recoveries are ranged from 95.5% to 105.5%, which is similar to that by HPLC, implying a good accuracy of this detection strategy for ampicillin detection. It was clearly demonstrates that this electrochemical technique can be applied for ampicillin detection in milk samples.

Conclusions

In summary, a sensitive electrochemical method with signal on model was reported for ampicillin detection based on DNA aptamer, DNA-AuNPs and ssDNA-BP. The employment of DNA-AuNPs and ssDNA-BP greatly improved the detection sensitivity. Though the electrode modification process is a little long, this electrochemical method still shows high detection sensitivity with low detection limit. Moreover, this detection strategy also possessed high detection selectivity, stability and reproducibility. The assay was successfully applied to quantify ampicillin in liquid milk sample, indicating its generality and potential in application.

Acknowledgements This work was supported by the National Key Research and Development Program of China (2016YFD0800304), the Natural Science Foundation of Shandong (ZR2017MD023), Project of the Distinguished Young Scholar of Shandong Agricultural University, Founds of Shandong "Double Tops" Program (SYL2017XTTD15).

Compliance with ethical standards The author(s) declare that they have no competing interests.

References

1. Tang Y, Li M, Gao X, Liu X, Ma Y, Li Y, Xu Y, Li J (2016) Preconcentration of the antibiotic enrofloxacin using a hollow molecularly imprinted polymer, and its quantitation by HPLC. Microchim Acta 183(2):589–596. https://doi.org/10.1007/s00604-015-1681-7
2. Gomaa A, Boye J (2015) Simultaneous detection of multi-allergens in an incurred food matrix using ELISA, multiplex flow cytometry and liquid chromatography mass spectrometry (LC-MS). Food Chem 175:585–592
3. Cheng C, Liu S, Xiao D, Hansel S (2010) The application of trichloroacetic acid as an ion pairing reagent in LC–MS–MS method development for highly polar aminoglycoside compounds. Chromatographia 72(1–2):133–139. https://doi.org/10.1365/s10337-010-1614-x
4. Zhou JL, Maskaoui K, Lufadeju A (2012) Optimization of antibiotic analysis in water by solid-phase extraction and high performance liquid chromatography-mass spectrometry/mass spectrometry. Anal Chim Acta 731:32–39. https://doi.org/10.1016/j.aca.2012.04.021
5. Wang Y, Ma T, Ma S, Liu Y, Tian Y, Wang R, Jiang Y, Hou D, Wang J (2017) Fluorometric determination of the antibiotic kanamycin by aptamer-induced FRET quenching and recovery between MoS_2 nanosheets and carbon dots. Microchim Acta 184(1):203–210. https://doi.org/10.1007/s00604-016-2011-4
6. Tang Y, Li M, Gao X, Liu X, Gao J, Ma T, Li J (2017) A NIR-responsive up-conversion nanoparticle probe of the $NaYF_4$:Er,Yb type and coated with a molecularly imprinted polymer for fluorometric determination of enrofloxacin. Microchim Acta 184(9):3469–3475. https://doi.org/10.1007/s00604-017-2387-9
7. Wang X, Zou M, Xu X, Lei R, Li K, Li N (2009) Determination of human urinary kanamycin in one step using urea-enhanced surface plasmon resonance light-scattering of gold nanoparticles. Anal Bioanal Chem 395(7):2397–2403. https://doi.org/10.1007/s00216-009-3134-9
8. Altria K, Chanter Y (1993) Validation of a capillary electrophoresis method for the determination of a quinolone antibiotic and its related impurities. J Chromatogr A 652(2):459–463. https://doi.org/10.1016/0021-9673(93)83266-U
9. Lai C, Liu X, Qin L, Zhang C, Zeng G, Huang D, Cheng M, Xu P, Yi H, Huang D (2017) Chitosan-wrapped gold nanoparticles for hydrogen-bonding recognition and colorimetric determination of the antibiotic kanamycin. Microchim Acta 184(7):2097–2105. https://doi.org/10.1007/s00604-017-2218-z
10. Lavaee P, Danesh NM, Ramezani M, Abnous K, Taghdisi SM (2017) Colorimetric aptamer based assay for the determination of fluoroquinolones by triggering the reduction-catalyzing activity of gold nanoparticles. Microchim Acta 184(7):2039–2045. https://doi.org/10.1007/s00604-017-2213-4
11. Bansod B, Kumar T, Thakur R, Rana S, Singh I (2017) A review on various electrochemical techniques for heavy metal ions detection with different sensing platforms. Biosens Bioelectron 94:443–455. https://doi.org/10.1016/j.bios.2017.03.031

