

Voltammetric detection of microcystis genus specific-sequence with disposable screen-printed electrode modified with gold nanoparticles

Minbo Lan^{1,2*}, Chen Chen¹, Qin Zhou¹, Yuanjie Teng¹, Hongli Zhao¹, Xiangheng Niu¹

¹Shanghai Key Laboratory of Functional Materials Chemistry, and Research Centre of Analysis and Test, East China University of Science and Technology, Shanghai 200237, P. R. China

²State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, P. R. China

*Corresponding author. Tel: (+86) 21 64253574; Fax: (+86) 21 64252947; E-mail: minbolan@ecust.edu.cn

Received: 28 July 2010, Revised: 10 Sept 2010 and Accepted: 20 Sept 2010

ABSTRACT

A disposable electrochemical biosensor for detection of DNA sequence related to *Microcystis* was described. After depositing gold nanoparticles (AuNPs) onto a screen-printed carbon electrode (SPCE) surface by electrodeposition, the DNA probe which is complementary to a specific gene sequence related to *Microcystis* was immobilized onto the AuNPs modified SPCE via a thiol linker at the 5' end. On the electrode surface the immobilized single-stranded DNA could undergo hybridization in the solution containing DNA specific-sequence of *Microcystis*. Using methylene blue as the indicator, DNA immobilization and hybridization were characterized by cyclic voltammetry and differential pulse voltammetry. Parameters, such as the deposition conditions of gold nanoparticle and the preconcentration time of MB, were optimized. In addition, the control experiments with non-complementary and single base mismatch sequences were investigated and results demonstrated the high selectivity of the biosensor. Copyright © 2010 VBRI press.

Keywords: Microcystis; water bloom; gold nanoparticles; screen printed carbon electrode.



Minbo Lan is a Professor of Applied Chemistry at the East China University of Science and Technology (ECUST), China. He received his Ph.D. in applied chemistry from the ECUST in 1993. His research interests include the organic functional materials and formulation and application of nanomaterials, in particular in electron paramagnetic resonance (EPR) and electrochemical biosensors, and the application of micro-nano-capsules in Traditional Chinese Medicine (TCM) and

novel analytic methods applied in the biomedicines. He has published over 60 research papers and patents and received many awards. He had held visiting fellowships in Japan and Belgium and has also been the Chief Scientist of the Shanghai Nanotechnology Promotion Centre. He collaborates with Australia, Belgium, Japan, UK, Russia and Germany in the areas of EPR and electrochemical biosensing, new materials, organic nanotechnology and surface functional films.

Chen Chen is a Ph.D. candidate of the East China University of Science and Technology. Her major is DNA biosensors, especially



basing on screen-printed electrode.

Qin Zhou is a graduate student of the East China University of Science and Technology. The current field of her interest is DNA biosensors.



Yuanjie Teng is a Ph.D. candidate in analytical chemistry of the East China University of Science and Technology. The current field of interest is enzyme biosensors, especially basing on screen-printed electrode.



Hongli Zhao received her Ph.D. in Pharmaceutical Engineering and Technology from the East China University of Science and Technology in 2007. At present, she is working as a lecture at ECUST. Her research focuses on the Electron Paramagnetic Resonance (EPR) and electrochemical biosensors.



Xiangheng Niu is a Ph.D. candidate of the East China University of Science and Technology. The current field of interest is DNA biosensor for heavy metal ion detection.

Introduction

Water bloom (also known as algal bloom) is a natural phenomenon that the population of algae increases or accumulates rapidly in an aquatic system and it is usually caused by eutrophication. Water bloom is a highly serious environmental problem and it has an enormous impact on society and human health especially when it happens in freshwater lakes or rivers [1]. Normally, the most common phytoplankton species is cyanobacteria and studies [1,2] have shown that *Microcystis* is the most normal type of cyanobacteria. *Microcystis* can release hepatotoxic microcystins in water which may cause serious illnesses in fish, birds and mammals [3,4]. Currently the main technologies used in *Microcystis* detection include microscopic count [5,6], pigment-fluorescence analysis, and other chemical molecular techniques [1]. However, there are always some defects in these methods, for instance, the microscopic count method is time-consuming and hard-controlled, and the pigment-fluorescence analysis cannot distinguish the *Microcystis* from other algae directly [5-7]. Therefore, it is important to establish a simple, less time-consuming and sensitive approach to detect the *Microcystis* in freshwater particularly in drinking-water source.

Electrochemical DNA sensor is one kind of biosensor which detects the specific DNA sequence by the electrochemical approach. This kind of sensor has a lot of advantages including high-sensitivity, fast-response, easy-handling, low-cost etc and it has been used in a broad area just as virus detecting and diseases diagnosis [8-10]. Recently the studies of electrochemical DNA sensor have aroused wide attention and researchers have developed many methods to ameliorate the effect of these biosensors. In this approach a kind of DNA sensor was reported to detect *Microcystis* and methylene blue (MB) was used as the redox indicator [4,11,12] to detect the hybridization of specific-sequence of *Microcystis* DNA.

SPCE attracts broad interest in the research area of electrochemical sensor because it is cheap, easy-made and disposable [13-16]. It can also make the commercialization and miniaturization of the biosensor a reality. Some high sensitive biosensors are based on SPCE and display good

performances [13,17-19], however the application of SPCEs in the DNA sensor is very limited [20-22]. In order to achieve the miniaturization and portability of the DNA sensor and aimed to provide the basis for the in situ determination of the *Microcystis*, the SPCEs were used as the base electrode in this approach.

