Ultrasensitive electrochemical microcystin-LR immunosensor using gold nanoparticle functional polypyrrole microsphere catalyzed silver deposition for signal amplification

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Abstract:

This work designed an ultrasensitive electrochemical immunosensor for microcystin-LR (MC-LR) detection using gold nanoparticle functional polypyrrole microsphere (AuNP/PPyMS) as a novel signal tag to induce silver enhancement for signal amplification. The dispersed polypyrrole microsphere (PPyMS) synthesized with a chemical oxidative polymerization was used to load AuNP for labeling signal antibodies, which enhanced the detection signal due to large surface area. The proposed MC-LR immunosensor was constructed by coating a polyethylene glycol film on a carbon nanotubes modified electrode and then immobilizing MC-LR antigen on the film. With a competitive immunoassay format, coated MC-LR competed for MC-LR antibody with added target MC-LR to form antibody-antigen immunocomplex. Subsequently, the immunocomplex reacted with AuNP/PPyMS labeled signal antibodies. The MC-LR immunoreaction monitoring was achieved by the electrochemical stripping signal of the deposited silver catalyzed by AuNP/PPyMS. AuNP/PPyMS-mediated silver enhancement along with carbon nanotubes-promoted electron transfer led to high detection sensitivity of MC-LR. Under optimal conditions, the MC-LR immunoassay had a wide linear range of 0.25 ng L⁻¹ to 50 µg L⁻¹, low detection limit of 0.1 ng L⁻¹, and good precision, reproducibility and stability. The designed amplification strategy has potential application in the ultrasensitive electrochemical detection of other algae toxins.

Keywords: Electrochemical immunosensor; Microcystin-LR; Silver deposition; Polypyrrole microsphere; Gold nanoparticle; Signal amplification

1. Introduction

The cyanobacterial bloom is an escalating problem to public health and environment because of the release of cyanotoxins into water supplies [1]. Microcystins (MCs), a kind of cyclic heptapeptide cyanotoxins, is the most common and toxic metabolite from cyanobacteria. MCs has shown a tumor promotion effect and potent inhibition of protein phosphatases 1 and 2A, two key enzymes in cellular processes [2,3], and also could be accumulated in aquatic organisms and transferred to higher trophic levels through the biological chain. Up to now, more than 90 congeners of MCs have been isolated and identified from lowly toxic to highly toxic with molecular weights from 900 to 1120 Da. Microcystin-LR (MC-LR, MW=995.2), containing five nonproteinogens and two substitutions of leucine (L) and arginine (R) at positions 2 and 4, is the most frequent and hepatotoxic congener among all microcystin variants [4-6], and the LD₅₀ of MC-LR is 43 $\mu\text{g kg}^{-1}$ [7]. In 1998, the World Health Organization (WHO) had set a maximum permitted level of 1 $\mu\text{g L}^{-1}$ for MC-LR in drinking water, and the acceptable daily intake value (ADI) for MC-LR was between 0 and 0.04 $\mu\text{g kg}^{-1}$ [8]. Therefore, a convenient, rapid and sensitive MC-LR detection method is urgent to public health.

Many analytical methods have been developed for MC-LR detection, such as high-performance liquid chromatography (HPLC) [9], liquid chromatography tandem mass spectrometry (LC-MS/MS) [10] and protein phosphatase inhibition assays [11]. Although these methods are well-proven and widely accepted, they require relatively expensive equipments, advanced technical expertise, high cost and are time-consuming. Thus, immunoassay techniques are of great interest in qualitative and quantitative detection of MC-LR due to their highly specific molecular recognition without the need for prior sample concentration or pretreatment. The best candidates for the on-site immunoassay of MC-LR are the electrochemical immunosensors due to the high sensitivity, simplicity, easy to

miniaturization, and low cost of both the sensors and the instrumentation. Commonly, electrochemical MC-LR immunoassay uses horseradish peroxidase [12-14] and glucose oxidase [15] labeled signal antibody coupling with an enzymatic reaction to trace the immunosensing events. However, the practical applications of this enzyme-based MC-LR immunoassay are limited due to ease of denaturation, leakage, and the time-consuming and costly preparation and purification process of enzymes.

