Int. J. Electrochem. Sci., 15 **(2020)** 7520 – 7530, doi:10.20964/2020.08.28

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Magnetic bead-based electrochemical aptasensor doped with multi-wall carbon nanotubes for the detection of ampicillin in milk

Fang Li[1,2,*], *Junya Zhu*[1], *Ruohan Li*, *Yunhong Liu*[1,*], *Zhaozhou Li*[1], *Huaibin Kang*[1]

[1] College of Food and Biological Engineering, Henan University of Science and Technology, Luoyang 471023, China
[2] National Experimental Teaching Demonstration Center of Food Processing and Security, Henan University of Science and Technology, Luoyang 471023, P. R. China
[*]E-mail: lifang182006@126.com ; lyunhong@126.com

Received: 8 April 2020 / *Accepted:* 11 May 2020 / *Published:* 10 July 2020

Given the ubiquitous presence of antibiotic residues in foodstuff, establishing an efficient method to determine antibiotic residuals is extremely urgent to ensure food safety. This work reports a magnetic bead-based electrochemical aptasensor doped with multi-wall carbon nanotubes for the determination of ampicillin, an important antibiotic. In this aptasesnsor, ampicillin-coated magnetic beads were used to compete with a sample for biotinylated aptamers and to recognize streptavidin–horse radish peroxidase. Multi-wall carbon nanotubes were introduced to simply mix with magnetic beads to improve electrochemical performance. A magnetic glass carbon electrode was employed to draw these magnetic beads for subsequent electrochemical detection. Under optimal conditions, the net peak current had a linear relationship with the logarithm of ampicillin concentration in the range of 1.0×10^{-13} mol/L to 1.0×10^{-8} mol/L and a detection limit of 1.0×10^{-13} mol/L (3σ). In addition, this aptasensor was used to detect ampicillin in milk with recoveries of 95.02% – 101.83%. The proposed aptasensor platform is easy to fabricate and process, can be produced pre-equipped with the aid of a magnet, and possesses great promise for antibiotic residue detection in the food safety field.

Keywords: Magnetic beads; Carbon nanotubes; Ampicillin; Electrochemical aptasensor

1. INTRODUCTION

Ampicillin is a broadspectrum β–lactam antibiotic, that has been widely applied to cure various bacterial infections in stockbreeding management [1]. However, the imprudent use of antibiotics may lead to trace residues in foods of animal origin (e.g., milk) and may damage consumer health by allergic reactions and antibiotic resistance [2-5]. According to the US Food and Drug Administration (FDA), the tolerance for ampicillin in milk is 28.6 nmol/L (10 μg/kg) [6]. The European Union recommends a

maximum residue limit (MRL) of 11.5 nmol/L (4 μg/kg) in milk [7]. Therefore, simple, quantitative, and reliable methodologies must be developed to monitor antibiotic residues in foods of animal origin.

Several methods, such as high-performance liquid chromatography [8], surface resonance [9], microbial screening [10], colorimetry [11], fluorescence [1], and molecular imprinted electrochemical sensors [12] have been developed to determine ampicillin. Although these methods are sensitive for ampicillin detection, they require sophisticated instruments, professional users, and complicated processes, which may limit their applications. Alternatively, the use of electrochemical aptasensors that have been widely used in bioassays given their high sensitivity, simple instrumentation, and excellent compatibility is a promising method for antibiotic detection. A factor that has drawn researchers' interests is the outstanding recognition element of electrochemical aptasensors. Aptamers are short, single-stranded DNA or RNA that can be screened by the systematic evolution of ligands by exponential enrichment to bind targets with high affinity and specificity similar to antigen–antibody interactions. Unlike antibodies, aptamers can be easily modified and produced at a low cost. Therefore, aptamers are highly promising molecular recognition elements to replace antibodies. To date, aptamers have been widely used to fabricate aptasensors for the detection of various targets, including metal ions [13], small molecules [14], viruses [15], bacteria [16], and whole cells [17].