On the other hand due to the non-functional of the carbon surface of SPCEs, AuNPs have been introduced to the electrode surface, for linking the thiolated-DNA which is complementary to the specific-sequence of *Microcystis* on the electrode by Au-S bonds. AuNPs are very popular in the field of biosensor especially DNA sensor because of their specific properties in electronics, optics, magnetism, and chemistry. They can be easily synthesized and used to promote the sensors' sensitivity remarkably [23,24]. Owing to the big surface area and high surface free energy, AuNPs are able to adsorb the DNA molecules strongly and increase the quantity of the DNA immobilized on the electrode [25,26], and finally enhance the electrochemical signals and detection sensitivity of DNA sensor. Compared with traditional gold disk electrode, the AuNPs modified SPCEs do not need polishing before use and thus greatly save time.

Consequently based on the SPCE, this approach developed a DNA electrochemical sensor modified with AuNPs and researched its performance in the detection of *Microcystis*.

Experimental

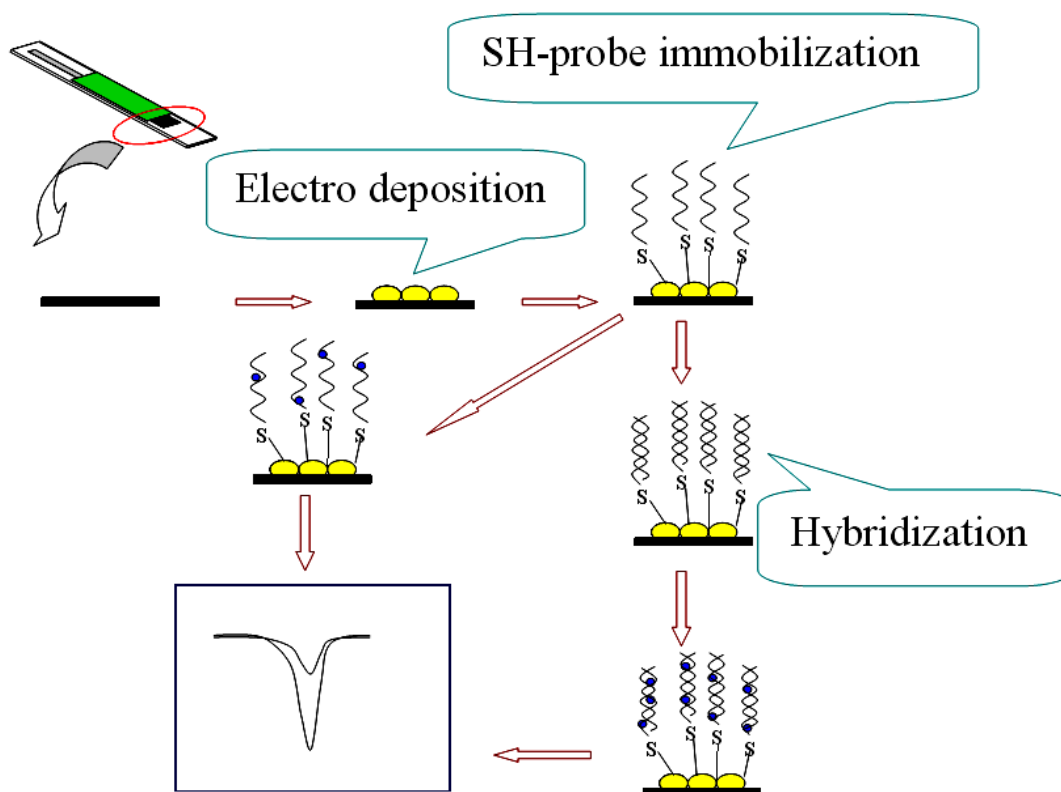
Reagents and solutions

All chemicals were of analytical-reagent grade and were used directly without further purification. Ultra-pure water (18.2 M Ω -cm) from Milli-Q purification system (Shanghai Hitech Instruments Co., Ltd) was used in all preparations (except the preparation of the simulative samples). TE (10 mmol/L Tris-HCl, 1 mmol EDTA, pH 8.0) (Tris is NH₂C(CH₂OH)₃ and EDTA is Ethylene Diamine Tetraacetic Acid) and 2 \times SSC (30 mmol/L of C₃H₅Na₃O₇ and 300 mmol/L of NaCl, pH 7.2) were used. Methylene blue (MB) and HAuCl₄·4H₂O were obtained from Sinopharm Chemical Reagent Co., Ltd.

Eighteen-base synthetic oligonucleotides were purchased from Shenggong Bioengineering Ltd. Company (Shanghai, China):

- 18-mer thiolated-DNA single sequence (ssDNA): 5'- SH-(CH₂)₆-CAA GCT CTT CTT CAG GCC-3';
- 18-mer complementary DNA sequence (cDNA): 5'- GGC CTG AAG AAG AGC TTG -3';
- 18-mer one-base mismatch sequence (oDNA): 5'- GGC CTG AAC AAG AGC TTG -3';
- 18-mer non-complementary sequence (ncDNA): 5' - ACA AAC TCC CCT CCA GCA -3'.