With the development of nanoscience and nanotechnology, quantum dot (QD) [16,17] and metallic nanoparticle [18-20] have been used as signal tags for signal enhancement and develop ultrasensitive enzyme-free MC-LR immunoassay. The QD-based detection requires harsh detection conditions, including nanocrystals dissolution, high potential accumulation, and deoxygenation, which is not suitable for application. As tracing tags, gold nanoparticle (AuNP) is the most used metallic nanoparticle in electrochemical immunosensors due to its unique abilities of good biocompatibility and easy functionalization with proteins [20]. Compared with the relatively positive electrochemical oxidization potential of nanogold, silver nanoparticle (AgNP) can be more easily oxidized for stripping analysis with a latively sharp peak [21]. AuNP could in situ catalyze the deposition of silver and be conveniently labeled to the signal antibody, and the immunoassay using AuNP-catalyzed silver deposition to amplify the anodic stripping signal has been quickly developed [22,23]. This work further combined the AuNP-catalyzed silver deposition with conducting polymer microspheres, synthesized well dispersed polypyrrole microsphere (PPyMS) to load AuNP for labeling signal antibodies and catalyzing silver deposition, and designed a dual signal amplified system for constructing ultrasensitive electrochemical immunosensor for MC-LR detection.

Polypyrrole (PPy), as one of attractive conducting polymers, is extensively applied to the design of efficient sensing elements due to its unique properties, such as low cost, easy preparation, tunable conductivity, and biocompatibility [24,25]. Polypyrrole microsphere

(PPyMS) obtained through simple oxidative polymerization possessed larger surface area and abundant active sites, indicating redox probes were easy to be immobilized on the surface of PPyMS, and PPyMS had become one of an ideal nanomaterial as immunoprobes [26,27]. This work synthesized a AuNP loaded PPyMS for labeling signal antibodies by electrostatic interaction, and designed a synergistic PPyMS-mediated silver enhancement process. This signal amplification process could be conveniently applied in sensitive immunosensing of MC-LR using a carbon nanotube (CNT)-enhanced electrochemical immunosensor.

This work constructed the MC-LR immunosensor by immobilizing MC-LR antigen on CNT modified electrode with the aid of polyethylene glycol (PEG) film, which not only assisted antigen immobilization, but also served as a potential stabilizer for binding sites of the antigen structure. After a competitive immunoassay format, the high-content AuNP on AuNP/PPyMS-labeled signal antibodies reacted with immune complexes catalyzed the chemical deposition of silver for electrochemical stripping analysis of target antigen MC-LR. Combined the AuNP/PPyMS-catalyzed silver deposition with carbon nanotube accelerated electron transfer, this MC-LR immunosensing showed a detection limit down to sub ng L^{-1} level, corresponding to enzyme and other nanomaterial amplification strategies (ng L^{-1}). This proposed method avoided the deoxygenation procedure for usual electrochemical detection and harmful solution for dissolution of tracing tag. The assay approach had acceptable stability, precision, and accuracy, showing potential applications in the detection of other algae toxins.

2. Experimental

2.1. Materials and reagents

CNT (purity $\geq 98\%$, diameter 60-80 nm, and length 1-2 μm) was purchased from Nanoport Co. Ltd. (Shenzhen, China). PEG ($M_w=20000 \text{ g}\cdot\text{mol}^{-1}$), Chloroauric acid ($\text{HAuCl}_4\cdot 4\text{H}_2\text{O}$),

Tween-20, and Pyrrole were obtained from Sinopharm Chemical Reagent Co. Ltd. (China). Trisodium citrate was purchased from Shanghai Reagent Company (Shanghai, China). Poly(diallyldimethylammonium chloride) (PDDA, 20%, w/w in water, $M_w=200000-350000$) and silver enhancer solutions A and B were purchased from Sigma-Aldrich (USA). MC-LR and MC-LR antibody (anti-MC-LR) solution with the concentration of 1 mg mL^{-1} were purchased from Express Technology Co., Ltd. (Beijing, China). Bovine serum albumin (BSA) and goat anti-mouse IgG (GaMIgG) were obtained from Wuhan Boster Biological Technology Co., Ltd. (Wuhan, China). All other reagents were of analytical grade. The blocking buffer was 0.01 M pH 7.4 phosphate buffer saline (PBS) containing 5% BSA. To minimize unspecific adsorption, 0.05% Tween-20 was spiked into PBS as wash buffer (PBST). Ultrapure water obtained from a Millipore water purification system ($\geq 18 \text{ M}\Omega$, Milli-Q, Millipore) was used in all assays. The mixture solution of silver enhancer solutions A and B was freshly diluted with ultrapure water for silver deposition enhancement. The polluted water samples were from Tai Lake in Wuxi, China.