Recently, some electrochemical aptasensors have been developed to detect antibiotics [18-20]. These aptasensors follow the classical electrochemical model, which involves immobilizing a probe (aptamer or target) on the surface of an electrode by using self-assembled monolayers and then electrochemically analyzing the same electrode surface. However, this model may suffer from nonspecific absorption and poor reproducibility. Magnetic bead-based electrochemical biosensors are an alternative strategy. They use magnetic beads (MBs) as the platform to bind recognition elements for the recognition reaction and then use a magnet to absorb MBs on the electrode surface for electrochemical detection; this platform allows recognition and detection processes to be performed on a different surface [21]. Moreover, MB-based electrochemical biosensors usually have low detection limit, and easy pre-treatment processing. Given these advantages, MB-based electrochemical biosensors have attracted many researchers' attention [22-25]. However, MBs and the biomolecules that surround them are commonly nonconductive. They can insulate the conductive support and perturb interfacial electron transfer between the electrode and electroactive species in a solution, leading to unsatisfactory performance.

Carbon nanotubes (CNTs) are allotropes of carbon that consist of a graphene sheet rolled into a nanoscale tube [26, 27]. CNTs exhibit unique electrical, chemical, mechanical, and thermal properties. Particularly, their exceptional high surface-to-volume ratio and electronic properties make them a very interesting surface modifier for the construction of electrochemical biosensors [28]. However, similar to a probe assembly process, using CNTs to modify electrode surface or to carry increased signal elements is generally complicated, sophisticated, and laborious. Finding a simple method that uses CNTs to improve the charge transfer of MB-based electrochemical biosensors is important.

Herein, we report a novel, simple MBs-based electrochemical aptasensor doped with multi-wall CNTs (MWCNTs) for the detection of ampicillin in milk. As shown in Figure 1, MBs modified with carboxyl groups were adopted to covalently couple with ampicillin. Then, the ampicillin-coated MBs were used to compete with a sample containing free ampicillin for biotinylated aptamers. Only

biotinylated aptamers that bound on the surfaces of MBs could be separated by magnetic force through ampicillin–aptamer interaction. Subsequently, the signal elements, streptavidin–horse radish peroxidase (SA–HRP), could attach on the surfaces of MBs on the basis of the very strong SA–biotin interaction ($K_d \sim 10^{-15}$) [29]. After simple doping with MWCNTs, the MBs were transferred on the electrode surface with the aid of magnetic electrodes. Electrochemical detection was carried out with the use of a H_2O_2–hydroquinone–HRP system. Electrochemical signal intensities were proportional to the concentration of HRP and further indicated the amount of ampicillin. MWCNTs greatly improved charge transfer and allowed communication between the active site of HRP, redox mediator, and magnetic electrode.

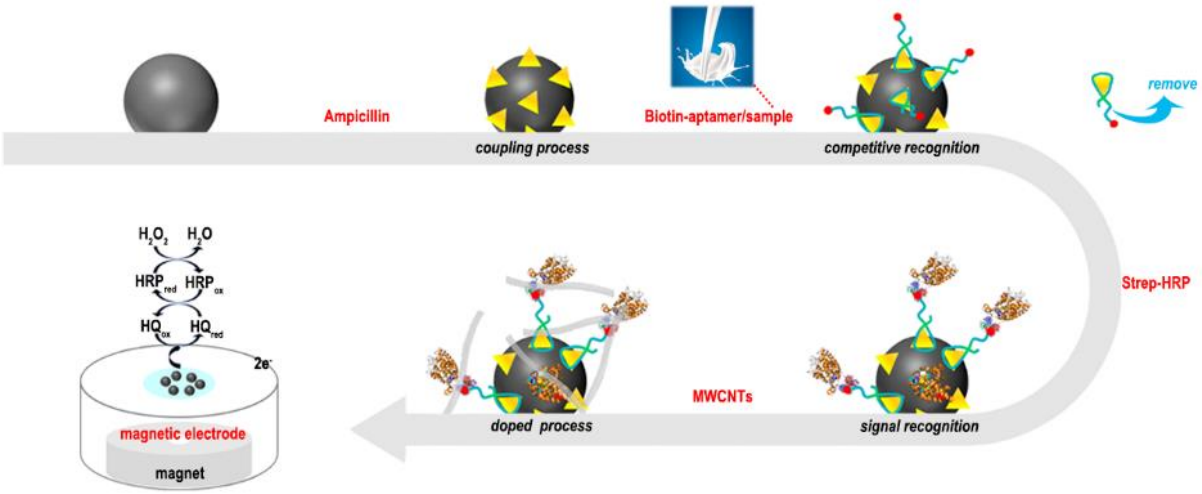

Figure 1. The schematic of the proposed aptasensor.