DNA oligonucleotide stock solutions (25 μ mol/L) were prepared in a TE buffer solution and kept frozen. More dilute solution of ssDNA was prepared in 50 mmol/L Tris-HCl, 20 mmol/L NaCl (pH 7.2), and other oligomers were diluted in 0.05 mol/L Phosphate buffer solution (PBS), containing 0.3 mol/L NaCl (pH 7.2).



Scheme 1: Illustration of the processes of the AuNPs electro deposition, ssDNA immobilization onto Au nano/SPCE and the detection of DNA.

Purity of oligonucleotides

The achievement of efficient immobilization of thiolated DNA probes depends on their purity. Thus the ssDNA solution should be detected by UV-vis absorption spectrometry before any experiment started. The ratio between the absorbance values obtained at 260 and 280 nm close to 1.8 indicated that DNA solution was sufficiently free of protein and mononucleotides, and ssDNA was suitable for electrochemical deposition on the electrode surface [8].

Preparation of SPCE and immobilization of ssDNA

SPCEs with the diameter of 4mm were fabricated by a TORCH T3244 manual screen-printer (Beijing Torch Co., Ltd., China). The scheme and fabrication steps were shown in our previous work [27]. Prior to the immobilization of ssDNA, the SPCEs should be electrodeposited with AuNPs. It was carried out in 0.5 M H₂SO₄ solution containing 0.01 M HAuCl₄ using chronoamperometry. The electrode potential was stepped from 0.76-0.16 V with the deposition time 5s. Then the electrode was electrochemically treated by cyclic potential scanning between 0 and 1.5 V in 0.5 M H₂SO₄ until the cyclic voltammogram became reproducible. After these pretreatments, 10 μ L, 1 μ M ssDNA probe solution was added onto the AuNPs modified SPCE (Au nano/SPCE) and after 2h incubation at room temperature, the modified electrodes were rinsed with 2 \times SSC for 5s

and then thoroughly rinsed with ultra-pure water. The processes of the AuNPs electro deposition, ssDNA immobilization onto Au nano/SPCE and the detection of DNA were shown in **Scheme 1**.

Hybridization with targets

In the qualitative experiments, when the ssDNA immobilized Au nano/SPCE (ssDNA/Au nano/SPCE) was prepared, 10 μ L hybridization solution containing 1 μ M complementary target (cDNA) was added onto the surface of the electrode. The hybridization was allowed to proceed for 1h. The biosensor was then rinsed for 10s with 2 \times SSC buffer and finally named cDNA/Au nano/SPCE. Control experiments were carried out with samples containing the same concentration of the non-complementary sequence (ncDNA) or the one-base mismatch sequence (oDNA), and then sensors were named ncDNA/Au nano/SPCE and oDNA/Au nano/SPCE, respectively.

In the quantitative experiments, hybridization solutions including different concentrations of cDNA ranged from 0.10 to 0.01 μ M were used in the same way mentioned above. In the simulative experiments, hybridization solutions were prepared by the water from Qingchun River in the campus of the East China University of Science and Technology. After deposition and filtration, the river water (pH \sim 7.1) was used instead of the ultra-pure water. All the other steps were as above.

Apparatus and measurement

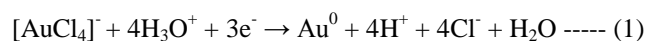
All electrochemical experiments were performed with a CHI 440A electrochemical workstation (CHI Instruments Inc., USA) at room temperature with a conventional three-electrode system. It consisted of a modified SPCE (working electrode), a saturated calomel electrode (SCE) (work as the reference electrode, saturated with KCl) and a counter electrode made of platinum wire.

UV-vis absorption spectroscopy was done by a UV-2102 (Unico (Shanghai) Instrument Co., Ltd., USA). The scanning electron microscopy (SEM) image of the Au nano/SPCE surface was recorded by a JSM-6360LV SEM (JEOL Ltd., Japan).

Results and discussion

Electrochemical characterization of Au nano/SPCE

Fig. 1 shows a scanning electron microscopy of Au nano/SPCE electrode microstructure. After applying a potential range of 0.16-0.76 V (vs. SCE) for 5 s in 4 mL, 0.5 M H₂SO₄ solution containing 0.01 M HAuCl₄, the SEM chart shows that there are masses of AuNPs cover on the surface of the electrode and the average diameter is around 100 nm. The mechanism can be concluded as **Equ. (1)**.



It also can be seen some AuNPs are agglomerated and the supposed reason is the surface of electrode is not plane enough. The AuNPs can enlarge the surface area of electrode and then increase the quantity of DNA molecules that binding to the electrode due to the Au-S bond.

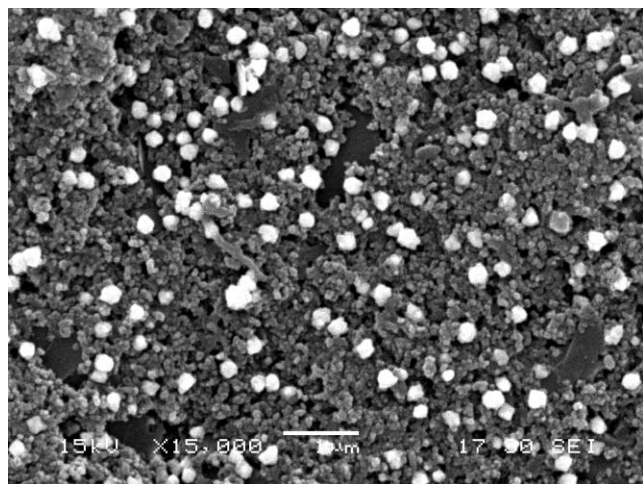


Fig. 1. SEM of AuNPs on SPCE.