2.2. Apparatus

Electrochemical experiments were performed with CHI660D electrochemical workstation (CH Instruments Inc., USA) with a conventional three-electrode system with a modified GCE as working, a platinum wire as auxiliary and a saturated calomel electrode as reference electrode, respectively. All the electrochemical measurements were carried out at 25°C . A pH-3C digital pH meter obtained from Shanghai INESA scientific instrument Co. Ltd. (Shanghai, China) with a combined glass electrode was used to adjust the pH value of the PBS buffer solution. Transmission electron microscopic (TEM) images were gained on a JEM-2100 transmission electron microscope (JEOL, Japan).

2.3. Preparation of well-dispersed CNT solution

50 mg CNT was dispersed in 50 mL 65% HNO₃ solution, and the resultant mixture was refluxed for 12 h at 110 °C [28]. The resulting suspension was centrifuged and the precipitate was washed thoroughly with water to get carboxylic group functionalized CNT. The black solid was collected and dried in a vacuum at 60 °C. The resulting CNT could well disperse in water for preparation of a 1 mg mL⁻¹ CNT solution, and the black homogeneous solution was stable for a long time (at least 3 months).

2.4. Preparation of PPyMS, AuNP/PPyMS, and Ab₂-AuNP/PPyMS

PPyMS was synthesized by a facile and one-step chemical oxidative polymerization approach [29]. Firstly, 1 mL of pyrrole was dispersed in 60 mL ultrapure water, and 0.1g FeCl₂ was added in the pyrrole/H₂O solution. Then, 5 mL 3% H₂O₂ was added in the pyrrole/FeCl₂/H₂O mixture and then polymerized at 25 °C for 12 h. The dark precipitated mixture was filtered, washed with water and freeze-dried for 12 h to yield the resulting PPyMS.

The AuNP was obtained by reducing chloroauric acid with trisodium citrate according to a previous report [30]. Briefly, 0.5 mL of 1% HAuCl₄·4H₂O solution was dissolved in 50 mL ultrapure water and brought to vigorous boiling with stirring, and a 1.75 mL-portion of 1% sodium citrate was added to the solution, followed by stirring for 10 min. The solution changed from colorless to deep red, indicating the formation of AuNP. After 10 min, the resulting solution was rapidly cooled in an ice water bath with stirring to produce a stable AuNP colloid solution.

Ab₂-AuNP/PPyMS was prepared according to the following procedure (Scheme 1A).

Firstly, 500 μL PDDA aqueous solution (5 mg mL^{-1}) containing NaCl (0.5 M) was added into the 5 mL as-prepared PPyMS dispersion (1 mg mL^{-1}), and the mixture was stirred for 4.5 h to get a homogenous suspension. Residual PDDA was removed by high speed centrifugation, and the complex was washed three times to get PDDA/PPyMS. Secondly, 0.5 mL PDDA/PPyMS solution dispersed with ultrapure water (1 mg mL^{-1}) was added into 1 mL AuNP solution to construct the AuNP/PPyMS composite, and the mixture was then allowed to stand overnight, followed by centrifugation for three times and redispersion in 1 mL ultrapure water to get AuNP/PPyMS dispersion. Thirdly, 25 μL 1.0 mg mL^{-1} secondary antibodies GaMIgG (Ab_2) solution was added to 1 mL AuNP/PPyMS solution adjusted to pH 9.0 with $0.1 \text{ M K}_2\text{CO}_3$, followed by incubation at room temperature with lightly shaking for 2 h. The conjugate was then centrifuged at 4500 g for 5 min. The supernatant was discarded and the soft sediment of immunocomplex was rinsed with PBS for three times, after centrifugation at 4500 g for another 5 min, the conjugate was resuspended in 250 μL PBS containing 1% BSA for immunoassay. The resulting AuNP/PPyMS labeled Ab_2 ($\text{Ab}_2\text{-AuNP/PPyMS}$) could be stored at 4°C for more than 2 weeks without loss of activity