12. Liu H, Weng L, Yang C (2017) A review on nanomaterial-based electrochemical sensors for H_2O_2, H_2S and NO inside cells or released by cells. Microchim Acta 184(5):1267–1283. https://doi.org/10.1007/s00604-017-2179-2
13. Han C, Li R, Li H, Liu S, Xu C, Wang J, Wang Y, Huang J (2017) Ultrasensitive voltammetric determination of kanamycin using a target-triggered cascade enzymatic recycling couple along with DNAzyme amplification. Microchim Acta 184(8):2941–2948. https://doi.org/10.1007/s00604-017-2311-3
14. Zhan X, Hu G, Wagberg T, Zhan S, Xu H, Zhou P (2016) Electrochemical aptasensor for tetracycline using a screen-printed carbon electrode modified with an alginate film containing reduced graphene oxide and magnetite (Fe3O4) nanoparticles. Microchim Acta 183(2):723–729. https://doi.org/10.1007/s00604-015-1718-y
15. Wu C, Han D, Chen T, Peng L, Zhu G, You M, Qiu L, Sefah K, Zhang X, Tan W (2013) Building a multifunctional aptamer-based DNA nanoassembly for targeted cancer therapy. J Am Chem Soc 135(49):18644–18650. https://doi.org/10.1021/ja4094617
16. Pavlov V, Xiao Y, Shlyahovsky B, Willner I (2004) Aptamer-functionalized au nanoparticles for the amplified optical detection of thrombin. J Am Chem Soc 126(38):11768–11769. https://doi.org/10.1021/ja046970u
17. Wang Y, Gao D, Zhang P, Gong P, Chen C, Gao G, Cai L (2014) A near infrared fluorescence resonance energy transfer based aptamer biosensor for insulin detection in human plasma. Chem Commun 50(7):811–813. https://doi.org/10.1039/C3CC47649A
18. Bala R, Kumar M, Bansal K, Sharma RK, Wangoo N (2016) Ultrasensitive aptamer biosensor for malathion detection based on cationic polymer and gold nanoparticles. Biosens Bioelectron 85: 445–449. https://doi.org/10.1016/j.bios.2016.05.042
19. Duan N, Wu S, Dai S, Miao T, Chen J, Wang Z (2015) Simultaneous detection of pathogenic bacteria using an aptamer based biosensor and dual fluorescence resonance energy transfer from quantum dots to carbon nanoparticles. Microchim Acta 182(5–6):917–923. https://doi.org/10.1007/s00604-014-1406-3
20. Hushegyi A, Pihíková D, Bertok T, Adam V, Kizek R, Tkac J (2016) Ultrasensitive detection of influenza viruses with a glycan-based impedimetric biosensor. Biosens Bioelectron 79:644–649. https://doi.org/10.1016/j.bios.2015.12.102
21. Li J, Sun M, Wei X, Zhang L, Zhang Y (2015) An electrochemical aptamer biosensor based on "gate-controlled" effect using β-cyclodextrin for ultra-sensitive detection of trace mercury. Biosens Bioelectron 74:423–426. https://doi.org/10.1016/j.bios.2015.06.061
22. Huang K-J, Liu Y-J, Zhang J-Z, Cao J-T, Liu Y-M (2015) Aptamer/au nanoparticles/cobalt sulfide nanosheets biosensor for 17β-estradiol detection using a guanine-rich complementary DNA sequence for signal amplification. Biosens Bioelectron 67:184–191. https://doi.org/10.1016/j.bios.2014.08.010