In this study, cyclic voltammetry over the potential ranged from 0 to 1.5 V in 0.5 M H₂SO₄ solution is chosen to demonstrate whether the AuNPs have been formed. After 10 repeated scans at a scan rate of 100 mV/s, there is no more change in peak height or shape. The cyclic voltammograms of the bare SPCE and the Au nano/SPCE are compared in **Fig. 2**. It can be seen clearly that Au

nano/SPCE (**curve a**) shows a typical cyclic voltammogram of gold, which contains a single sharp reduction peak located at ~0.9 V and multiple overlapping oxidation peaks in the range of 1.2-1.5 V. By contraries the bare SPCE (**curve b**) shows none of the peaks mentioned above. As a consequence it indicates out that the AuNPs can be deposited on the electrode by this electrochemical method.

The stability of the Au nano/SPCE was tested by comparing the cyclic voltammogram detected 2 days later with the one detected immediately after prepared. After the 10 scans carried out in 0.5 M H₂SO₄ and then 2 days kept in silicagel desiccator, the Au nano/SPCE did not show any difference in the cyclic voltammogram (data not shown) obtained with the same parameters in 0.5 M H₂SO₄. The result indicates the AuNPs deposited on the Au nano/SPCE are stable and steady.

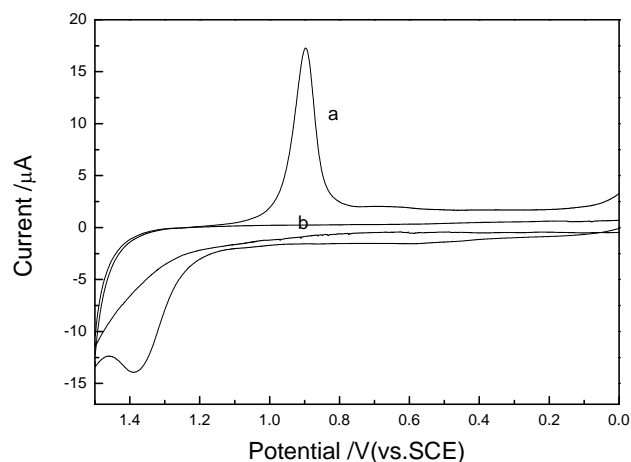


Fig. 2. Cyclic voltammogram for bare SPCE and gold nanoparticle modified SPCE in a 0.5 M H₂SO₄ solution: bare SPCE (a); Au nano/SPCE (b). Scan rate: 100 mV/s.

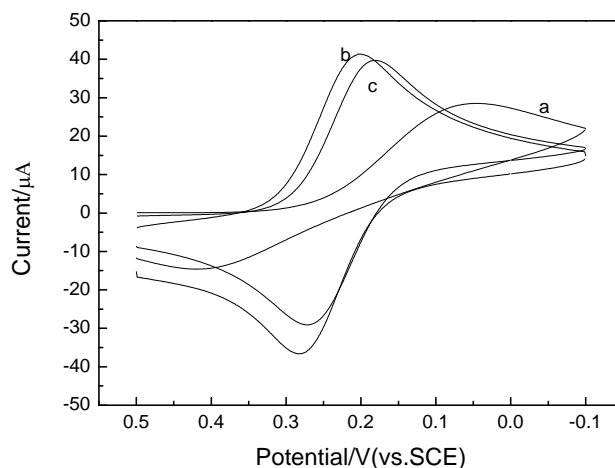


Fig. 3. Cyclic voltammograms measured with bare SPCE (a), Au nano/SPCE (b) and ssDNA/Au nano/SPCE (c) in the 2 mM ferricyanide, 0.5 M KCl aqueous solution, with a scan rate of 50 mV/s.

Electrochemical characterization of DNA-modified Au nano/SPCE

Electroactive substance K₃[Fe(CN)₆] was used to check out whether the DNA molecules have been modified on the

electrode in this approach. Three SPCEs were chosen and then AuNPs were electrodeposited in two of them. One Au nano/SPCE was modified with ssDNA and named ssDNA/Au nano/SPCE. The bare SPCE, Au nano/SPCE and ssDNA/Au nano/SPCE prepared above were carried out with cyclic voltammetry in 2 mM $K_3Fe(CN)_6$, 0.5 M KCl solution. As showed in **Fig. 3**, due to the inert surface and low electrode transport rate, the cyclic voltammogram of SPCE does not display the typical redox curve (**curve a**). Owing to that gold is propitious to transport the electrodes, when the AuNPs are deposited on the surface of the electrode (**curve b**), the anodic and cathodic peaks of $K_3Fe(CN)_6$ are symmetrical and the peak currents are almost the same ($i_{pc}/i_{pa} = 1$, i_{pc} is the peak current of cathodic peak and i_{pa} is the peak current of anodic peak). The electric potential difference ΔE is 80 mV. It demonstrates that the redox reaction happened in Au nano/SPCE is a quasi-reversible process and the result indicates again that the AuNPs can be deposited on the SPCE through the electrochemical method. After modifying the ssDNA molecules on the Au nano/SPCE (curve c), the difference of the peak currents (Δi_p) of the redox curve decreases and the ΔE increases (91 mV). The main reason is the repeating structure of phosphate backbone makes the DNA molecules electronegative thus repulse the $[Fe(CN)_6]^{3-}$. The other reason is that the steric hindrance of the DNA molecules may reduce the electron transport rate and therefore impacts the cyclic voltammogram. Based on **Fig. 3** and all the analysis, it can be known clearly that the ssDNA have been successfully modified on the electrode.