2. MATERIALS AND METHOD

2.1 Materials and equipment

Monodispersed COOH-coated MBs (2.5 μm in diameter) were supplied by Invitrogen–Thermo Fisher (USA). Ampicillin (≥98.89%) was purchased from Dr. Ehrenstorfer GmbH Inc. (Germany) and used as received. Biotinylated aptamer (5′–biotin–GCG GGC GGT TGT ATA GCG G–3′) was synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). SA–HRP was purchased from Bioss Biotech Co., Ltd. (Beijing, China). Other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) were performed in a CHI 620E electrochemical analyzer (Shanghai Chenhua Instruments, Shanghai, China). All measurements were carried out at room temperature (25 ± 1 °C) in a 5 mL electrochemical cell with a normal three-electrode configuration. A homemade magnetic carbon glass electrode was used as a working electrode. A platinum wire counter electrode and an Ag/AgCl reference electrode were used in the three-electrode configuration. A JEM 1200EX transmission electron microscope (TEM, JEOL Corporation, Japan) was used to characterize the morphology of the MBs doped with MWCNTs.

2.2 Synthesis of ampicillin-modified MBs

The amino groups (NH_2) of ampicillin were covalently coupled with the carboxyl groups (COOH) on the surface of the MBs. In brief, 1 mg of MBs was transferred into a tube and thoroughly washed twice with 15 mmol/L 2–(N–morpholino) ethanesulfonic acid (MES) solution (pH = 6.0) to balance the salt concentration. The MBs were activated with 100 μL of MES containing 5 mg/mL 1–ethyl–3–(3–dimethylaminopropyl) carbodiimide for 30 min and then mixed with 500 μL of 1 mg/mL ampicillin. The reaction was left to proceed for 4 h at room temperature (25 ± 1 °C) in the dark under stirring. Subsequently, the modified MBs were rinsed three times with PBS–Tween (0.01 mol/L PBS, 0.05% Tween, pH = 7.4) to remove the residual ampicillin. Approximately 500 μL of 0.5 mol/L ethanolamine solution was added to block the ampicillin-coated MBs at room temperature for 2 h under stirring. After washing with PBS–Tween three times, the MBs were resuspended in 1 mL of 0.01 mol/L PBS (pH = 7.4) and stored in the dark at 4 °C until use.

2.3 Sandwich aptasensor process

Approximately 50 μg of ampicillin-coated MBs was mixed with 100 μL of ampicillin standard solutions or samples and then added with 100 μL of biotinylated aptamer. The incubation was allowed to proceed with rotation at room temperature for 90 min, followed by washing three times with PBS–Tween (0.01 mol/L PBS, pH = 7.4). Through SA–biotin recognition, the MBs were dispersed in 200 μL of 1:1000 diluted SA–HRP and then mixed and left for 1 h at room temperature. The MBs were washed with PBS–Tween (0.01 mol/L PBS, pH = 7.4) for three times and then mixed with carboxylated MWCNTs. The mixture was gently washed to remove poorly wrapped MWCNTs and resuspended in 20 μL of 0.01 mol/L PBS pH 7.4 for subsequent electrochemical measurements.

2.4 Electrochemical measurement process

Homemade magnetic glass carbon electrode (3 mm in diameter) was polished with alumina slurry and then sequentially cleaned under bath sonication with ultrapure water, ethanol, and ultrapure water. Subsequently, the magnetic glass carbon electrode was cleaned in 0.5 mol/L H_2SO_4 by performing CVs from −0.2 V to 1.5 V (vs. Ag/AgCl) at a scan rate of 100 mV/s for 20 cycles. After thoroughly rinsing with water and drying with high-purity nitrogen gas (99.999%), the electrode was ready for the following detection.

The entire suspension of MBs doped with MWCNTs was pipetted on the surface of the magnetic glass carbon electrode. Then, the magnetic glass carbon electrode was gently immersed into an electrochemical cell. CVs were carried out in 0.1 mol/L KCl solutions containing 1.0 mmol/L $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and recorded at a potential range of −0.1 V to 0.6 V (vs. Ag/AgCl) at a scan rate of 50 mV/s. LSV was performed in 0.01 mol/L PBS solution (pH = 7.4) containing 1.0 mmol/L hydroquinone and 1.0 mmol/L H_2O_2 and recorded at a potential range of −0.3 V to 0.1 V (vs. Ag/AgCl). Ampicillin was quantified by measuring the net current intensity ΔI (the difference of the current intensities with and without the addition of ampicillin).