2.5. Preparation of MC-LR Immunosensor

The glassy carbon electrode (GCE) with 3 mm diameter was polished successively with 0.3 and 0.05 μm alumina slurry (Beuhler), followed by rinsing thoroughly with doubly distilled water. After successive sonication in 1:1 nitric acid, ethanol, and deionized water, the electrode was rinsed with deionized water and allowed to dry at room temperature. 5 μL of 1.0 mg mL^{-1} carboxylic group functionalized CNT suspension was dropped on the pretreated GCE and dried in a desiccator. Then, 5 μL of 2.0 mg mL^{-1} PEG solution was dropped onto the CNT modified GCE and the modified GCE was incubated in water-vapor-saturated environment at 4°C overnight to allow the passive adsorption of PEG

onto CNT modified GCE surface. After washing with water, 5 μL of 250 $\mu\text{g}\cdot\text{L}^{-1}$ MC-LR was immediately dropped on the modified GCE surface and incubated sequentially for 1 h to form MC-LR/PEG/CNT/GCE. Subsequently, excess antigen was removed by washing with 0.01 M pH 7.4 PBST. Finally, 0.01 M pH 7.4 PBS containing 5% BSA was dropped on the electrode surface and incubated for 1 h to block possible remaining active sites against nonspecific adsorption. The preparation of the MC-LR immunosensor is shown in [Scheme 1B](#). After thoroughly washing with 0.01M pH 7.4 PBST, the obtained immunosensor was stored at 4 $^{\circ}\text{C}$ prior to use.

2.6. Analytical Procedure

To carry out the immunoreaction and electrochemical measurements, 5 μL MC-LR solutions with different concentrations or water samples were mixed with 5 μL of 1:120 diluted MC-LR antibody to obtain the incubation solution. As shown in [Scheme 1B](#), the incubation solution was dropped on the MC-LR immunosensor and incubated for 1 h at 25 $^{\circ}\text{C}$ and then washed carefully with 0.01 M pH 7.4 PBST to obtain anti-MC-LR/MC-LR/PEG/CNT/GCE. Secondly, 10 μL Ab₂-AuNP/PPyMS was casted on the immunosensor incubated for 1 h at 25 $^{\circ}\text{C}$ to form Ab₂-AuNP/PPyMS /anti-MC-LR/MC-LR/PEG/CNFs/GCE. During the incubation process, the immunosensor was placed in a container to avoid the evaporation of incubation solution. After washing with PBST and water, silver deposition enhancement was performed using a 5 μL mixture of silver enhancer solutions for 4 min at 25 $^{\circ}\text{C}$ in a dark incubator. The immunosensor was finally rinsed with water to perform linear sweep voltammetry (LSV) detection from -0.1 to 0.2 V at 50 mV s^{-1} in 1.0 M KCl solution. The data for condition optimization and the calibration curve were the average of three measurements.

3. Results and discussion

3.1. Morphology characterization of PPyMS and AuNP/PPyMS

The morphologies of PPyMS were characterized by TEM. As shown in Fig. 1A, the as-synthesized PPyMS showed a uniform size of approximately 500 nm. The PPyMS could be used as excellent carriers for loading different signal molecules or tags because of their good monodispersity, large surface area, and accepted conductivity. After using positively charged PDDA as a bridge, negatively charged AuNP could electrostatically attach onto the surface of PPyMS to form a compact nanoparticle layer (Fig. 1B). The AuNP created an enriched aggregation on the PDDA/PPyMS surface. The AuNP/PPyMS hybrids could produce a synergic effect among electrochemical activity, conductivity, and biocompatibility for labeling of biomacromolecules and promoting the amplification of tracing signal.

3.2. Strategy, signal amplification and nonspecific adsorption of the proposed immunosensing

The preparation process of the MC-LR immunosensor and the detection strategy with competitive electrochemical immunoassay are shown in Scheme 1B. After competitive immunoreactions, Ab₂-AuNP/PPyMS can be captured on the immunosensor surface by the formation of immunocomplex. Silver enhancement solution was prepared by mixing solution A (Ag iron) and solution B (hydroquinone) in silver enhancement kit with 1:1 ratio. After the addition of silver enhancer solution onto the electrode surface, the attached AuNP could act as nuclei and catalysts to induce the reduction reaction of silver ion in the present of hydroquinone [31,32]. The quantitatively deposited silver nanoparticle could be easily detected by anodic stripping voltammetric analysis with a sharp stripping peak in the presence of Cl⁻ [33]. The possible reaction and detection principle could be explained by the following equations:

The LSV peak current decreased with the increasing concentration of antigen used in the competitive immunoreaction, and could thus be used for immunoassay. When using the signal antibody Ab₂ for the competitive immunoreaction, the silver deposition led to a stripping voltammetric peak at 0.32 V, and the peak current was 12.4 μ A (curve a, Fig. 2). This response resulted from the chemical reduction of Ag⁺ in the absence of the catalysis, AuNP/PPyMS, thus could be thought as a background signal of this method. After using Ab₂-AuNP to replace Ab₂ for the immunoreactions, the silver deposition produced a higher stripping peak current of 18.2 μ A (curve b, Fig. 2), indicating AuNP could catalyze the silver deposition to produce more silver nanoparticle on the immunosensor surface. With the same conditions Ab₂-AuNP/PPyMS probe produced a response of 40.1 μ A (curve c, Fig. 2), which was about two times higher than that of Ab₂-AuNP, indicating the synergistic silver enhancement of AuNP and PPyMS.

In order to obtain the minimum nonspecific adsorption and good biocompatibility, PEG was selected as a bridge or antigen immobilization because of its known capacity to undergo a variety of interactions with proteins including bovine serum albumin (BSA), which acted to suppress the possible remaining active sites against nonspecific adsorption. The nonspecific adsorption of the proposed MC-LR immunosensor was also studied in our experiment. The control MC-LR immunoassay was prepared with the representation of the preparation and detection procedure only in the absence of MC-LR antibody, and the stripping peak current of the control MC-LR immunoassay was 13.3 μ A (curve d, Fig. 2). The peak current of the

control immunoassay was almost the same with the background signal of the designed method (12.4 μA) (curve a, Fig. 2), indicating the elimination of nonspecific adsorption on the electrode surface by BSA, and much smaller than the signal of proposed immunoassay (40.1 μA) (curve c, Fig. 2). Thus, the proposed immunosensor showed an excellent performance with high sensitivity of LSV signal, good biocompatibility to retain their specific immunorecognition, and a feasible ability against nonspecific adsorption.

3.3. Optimization of Conditions for Electrochemical Detection

The concentration of silver enhancer solution is the important parameter to the analytical performance of the immunosensor. As shown in Fig. 3A, the amount of deposited silver at the immunosensor surface increased with the increasing concentration of silver enhancer solution, however, higher concentration of silver enhancer solution produced higher background current, which was measured in absence of the tracing tag due to the direct reduction of Ag^+ by the reducing reagent in silver enhancer solution. To obtain higher sensitivity, this work used the signal to noise ratio (S/N) to optimize the deposition condition. Upon the successive dilution of silver enhancer solution, the S/N quickly increased and trended to a maximum value at 120-fold dilution (Fig. 3A inset). Therefore, silver enhancer solution with 120-fold dilution was used for silver deposition.

The influence of silver deposition time on the analytical performance of immunoassay was also investigated in our work. With the increasing silver deposition time from 1 min to 5 min, the stripping peak current for the MC-LR immunosensor increased. However, longer silver deposition time also produced higher background current (Fig. 3B). As shown in Fig. 3B inset, the S/N of the MC-LR immunosensor increased continuously as the silver deposition time increasing from 1 min to 4 min, and reached the maximum value at 4 min,

following by a large decrease at the incubation time of 5 min. Thus, 4 min was chosen as the optimal silver deposition time.

3.4. Optimal Conditions for Immunoreaction

Under optimal detection conditions, the electro-oxidize response of the deposited silver nanoparticle depended on the amount of Ab₂-AuNP/PPyMS on the electrode surface, which was decided by the concentration of MC-LR antibody in the incubation solution. In order to obtain the optimal concentration of the MC-LR antibody, the MC-LR/PEG/CNT/GCE was incubated in MC-LR antibody solutions with different concentrations. As shown in Fig. 4A, with the decreasing dilution times or increasing concentration of MC-LR antibody the stripping peak current increased and trended to the plateau value at 120-fold dilution for MC-LR antibody, which indicated all of the specific sites of the immobilized antigen were occupied. Thus, anti-MC-LR solution with 120-fold dilution was chosen as the working antibody solution.

The concentration of Ab₂-AuNP/PPyMS solution had been studied in this work. As shown in Fig. 4B, the stripping peak current of the MC-LR immunoassay increased with the decreasing dilution times or increasing concentration of Ab₂-AuNP/PPyMS solution, and trended to a plateau value at 2-fold dilution. Therefore, Ab₂-AuNP/PPyMS solution with 2-fold dilution was chosen as the working solution.