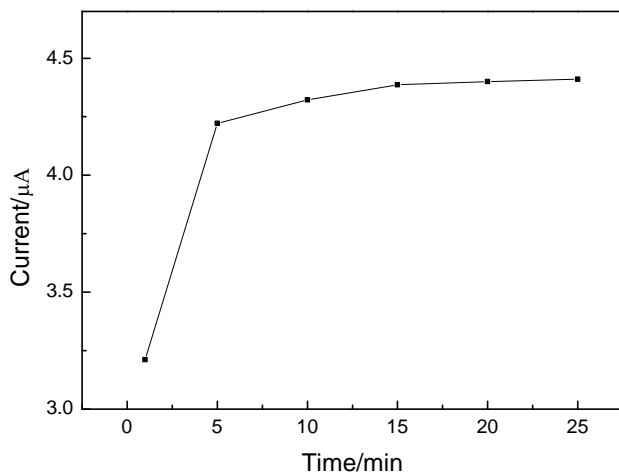


Fig. 4. Plot of the reduction peak currents of MB with CV for ssDNA/Au nano/SPCE as a function of MB accumulation time.

Optimization of the experimental parameters

Effect of the preconcentration time of MB: The **Fig. 4** shows the relation curve of the reduction peak current of the MB against the preconcentration time of MB. According the graph the signal value of the MB shows an exponentially increase with time in the first 5 min, and then its growth rate reduces. After 15 min the signal value of MB reaches a plateau and it means the amount of MB

molecules embedded in the ssDNA have been saturated. Consequently 15 min is chosen to be the best preconcentration time of MB.

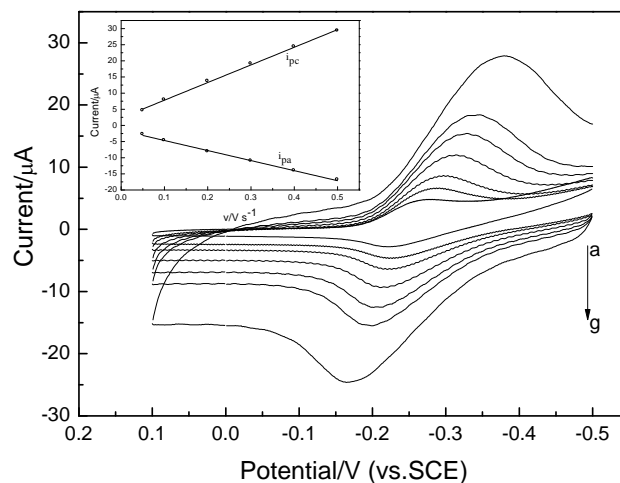


Fig. 5. Cyclic voltammograms of the ssDNA/Au nano/SPCE in a TE solution containing 2×10^{-5} M MB a~g: 100, 200,300,500,750,1000,2000 mV/s, respectively. Insert: Plots of cathodic (i_{pc}) and anodic (i_{pa}) peak currents versus scan rates.

Effect of scanning rate: In order to investigate the effect of the scanning rate, the ssDNA/Au nano/SPCEs were applied with cyclic voltammetry in TE buffer solution (pH 8.0) containing 2×10^{-5} M MB at different scanning rates (0.05 V/s ~0.5 V/s) and the results are shown in **Fig. 5**. It can be seen that after scanning for 7 times the shape of peaks in the cyclic voltammograms is still kept good and it indicates that the modified electrode is stable. Furthermore the figure insert demonstrates that the anodic peak current (i_{pa}) and cathodic peak current (i_{pc}) are linear with scanning rate respectively in the range of scanning rate investigated, and it means there is an interaction between MB and ssDNA/Au nano/SPCE and the process is mainly controlled by the effect of adsorption.

Effect of the dissolution of adsorbed MB: After scanning in MB solution, there will be a large number of MB molecules adsorbed on the surface of ssDNA/Au nano/SPCE. To avoid MB molecules' effect on hybridization, it is necessary to remove the adsorbed MB. In the study, it is found that if the ssDNA/Au nano/SPCE is transferred into TE buffer solution (pH = 8.0) immediately after scanning in MB solution, the peak value of MB decreases nearly 8% in 1 min and nearly 40% in 6 min. If the immersing time increases, the peak value of MB will keep reducing until it can be ignored. **Fig. 6** shows the relation curve between reduction peak current of MB and the immersing time. This graph shows that the MB adsorbed in the modified electrode will resolve in TE buffer solution in a few minutes. Therefore in this study we immerse the electrodes which affected with MB in TE buffer solution for 30 min to remove the adsorbed MB.