2.5 Sample pretreatment

Artificially contaminated milk was prepared by spiking standard ampicillin solutions into ampicillin-free milk to identify the validity of the proposed method for the detection of ampicillin in complex samples. The milk samples were briefly pretreated as previously described [30]. First, 20% acetic acid was added drop by drop to the specimens to adjust the pH to 4.6. The samples were incubated at 45 °C for 10 min and then centrifuged at 10 000 rpm for 25 min. Finally, the supernatant was collected and filtered with a 0.22 μm membrane for subsequent detection.

3. RESULTS AND DISCUSSION

3.1 Characterization of MB-based electrochemical aptasensor

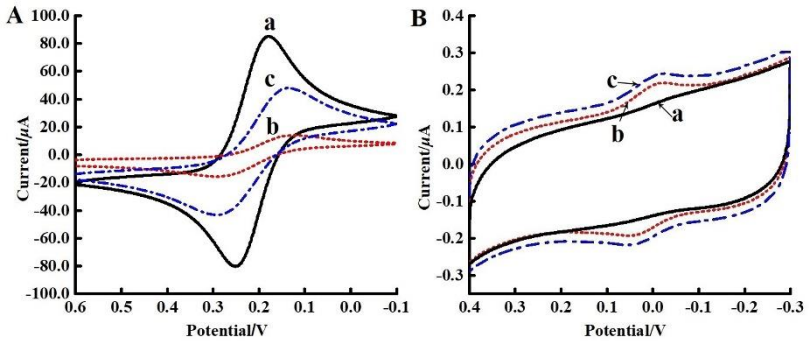

Figure 2. Cyclic voltammograms of the electrode at different stages in a **(A)** $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 mol/L KCl and a **(B)** 0.01 mol/L PBS solution (pH = 7.4) containing 1.0 mmol/L hydroquinone and 1.0 mmol/L H_2O_2 ; scan rate, 50 mV/s. (a) Bare glass carbon electrode, (b) MBs, (c) MBs doped with MWCNTs.

CV was performed for the electrochemical characterization of aptasensor electrodes during the stepwise construction process. The CVs of the electrodes at different stages were observed in a 10 mmol/L $[Fe(CN)_6]^{3-/4-}$ solution. A typical redox peak was captured for the bare magnetic glass carbon electrode (curve a in Figure 2a). When the magnetic glass carbon electrode captured the MBs on its surface, the redox peak had a drastic decline (curve b in Figure 2a) because the MBs were modified with an inferior conductivity of biomolecules that would insulate and block electrons between redox mediator and electrode surface. However, the electrode showed a significant increase in the peak current after MWCNTs were doped with the MBs (curve c in Figure 2a) due to the doped MWCNTs. The same phenomena were also observed in the PBS solution containing hydroquinone and H_2O_2, where hydroquinone served as an electron transfer mediator and H_2O_2 as the enzyme substrate (Figure 2b). The doped MWCNTs produced an enhanced electrochemical response (Figure 2b curve c), which was attributed to the unique character of MWCNTs. The high conductivity of CNTs can improve electrochemical signal transduction, and its nano–architecture imposes electron contact between redox centers deeply laid in the enzyme structure and the electrode surface [31-34]. The TEM images of the

MBs before and after doping with MWCNTs are shown in Figure 3. Figure 3B shows that MWCNTs twin around the MBs, indicating that the MBs were successfully doped with MWCNTs.

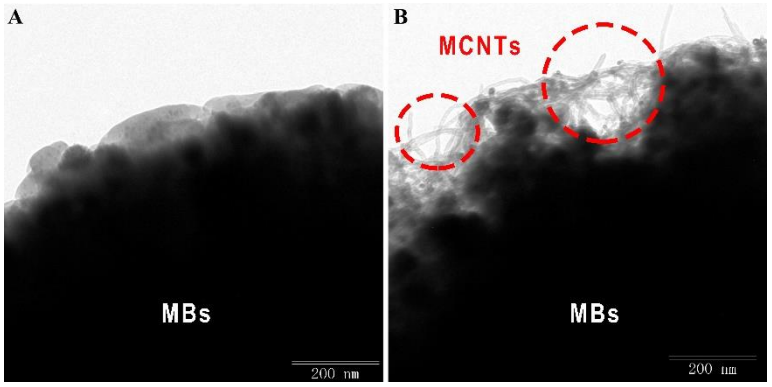

Figure 3. TEM images of the MBs before **(A)** and after **(B)** doping with MWCNTs.