3.5. Immunoassay performance of the immunosensor to MC-LR

For the measurement of MC-LR, a competitive assay configuration was applied under optimized conditions. A standard solution of MC-LR with a known concentration was added into the incubation solution containing a certain concentration of MC-LR antibody. The MC-LR in the incubation solution competed with the immobilized MC-LR on the

immunosensor surface to bind the limited binding sites of the MC-LR antibody to form the immunocomplex. Subsequently, the immunocomplex reacted with Ab₂-AuNP/PPyMS, and the bound AuNP/PPyMS induced the reduction reaction of silver ion in the presence of Cl⁻. The anodic stripping LSV peak current of deposited silver nanoparticle decreased with the increasing MC-LR concentration in the incubation solution (Fig. 5A). As shown in Fig. 5B, the decrease of LSV peak current was proportional to the logarithm value of MC-LR concentration in the range of 0.00025-50 $\mu\text{g L}^{-1}$ with a correlation coefficient of 0.999. The detection limit was calculated to be 0.1 ng L^{-1} at a signal-to-noise ratio of 3. The performance of the MC-LR immunosensor fabricated in this work was compared with other electrochemical immunosensors (Table 1) and already existing methodologies (Table 2). As shown in Table 1 and Table 2, the detection limit of the proposed immunosensor (sub ng L^{-1}) is much lower than those of immunosensors based on enzyme and other nanomaterial amplification strategies (ng L^{-1}), and also much lower than already existing methodologies and the WHO provisional guideline limit of 1 $\mu\text{g L}^{-1}$ for MC-LR in drinking water. The linear range over 5 orders of magnitude was the widest of all immunosensors, which was also very significant for practical application. According to the analytical process, the detectable linear ranges in the sample with a dilute factor of 2 by adding 5 μL MC-LR antibody were 0.0005-100 $\mu\text{g L}^{-1}$ with the detection limit of 0.2 ng L^{-1} . Thus the proposed method was enough for practical application.

3.6. Precision, reproducibility, stability and specificity of MC-LR Immunosensor

The intra-assay variation coefficients for five replicative measurements with this method at MC-LR concentrations of 0.5 and 5 $\mu\text{g L}^{-1}$ were 2.6% and 0.9%, respectively, showing a good precision. While the inter-assay variation coefficients at these concentrations on five immunosensors made independently were 1.1% and 1.0%, respectively, indicating excellent

fabrication reproducibility. When the immunosensor was not in use, it was stored at 4 °C. 96.3% of the initial response of the immunosensor for MC-LR was remained after one week and 85.4% of the initial response was remained after four weeks. These results indicated the immunosensor had acceptable stability.

To evaluate the specificity of the electrochemical MC-LR immunosensor, the LSV peak currents of the immunosensor in the presence of 50 $\mu\text{g L}^{-1}$ MC-LR, its isomer microcystin-RR (MC-RR) and their mixture (MC) were recorded, respectively. Compared with the signal produced by control assay, LSV peak current induced by the specific binding of the immunosensor probes with MC-RR did not show any obvious decrease, while a significant decrease of LSV peak current induced by the specific binding of the immunosensor probes with MC-LR or MC mixture were observed (Fig. 6). Moreover, there were no apparent differences in LSV peak current for the mixture (MC), even though the same concentration level of the MC-RR coexisted in the detection. Additionally, when the interferences of cations, anions and other compounds in natural water samples, such as MgSO_4 , NaNO_3 and KCl , were assessed by the proposed MC-LR immunosensor. As shown in Fig. 6, the compounds had no influence on the response signals of MC-LR immunosensor. These results demonstrated the high selectivity of the developed immunosensing strategy.

3.7. Analysis of polluted water samples

To evaluate the analytical reliability and application potential of the proposed method, 5 μL of water samples without any need of sample pretreatment appropriate dilution except for an appropriate dilution was mixed with 5 μL MC-LR antibody, and the mixture was dropped on the MC-LR immunosensor, subsequently, 10 μL $\text{Ab}_2\text{-AuNP/PPyMS}$ solution was casted on the immunosensor. The MC-LR concentrations of two polluted water samples determined with this method were $0.530 \pm 0.005 \mu\text{g L}^{-1}$ and $6.8 \pm 0.6 \text{ ng L}^{-1}$. Recovery testing was

carried out to demonstrate the validity of the proposed method, after 5-fold concentrations of MC-LR added to two polluted water samples, and the obtained recoveries of MC-LR were $105.7 \pm 1.5\%$ and $94.1 \pm 2.1\%$, respectively (five measurements), which indicated acceptable accuracy. Thus, the present method could satisfy the need for immunoassay of MC-LR in water samples.