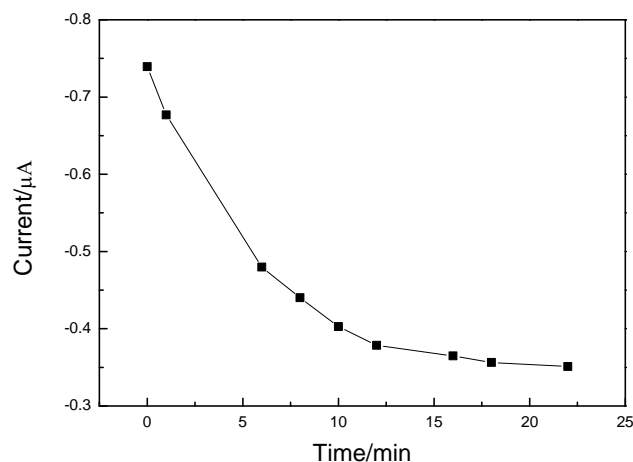


Fig. 6. Plot of the reduction peak currents of MB with DPV for ssDNA/Au nano/SPCE as a function of MB solution time in TE buffer solution.

Electrochemical detection of the DNA specific-sequence of *Microcystis*

Differential pulse voltametry (DPV) was used to illustrate the electrochemical effect between MB molecules and the bare SPCE, Au nano/SPCE, ssDNA/Au nano/SPCE, cDNA/Au nano/SPCE, respectively, and **Fig. 7** and **Fig. 8** show the results.

Fig. 7a shows that the electrochemical signal of MB on bare SPCE is small. It can be attributed that the interaction between bare SPCE and MB is not strong enough and the MB molecules can only be accumulated by adsorbing to the small space on the carbon electrode. However, after the AuNPs have been deposited on the surface of SPCE, the amount of the MB accumulated on the Au nano/SPCE electrode as well as the output signal (**Fig. 7b**) increases. This is due to the great deal of spacing among the nanoparticles though it is hard to avoid the agglomeration.

Latest studies [12,28,29] demonstrated that the main interactions between DNA and MB are the insertion action and electrostatic action. In addition T-shaped, non-stacked and face-to-face are found to be the three modes in which the MB⁺- guanine complexes orientate. According to the results obtained in this study, the insertion action plays the dominant role in the connection between ssDNA and MB. It is speculated that the reduction peak of ssDNA/Au nano/SPCE (**Fig. 7c**) is bigger than the bare SPCE and the Au nano/SPCE for two reasons: the first is the electronegative phosphate backbone of ssDNA has static sorption on the MB cations, and the second one is MB molecules have a strong affinity for the free guanines of ssDNA which will be more exposed when the ssDNA molecules are immobilized [29-31]. After the hybridization with complementary DNA (**Fig. 7d**), the double helix of DNA (also known as double sequence, dsDNA) will form on the surface of the ssDNA/Au nano/SPCE and it will be much easier for MB molecules to insert into the groove of DNA. Then the quantity of the bound MB molecules and the electrochemical signal will increase as a consequence.

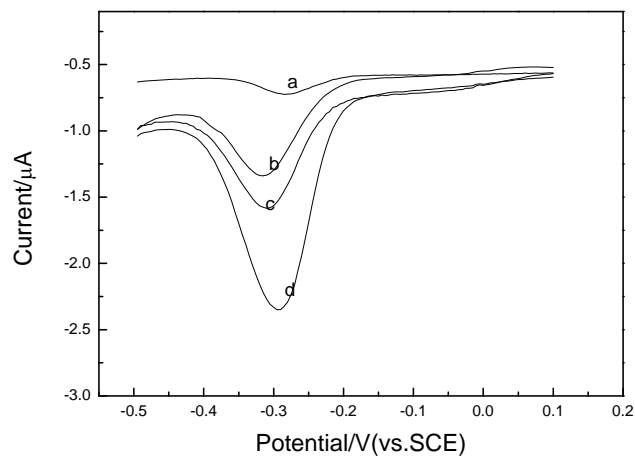


Fig. 7. Differential pulse voltammograms of MB accumulated on the bare SPCE (a), Au nano/SPCE (b), ssDNA/Au nano/SPCE (c), cDNA/Au nano/SPCE (d) in a 0.05 M K₂HPO₄/KH₂PO₄, 0.3 M NaCl (pH 7.2).

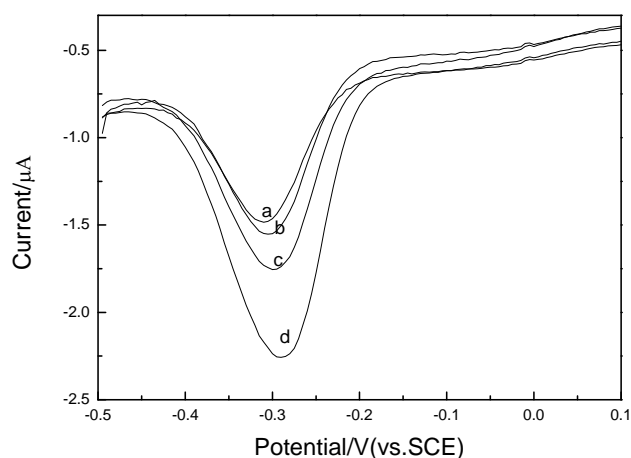


Fig. 8. Differential Pulse Voltammograms using 2.0×10^{-5} M MB as the redox indicator for ssDNA/Au nano/SPCE (a), ncDNA/Au nano/SPCE (b), oDNA/Au nano/SPCE (c), cDNA/Au nano/SPCE (d).