3.2. Optimization of working variables

The optimal electrochemical performance shows the sensitivity of the present electrochemical aptasensor. Therefore, some factors, such as the amount of the doped MWCNTs, hydroquinone and H_2O_2 were carefully investigated.

The amount of doped MWCNTs influences charge transfer and, thus further affects electrochemical performance. Therefore, the amount of the doped MWCNTs was examined in the range of 0–3.0 μg. As shown in Figure 4A, cathodic current in the presence of ampicillin increased with MCNT concentration increasing from 0 μg to 1.5 μg and then reduced beyond 1.5 μg. The same trend was also observed in Figure 4B, that is, the maximum ΔI was obtained at 1.5 μg. The presence of too many MWCNTs causes their twininge or folding with each other, inhibiting the formation of a good network with MBs to provide a good current response [31]. Therefore, 1.5 μg of MWCNTs was mixed with MBs for subsequent electrochemical detection.

The influence of hydroquinone concentration on net current responses was determined in the range of 0.1–2.0 mmol/L (Figure 4B). ΔI increased sharply with increasing hydroquinone concentration to the maximal value of 1.0 mmol/L and then tended to gradually decrease. Hydroquinone serves as an electron transfer mediator in detection systems. Although a high mediator concentration can provide high sensitivity, it may produce a large background current. Thus, 1.0 mmol/L of hydroquinone was selected for further work. Furthermore, the influence of H_2O_2 concentration was optimized (Figure 4C). With increasing H_2O_2 concentration from 0.1 mmol/L to 2.0 mmol/L, ΔI first dramatically grew and then reduced to over 1.0 mmol/L. H_2O_2 at 1.0 mmol/L was used in subsequent experiments to ensure that the enzymatic reaction rate only depends on HRP concentration and avoid excess H_2O_2 denaturalizing HRP.

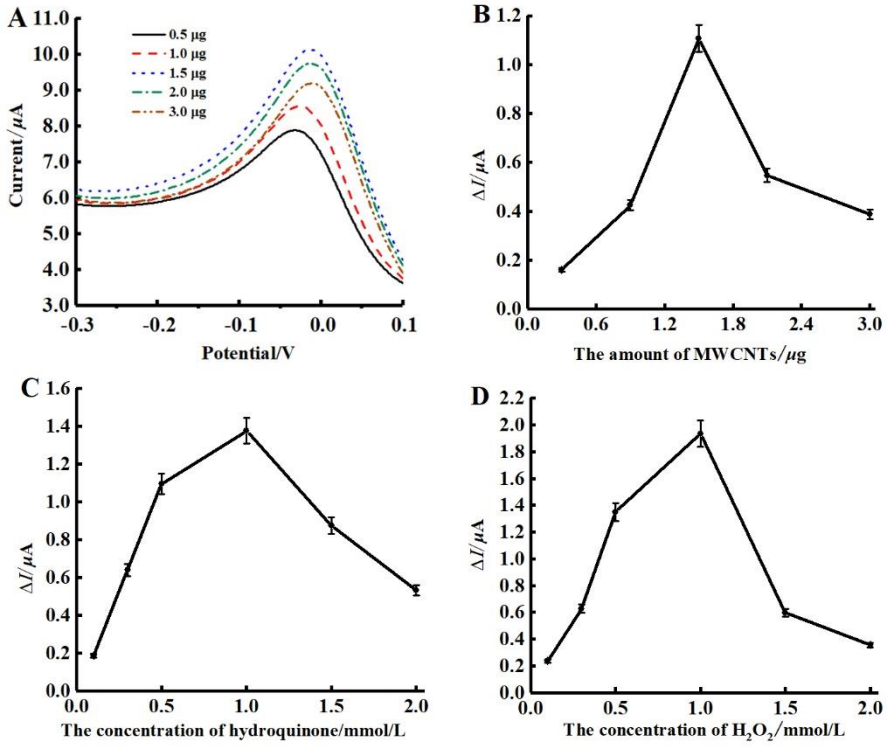

Figure 4. (A) LSV responses of the aptasensor to different amounts of doped MCNTs;**(B)** effect of different amounts of the doped MWCNTs on Δ*I*; **(C)** effect of different hydroquinone concentrations on Δ*I*; **(D)** effect of different H_2O_2 concentrations on Δ*I*.