4. Conclusion

A novel ultrasensitive electrochemical MC-LR immunosensor was developed with AuNP/PPyMS-catalyzed silver deposition as a signal amplification and CNT as an immunosensor platform. The MC-LR immunosensor was constructed with a competitive immunoassay format, and the MC-LR immunoreaction monitoring was achieved by electrochemical stripping signal of the deposited silver nanoparticle catalyzed by AuNP/PPyMS at CNT modified GCE. Combined with PPyMS carried AuNP as tracing tag to label signal antibody and AuNP catalyzed silver deposition for anodic stripping analysis, and CNT accelerated the electron transfer of immunosensor surface, the dual amplification led to an extremely high sensitivity with a low detection limit (sub ng L^{-1}) for MC-LR immunosensor. The proposed immunosensor had a wide linear range spanned over 5 orders of magnitude, acceptable precision and fabrication reproducibility, and excellent stability. It had been successfully applied in the detection of MC-LR in polluted water samples. The designed immunoassay can be easily applied to other harmful toxins in water pollution, and has a great potential application in environmental monitoring.

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FIGURE CAPTION

Scheme 1. Schematic representation of (A) the preparation of tracing tag and labeled Ab₂, and (B) MC-LR immunosensor fabrication and competitive immunoassay procedure.

Fig. 1. TEM images of PPyMS (A) and AuNP/PPyMS (B).

Fig. 2. Linear sweep stripping voltammetric curves of the immunosensor in 1.0 M KCl after incubating with MC-LR antibody and Ab₂ (a), Ab₂-AuNP (b), Ab₂-AuNP/PPyMS (c), and the linear sweep stripping voltammetric curve of the immunosensor in 1.0 M KCl after incubating with Ab₂-AuNP/PPyMS in the absence of MC-LR antibody (d).

Fig. 3. Effects of different dilution times of silver enhancer solutions (A) and silver deposition time (B).

Fig. 4. Effects of the concentrations of MC-LR antibody (A) and Ab₂-AuNP/PPyMS (B) on the stripping current peak of the immunosensor in 1.0 M KCl.

Fig. 5. Linear sweep stripping voltammetric curves of silver nanoparticle deposited at the immunosensor in 1.0 M KCl after incubated with 0.00025, 0.0005, 0.0025, 0.025, 0.25, 2.5, 25 and 50 $\mu\text{g L}^{-1}$ MC-LR (from highest to lowest peak currents) (A) and calibration curve for MC-LR immunoassay (B).

Fig. 6. Comparison of LSV peak currents of the proposed MC-LR immunosensor after incubation with MC-LR antibody and none (a), 50 $\mu\text{g L}^{-1}$ MC-RR (b), mixture of 500 $\mu\text{g L}^{-1}$ MgSO₄, NaNO₃ and KCl (c), mixture of 50 $\mu\text{g L}^{-1}$ MC-RR and 500 $\mu\text{g L}^{-1}$ MgSO₄, NaNO₃, KCl (d), 50 $\mu\text{g L}^{-1}$ MC-LR (e), mixture of 50 $\mu\text{g L}^{-1}$ MC-LR and 50 $\mu\text{g L}^{-1}$ MC-RR (f), mixture of 50 $\mu\text{g L}^{-1}$ MC-LR and 500 $\mu\text{g L}^{-1}$ MgSO₄, NaNO₃, KCl (g), mixture of 50 $\mu\text{g L}^{-1}$ MC-LR, 50 $\mu\text{g L}^{-1}$ MC-RR and 500 $\mu\text{g L}^{-1}$ MgSO₄, NaNO₃, KCl (h).

Graphical Abstract:

Ultrasensitive electrochemical microcystin-LR immunosensor using gold nanoparticle functional polypyrrole microsphere catalyzed silver deposition for signal amplification

By Jing Zhang, Zhanbing Xiong, Zhidong Chen

We designed an ultrasensitive electrochemical microcystin-LR immunosensor using gold nanoparticle functional polypyrrole microsphere catalyzed silver deposition for signal amplification, and the proposed immunosensor with a detection limit down to sub ng L⁻¹ level was successfully detect microcystin-LR in polluted water samples.