In control experiments, ssDNA/Au nano/SPCEs were added with the solution of the non-complementary sequence and one-base mismatch sequence respectively, and then be detected in the same way. The **Fig. 8** shows that compared with the ssDNA/Au nano/SPCE (**Fig. 8a**), the cathodic reduction signal of ncDNA/Au nano/SPCE (**Fig. 8b**) is almost unchanged. This phenomenon demonstrates that the hybridization reaction does not occur on the surface of the electrode. On the other hand, the cathodic reduction signal of oDNA/Au nano/SPCE (**Fig. 8c**) is bigger than the ncDNA/Au nano/SPCE but smaller than the cDNA/Au nano/SPCE (**Fig. 8d**), which means when the bases do not match absolutely, the hybridization cannot carry out thoroughly either. Accordingly, based on the DNA sensor proposed in this study, the qualitative detection of distinguish the DNA sequence related to *Microcystis* can be achieved by analyzing the signal of the MB bonded to the modified Au nano/SPCE.

As shown in the **Fig. 9**, the calibration curve of the DNA biosensor is researched by changing the concentration of the target complementary DNA (cDNA). After hybridization with cDNA of different concentrations, the reduction peak currents in the DPV are measured. In

addition, considering the background interference of the solution, blank background ($c_{\text{cDNA}} = 0 \mu\text{M}$) is subtracted from the original curves. It can be clearly seen from the insert figure that the reduction peak currents of MB are linear with respect to the concentrations of cDNA over a range from 10^{-7} to 10^{-8} M. The regression equation is $i_{\text{pc}} = -8.777 c_{\text{cDNA}} - 8.113 \times 10^{-2}$ (where i_{pc} represents the DPV peak current for MB in μA and c_{cDNA} represents the concentration of cDNA in $\mu\text{mol L}^{-1}$), with a correlation coefficient of 0.9961.

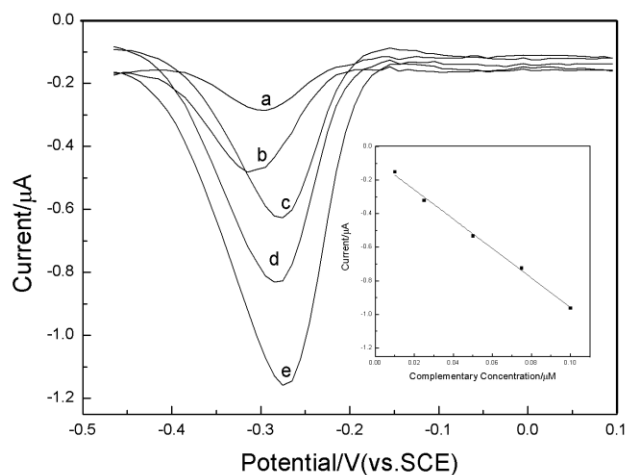


Fig. 9. Background-corrected Differential Pulse Voltammograms using 2.0×10^{-5} M MB as the redox indicator for cDNA/Au nano/SPCE with different concentrations of cDNA: 0.010 μM (a), 0.025 μM (b), 0.050 μM (c), 0.075 μM (d), 0.10 μM (e). Insert: Relationship between the peak currents (i_{pc}) of methylene blue using cDNA/Au nano/SPCEs and the concentrations (c_{cDNA}) of the target cDNA. The regression equation of the calibration line is $i_{\text{pc}} = -8.777c_{\text{cDNA}} - 8.113 \times 10^{-2}$ (where the peak current, i_{pc} , is in μA and the concentration, c_{cDNA} , of cDNA is in μM), with $R^2 = 0.9961$.

Simulative samples are prepared to test the application of the proposed biosensor. After deposition and filtration, the water (pH ~ 7.1) from Qingchun River is used instead of the ultra-pure water. **Fig. 10** clarifies that though there is no cDNA in the hybridization solution, the peak is still obvious (curve a). It means the background cannot be neglected for the existing signal. After the subtraction of the background, the current is $-0.9568 \mu\text{A}$ and the recovery of the cDNA are calculated to be 99.79% in the sample including 0.10 μM cDNA. These results obtained show that the measurement will not be affected though the environmental solution is complicated as real samples and then prove again the present sensor is a very effective analysis system. In the future it is hoped to detect the real samples from the freshwater where water boom has happened.

Conclusion

AuNPs have big specific surface area and good biocompatibility, and they can bond with thiolated-DNA due to the Au-S binding. Based on AuNPs modified SPCE, the cost of the biosensor is much lower than other approaches and the amount of DNA molecules immobilized on the electrode increases markedly. The DNA biosensor proposed in this study realizes the detection of the specific-

sequence of *Microcystis* genus and presents an important method to achieve the low-cost, high-sensitive, and dependable detection of *Microcystis* in the future.

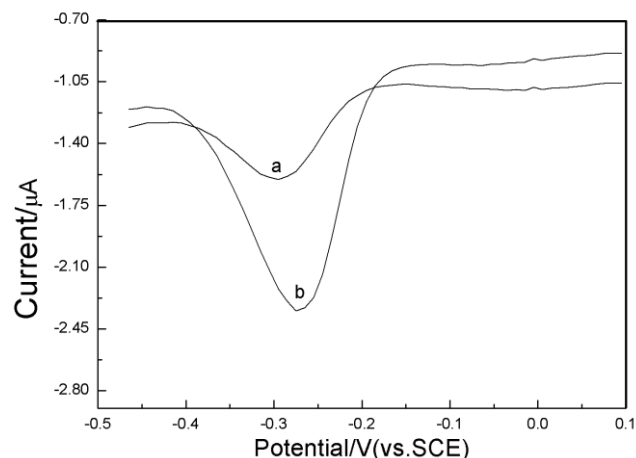


Fig. 10. Differential Pulse Voltammograms using 2.0×10^{-5} M MB as the redox indicator for cDNA/Au nano/SPCE with different concentrations of cDNA in the water from Qingchun River: 0 μM (a), 0.10 μM (b).