3.3. Analytical and operational performance

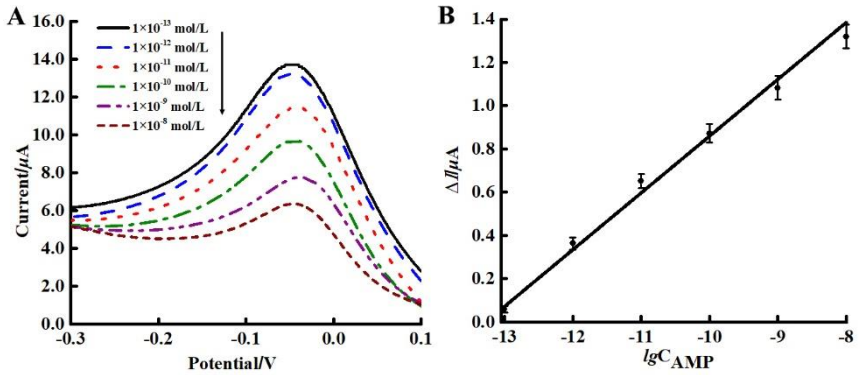

Figure 5. (A) LSV responses of the aptasensor to different concentrations of ampicillin; **(B)** Calibration curve of Δ*I* versus the logarithm of ampicillin concentrations.

Under optimal conditions, LSV was performed to quantitatively analyze different concentrations of ampicillin. As shown in Figure 5A, cathodic currents increased with increased ampicillin concentration. The plot of net peak current versus the logarithm of ampicillin concentration shows a linear relationship in the range of 1.0×10^{-13} mol/L to 1.0×10^{-8} mol/L (Figure 5B). The linear regression equation is as follows:

$\Delta I = 3.3286 + 0.2481\ lg\ C_{AMP}$ ($R^2 = 0.9943$),

where ΔI is the increase of cathodic current and C_{AMP} is the concentration of ampicillin. The limit of detection (LOD) as defined by the 3σ–rule is 1.0×10^{-13} mol/L. The performance characteristics of the other methods for ampicillin are summarized in Table 1. The performance of the proposed method is comparable and even better than that of most methods. Although the LOD of the as-proposed method is not the lowest in comparison with that of other methods, it is sensitive enough for ampicillin detection, as shown by the MRL of the official standard issued by the United Nations Food and Agricultural Organization or the US FDA. Moreover, the present sensor platform is easy to fabricate and process, and can be produced pre-equipped with the aid of a magnet for coupling, competitive recognition, signal recognition and doped procedure.

Table 1. Comparison of the proposed method with other methods

Methods	Linear range (mol/L)	Detection limit (mol/L)	Sample	Ref.
fluorescence	4.19×10^{-9}–2.09×10^{-7}	2.09×10^{-9}	milk	[1]
HPLC	1.67×10^{-6}–8.36×10^{-5}	3.3×10^{-7}	meat, liver, kidney, eggs	[7]
SPR	2.5×10^{-6}–1.0×10^{-3}	1.0×10^{-6}	water	[8]
colorimetry	6.20×10^{-8}–2.98×10^{-6}	2.48×10^{-8}	human urine	[10]
electrochemistry	1.0×10^{-8}–5.0×10^{-6}	1.0×10^{-9}	milk; animal feed; egg	[11]
photoelectrochemistry	1.0×10^{-10}–2.0×10^{-7}	9.3×10^{-11}	water; milk	[35]
electrochemistry	2.86×10^{-15}–5.72×10^{-9}	6.21×10^{-16}	human urine; water; milk	[36]
This work	1.0×10^{-13}–1.0×10^{-8}	1.0×10^{-13}	milk	

The reproducibility and repeatability of the proposed method was evaluated using five intra and extra-batch prepared ampicillin-coated MBs to respectively detect 1.0×10^{-12}, 1.0×10^{-10}, and 1.0×10^{-8} mol/L ampicillin under the optimal conditions. The relative standard deviations toward the above-mentioned concentrations are calculated to be 3.9%, 4.2%, 4.5% for intra-batch and 4.2%, 4.7%, 5.1% for extra-batch. The results showed the good reproducibility and repeatability of the LSV measurements made with the magnetic electrochemical aptasensor.

3.4 Selectivity

The specificity of the present method for ampicillin was also investigated by the addition of ampicillin (1.0×10^{-8} mol/L) and four interfering substances (1.0×10^{-6} mol/L), including amoxicillin, tetracycline, kanamycin, and erythromycin. As shown in Figure 6, the net current in the presence of

interference was significantly lower than that in the absence of ampicillin, demonstrating the satisfied specificity of the proposed method toward ampicillin.