23. Abnous K, Danesh NM, Alibolandi M, Ramezani M, Taghdisi SM, Emrani AS (2017) A novel electrochemical aptasensor for ultrasensitive detection of fluoroquinolones based on single-stranded DNA-binding protein. Sensors Actuators B Chem 240: 100–106. https://doi.org/10.1016/j.snb.2016.08.100
24. Liu J, Lu Y (2006) Preparation of aptamer-linked gold nanoparticle purple aggregates for colorimetric sensing of analytes. Nat Protoc 1(1):246–252. https://doi.org/10.1038/nprot.2006.38
25. Yin H, Zhou Y, Zhang H, Meng X, Ai S (2012) Electrochemical determination of microRNA-21 based on graphene, LNA integrated molecular beacon, AuNPs and biotin multifunctional bio bar codes and enzymatic assay system. Biosens Bioelectron 33(1):247–253. https://doi.org/10.1016/j.bios.2012.01.014
26. Song K-M, Cho M, Jo H, Min K, Jeon SH, Kim T, Han MS, JK K, Ban C (2011) Gold nanoparticle-based colorimetric detection of kanamycin using a DNA aptamer. Anal Biochem 415(2):175–181. https://doi.org/10.1016/j.ab.2011.04.007
27. Blanchaert B, Jorge EP, Jankovics P, Adams E, Van Schepdael A (2013) Assay of kanamycin a by HPLC with direct UV detection. Chromatographia 76(21–22):1505–1512. https://doi.org/10.1007/s10337-013-2440-8
28. Kim B-H, Kim Y-K, Ok JH (2001) Development of liquid chromatographic method for the analysis of kanamycin residues in varicella vaccine using phenylisocyanate as a derivatization reagent. J Chromatogr B 752(1):173–177. https://doi.org/10.1016/S0378-4347(00)00506-5
29. Zhu Y, Chandra P, Song K-M, Ban C, Shim Y-B (2012) Label-free detection of kanamycin based on the aptamer-functionalized conducting polymer/gold nanocomposite. Biosens Bioelectron 36(1):29–34. https://doi.org/10.1016/j.bios.2012.03.034
30. Wang C, Liu C, Luo J, Tian Y, Zhou N (2016) Direct electrochemical detection of kanamycin based on peroxidase-like activity of gold nanoparticles. Anal Chim Acta 936:75–82. https://doi.org/10.1016/j.aca.2016.07.013
31. Li H, Sun D-e, Liu Y, Liu Z (2014) An ultrasensitive homogeneous aptasensor for kanamycin based on upconversion fluorescence resonance energy transfer. Biosens Bioelectron 55:149–156. https://doi.org/10.1016/j.bios.2013.11.079
32. Yang W-C, A-M Y, Chen H-Y (2001) Applications of a copper microparticle-modified carbon fiber microdisk array electrode for the simultaneous determination of aminoglycoside antibiotics by capillary electrophoresis. J Chromatogr A 905(1–2):309–318. https://doi.org/10.1016/S0021-9673(00)00985-7
33. Ge S, Tang W, Han R, Zhu Y, Wang Q, He P, Fang Y (2013) Sensitive analysis of aminoglycoside antibiotics via hyphenation of transient moving substitution boundary with field-enhanced sample injection in capillary electrophoresis. J Chromatogr A 1295:128–135. https://doi.org/10.1016/j.chroma.2013.04.049
34. Leung K-H, He H-Z, Chan DS-H, Fu W-C, Leung C-H, Ma D-L (2013) An oligonucleotide-based switch-on luminescent probe for the detection of kanamycin in aqueous solution. Sensors Actuators B Chem 177:487–492. https://doi.org/10.1016/j.snb.2012.11.053