Acknowledgement

This work was supported by Science and Technology Commission of Shanghai Municipality (STCSM, contract No. 10dz2220500, No.09391911500 and 10391901600).

References

- Saker, M. L.; Vale, M.; Kramer, D.; Vasconcelos, V. M. *Appl. Microbiol. Biotechnol.* **2007**, *75*, 441.
- Fallon, R. D.; Brock, T. D. *Freshwater. Biol.* **2006**, *11*, 217.
- Wang, J.; Palecek, E.; Nielsen, P. E.; Rivas, G.; Cai, X.; Shiraishi, H.; Dontha, N.; Luo, D.; Farias, P.A.M. *J. Am. Chem. Soc.* **1996**, *118*, 7667.
- Erdem, A.; Kerman, K.; Meric, B.; Ozkan, D.; Kara, P.; Onsoz, M. *Turk. J. Chem.* **2002**, *26*, 851.
- Jost, M.; Jones, D. D.; Weathers, P. J. *Protoplasma.* **1971**, *73*, 329.
- Tsujimura, S.; Tsukada, H.; Nakahara, H.; Nakajima, T.; Nishino, M. *Hydrobiologia.* **2000**, *434*, 183.
- Cao, H. S.; Kong, F. X.; Tan, J. K.; Zhang, X. F.; Tao, Y.; Yang, Z. *Int. Rev. Hydrobiol.* **2005**, *90*, 347.
- Loaiza, O.; Campuzano, S.; Pedrero, M.; Pingaron, J. M. *Electroanalysis.* **2008**, *20*, 1397.
- Susmel, S.; Guilbault, G. G.; O'Sullivan, C. K. *Biosens. Bioelectron.* **2003**, *18*, 881.
- Wang, J.; Rivas, G.; Cai, X.; Palecek, E.; Nielsen, P.; Shiraishi, H.; Dontha, N.; Luo, D.; Parrado, C.; Chicharro, M. *Anal. Chim. Acta.* **1997**, *347*, 1.
- Ju, H.; Zhou, J.; Cai, C.; Chen, H. *Electroanalysis.* **1995**, *7*, 1165.
- Wang, J.; Wang, F.; Dong, S. *J. Electroanal. Chem.* **2009**, *626*, 1.
- Teng, Y. J.; Zuo, S. H.; Lan, M. B. *Biosens. Bioelectron.* **2009**, *24*, 1353.
- Cardosi, M. F.; Birch, S. W. *Anal. Chim. Acta.* **1993**, *276*, 69.
- Millan, K. M.; Saraullo, A.; Mikkelsen, S. R. *Anal. Chem.* **1994**, *66*, 2943.
- Lucarelli, F.; Kicela, A.; Palchetti, I.; Marrazza, G.; Mascini, M. *Bioelectrochemistry.* **2002**, *58*, 113.
- Gan, N.; Yang, X.; Xie, D.; Wu, Y.; Wen, W. *Sensors.* **2010**, *10*, 625.
- Radi, A. E.; Mu oz-Berbel, X.; Cortina-Puig, M.; Marty, J. L. *Electroanalysis.* **2009**, *21*, 1624.
- Yang, T. H.; Hung, C. L.; Ke, J. H.; Zen, J. M. *Electrochem. Commun.* **2008**, *10*, 1094.
- Yang, W.; Gerasimov, J.Y.; Lai, R.Y. *Chem. Commun.* **2009**, 2902.
- Liu, J.; Su, B.; Lagger, G.; Tacchini, P.; Girault, H. H. *Anal. Chem.* **2006**, *78*, 6879.

22. Abbaspour, A.; Baramakeh, L.; Nabavizadeh, S. M. *Electrochim. Acta.* **2007**, 52,4798.
23. Rashid, M. H.; Bhattacharjee, R. R.; Kotal, A.; Mandal, T. K. *Langmuir.* **2006**, 22, 7141.
24. Guo, S.; Wang, E. *Anal. Chim. Acta.* **2007**, 598, 181.
25. Cai, H.; Xu, Y.; Zhu, N.; He, P.; Fang, Y. *Analyst.* **2002**, 127, 803.
26. Zhuo, Y.; Yuan, R.; Chai, Y.; Tang, D.; Zhang, Y.; Wang, N.; Li, X.; Zhu, Q. *Electrochem. Commun.* **2005**, 7, 355.
27. Zuo, S.; Teng, Y.; Yuan, H.; Lan, M. *Sens. Actuators, B* **2008**, 133, 555.
28. Feng, K.; Sun, C.; Kang, Y.; Chen, J.; Jiang, J. H.; Shen, G. L.; Yu, R. Q. *Electrochem. Commun.* **2008**, 10, 531.
29. Yang, W.; Ozsoz, M.; Hibbert, D. B.; Gooding, J. J. *Electroanalysis.* **2002**, 14, 1299.
30. Erdem, A.; Kerman, K.; Meric, B.; Akarca, U. S.; Ozsoz, M. *Anal. Chim. Acta.* **2000**, 422, 139.
31. Brunberg, A. K. *Freshwater. Biol.* **2006**, 33, 541.