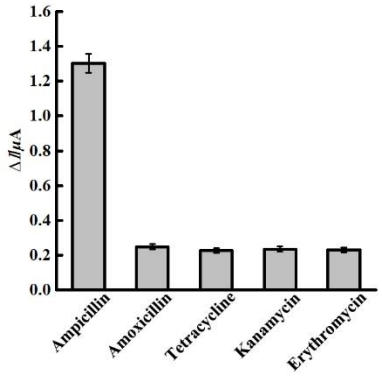

Figure 6. Specificity of the proposed method. The concentration of ampicillin is 1.0×10^{-10} mol/L, and the concentration of amoxicillin, tetracycline, kanamycin and erythromycin is 1.0×10^{-8} mol/L.

3.5 Detection of ampicillin in milk

To further validate the feasibility and reliability of the present method, milk specimens were spiked with different amounts of ampicillin and measured by the proposed aptasensor. As shown in Table 2, the recoveries of spiked milk samples were between 95.02% and 101.83%, indicating that the proposed method might hold great potential for real sample analysis.

Table 2. Detection results of ampicillin in milk samples using the proposed aptasensor (*n*=5).

Sample	Added(nmol/L)	Found(nmol/L)	Recovery(%)	RSD(%)
1	2.50×10^{-9}	2.47×10^{-9}	98.82	3.03
2	2.00×10^{-9}	2.01×10^{-9}	100.50	2.47
3	1.50×10^{-9}	1.48×10^{-9}	98.62	2.93
4	1.00×10^{-9}	9.95×10^{-10}	99.50	1.73
5	5.00×10^{-10}	4.79×10^{-10}	95.83	2.42
6	2.50×10^{-10}	2.44×10^{-10}	97.65	3.74
7	2.00×10^{-10}	2.01×10^{-10}	100.53	1.47
8	1.50×10^{-10}	1.43×10^{-10}	95.02	2.53
9	1.00×10^{-10}	9.77×10^{-11}	97.71	5.41
10	5.00×10^{-11}	5.09×10^{-11}	101.83	2.80

4. CONCLUSIONS

In summary, we developed an MB-based electrochemical aptasensor doped with MWCNTs for the sensitive determination of ampicillin. The aptasensor uses MBs as platforms and magnetic glass carbon electrodes as electrochemical transducers. The doped MWCNTs could effectively enhance

charge transfer and improve electrochemical performance. Under the optimal conditions, the fabricated aptasensor exhibited a broad linear range and a low detection limit. Furthermore, this aptasensor was successfully applied for ampicillin detection in milk. The proposed sensor platform is easy to fabricate and process, can be produced pre-equipped with the aid of a magnet, and shows application potential for antibiotic residue detection and related food analysis.

ACKNOWLEDGEMENTS
This work was financially supported by the National Natural Science Foundation of China (Grant No. 31501563, No. 31701694) and Science and Technology Project of Henan Province (Grant No. 192102310139).

References

1. K.M. Song, E. Jeong, W. Jeon, M. Cho, C. Ban, *Anal. Bioanal. Chem.,* 402(2012) 2153.
2. P. Fei, Y. Jiang, S. Gong, R. Li, Y. Jiang, X. Yuan, Z. Wang, H. Kang, M.A. Ali, *J. Food Protect.,* 81(2018) 456.
3. T.M.D. Prado, M.V. Foguel, L.M. Gonçalves, M.d.P.T. Sotomayor, *Sens. Actuator B-Chem.,* 210(2015) 254.
4. H. Xi, Y. Liu, L. Guo, R. Hu, *Food Sci. Technol.,* 29(2020) 93.
5. Y. Liu, Q. Wang, X. Gao, A. Xie, *J. Food Process. Eng.,* 42(2019) 13224.
6. Z.G. Yu, A.L. Sutlief, R.Y. Lai, *Sens. Actuator B-Chem.,* 258(2018) 722.
7. A. Aghaei, M. Erfani Jazi, T. E Mlsna, M.A. Kamyabi, *J. Sep. Sci.,* 42(2019) 3002.
8. F.A. Ibrahim, J.J.M. Nasr, *Anal. Methods,* 6(2014) 1523.
9. A. Blidar, B. Feier, M. Tertis, R. Galatus, C. Cristea, *Anal. Bioanal. Chem.,* 411(2019) 1053.
10. K. Chakraborty, B. Thilakan, V.K. Kizhakkekalam, *J. Appl. Microbiol.,* 124(2018) 108.
11. K. Shrivas, J. Sahu, P. Maji, D. Sinha, *New J. Chem.,* 41(2017) 6685.
12. S.L. Wei, Y. Liu, T. Hua, L. Liu, H.W. Wang, *J. Appl. Polym. Sci.,* 131(2014) 8.
13. Y. Liu, Y. Deng, T.T. Li, Z. Chen, H. Chen, S. Li, H.N. Liu, *J. Biomed. Nanotechnol.,* 14(2018) 2156.
14. C. Wang, Q. Zhao, *Anal. Bioanal. Chem.,* 411(2019) 6637.
15. P. H. Lu, Y. D. Ma, C. Y. Fu, G. B. Lee, *Lab Chip,* 20(2020) 789.
16. S. Zhou, C. Lu, Y. Li, L. Xue, C. Zhao, G. Tian, Y. Bao, L. Tang, J. Lin, J. Zheng, *ACS sensors,* 5 (2020) 588.
17. L.H. Ding, Y.J. Wu, W. Liu, L. Liu, F. Yu, S.C. Yu, Y.M. Tian, J.D. Feng, L.L. He, *Talanta,* 205(2019) 7.
18. Z.H. Yang, X.F. Ding, Q. Guo, Y. Wang, Z.W. Lu, H.C. Ou, Z.F. Luo, X.H. Lou, *Sens. Actuator B-Chem.,* 253(2017) 1129.
19. B.S. He, L. Wang, X.Z. Dong, X.H. Yan, M. Li, S.S. Yan, D.D. Yan, *Food. Chem.,* 300(2019) 6.
20. X.K. Liu, M.Y. Hu, M.H. Wang, Y.P. Song, N. Zhou, L.H. He, Z.H. Zhang, *Biosens. Bioelectron.,* 123(2019) 59.
21. E. Palecek, M. Fojta, *Talanta,* 74 (2007) 276.
22. F. Li, R. Zhou, K. Zhao, H. Chen, Y. Hu, *Talanta,* 87(2011) 302.
23. S. Liu, L. Dou, X. Yao, W. Zhang, B. Zhao, Z. Wang, Y. Ji, J. Sun, B. Xu, D. Zhang, J. Wang, *Food Chem.,* 315(2020) 126310.
24. K. Zeng, D. Wei, Z. Zhang, H. Meng, Z. Huang, X. Zhang, *Sens. Actuator B-Chem.,* 292(2019) 196.
25. C. Munoz-San Martin, M. Gamella, M. Pedrero, A. Montero-Calle, R. Barderas, S. Campuzano, J.M. Pingarron, *Sens. Actuator B-Chem.,* 307 (2020)127623.

26. Y. Zhou, Y. Fang, R.P. Ramasamy, *Sensors,* 19 (2019) 392.
27. H. Beitollahi, F. Movahedifar, S. Tajik, S. Jahani, *Electroanalysis,* 31(2019) 1195.
28. Y. Si, J.W. Park, S. Jung, G.S. Hwang, E. Goh, H.J. Lee, *Biosens. Bioelectron.,* 121(2018) 265.
29. L. Bonel, J.C. Vidal, P. Duato, J.R. Castillo, *Biosens. Bioelectron.,* 26(2011) 3254.
30. N.D. Zhou, J. Zhang, Y.P. Tian, *Anal. Methods,* 6(2014) 1569.
31. Y. Zare, K.Y. Rhee, *Compos. Sci. Technol.,* 155(2018) 252.
32. A. Ben Tahar, K. Zelechowska, J.F. Biernat, E. Paluszkiewicz, P. Cinquin, D. Martin, A. Zebda, *Biosens. Bioelectron.,* 151(2020) 111961.
33. M.V. Jose, S. Marx, H. Murata, R.R. Koepsel, A.J. Russell, *Carbon,* 50(2012) 4010.
34. A.H. Pourasl, M.T. Ahmadi, M. Rahmani, H.C. Chin, C.S. Lim, R. Ismail, M.L.P. Tan, *Nanoscale Res. Lett.,* 9(2014) 7.
35. J. Gao, Y. Chen, W. Ji, Z. Gao, J. Zhang, *Analyst,* 144(2019) 6617.
36. X. Liu, M. Hu, M. Wang, Y. Song, N. Zhou, L. He, Z. Zhang, *Biosens. Bioelectron.,* 123(2019) 59.