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Detection of trace microcystin-LR on a 20 MHz QCM sensor coated with in situ self-assembled MIPs

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ABSTRACT

A 20 MHz quartz crystal microbalance (QCM) sensor coated with in situ self-assembled molecularly imprinted polymers (MIPs) was presented for the detection of trace microcystin-LR (MC-LR) in drinking water. The sensor performance obtained using the in situ self-assembled MIPs was compared with traditionally synthesized MIPs on 20 MHz and normal 10 MHz QCM chip. The results show that the response increases by more than 60% when using the in situ self-assembly method compared using the traditionally method while the 20 MHz QCM chip provides four-fold higher response than the 10 MHz one. Therefore, the in situ self-assembled MIPs coated on a high frequency QCM chip was used in the sensor performance test to detect MC-LR in tap water. It showed a limit of detection (LOD) of 0.04 nM which is lower than the safety guideline level (1 nM MC-LR) of drinking water in China. The low sensor response to other analogs indicated the high specificity of the sensor to MC-LR. The sensor showed high stability and low signal variation less than 2.58% after regeneration. The lake water sample analysis shows the sensor is possible for practical use. The combination of the higher frequency QCM with the in situ self-assembled MIPs provides a good candidate for the detection of other small molecules.

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1. Introduction

Water bloom is a phenomenon wherein various kinds of alga proliferate rapidly as a result of water eutrophication. As water bloom occurs, some alga produce harmful cyclic peptide toxins such as microcystins [1,2]. The chemical and physical properties of microcystins are studied by many researchers [3]. Drinking water contains small amounts of microcystins that could cause harm to human health [4]. More than 50 different kinds of microcystins have been isolated. Among the different kinds of microcystins, microcystin-LR (MC-LR) is the most harmful and widespread one [5].

Currently, the qualitative and quantitative analyses of microcystins are mainly based on high-performance liquid chromatography (HPLC) [6] and thin layer chromatography (TLC) [7]. Immunological methods, such as enzyme-linked immunosorbent

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http://dx.doi.org/10.1016/j.talanta.2014.07.071 0039-9140/© 2014 Elsevier B.V. All rights reserved. assay (ELISA) [8] and protein phosphatase inhibition assay (PPIA) [9], are also used in the detection of microcystins. They are highly specific, simple and suitable for large number of samples. However, these technologies are time-consuming and rely on bulky instruments that require well-trained operators and costly chemical procedures. Furthermore, the widespread use of plastic additives can interfere the HPLC results[10]. Therefore, more stable, easy-to-use, rapid and specific methods for microcystins detection have been developed [11].

A biosensor typically relies on an active recognition element and a transducer [12]. Of most transducers used in biosensors, the quartz crystal microbalance (QCM) is a simple, inexpensive, portable and sensitive gravimetric sensor [13]. Due to its high sensitivity down to mass change of nanogram level, it has been widely used for detection of drugs [14], nucleic acids [15], peptides [16] and so on [17].

In most studies, AT-cut 5 MHz or 10 MHz crystal chips are often used [14,18]. Based on the Sauerbrey equation [19], a higher resonant frequency of the QCM increases the sensitivity of the sensor. However, studies on the use of QCM with frequency higher







than 10 MHz for sensors are scarce [20]. The lack of studies on the use of QCM with frequency higher than 10 MHz is attributed to the requirement of extremely fragile thin quartz chips. In this study, the sensor performance of the sensor with the AT-cut 20 MHz quartz crystals is compared with that with the normal 10 MHz ones.

In addition, the recognition element of the sensor is a key factor for the specificity of the sensors. In the past few decades, immunoassay techniques based on antibody have been widely used in the active sensing unit because of its high specificity and sensitivity for target molecules [21,22]. However, poor stability, high cost and time-consuming production still pose problems for the use of immunoassay techniques. Therefore, the development of a synthetic alternative that can mimic the recognition elements of target molecules with high stability and competitive specificity are needed [23].

Molecular imprinting is an attractive technique for the development of artificial receptors [24,25]. It is a promising technique for the preparation of polymers with pre-designed recognition sites which have the right shape and functionality to specifically capture template molecules [26,27]. To achieve this, the template, functional monomer, cross-linker and initiator are copolymerized into three-dimensional cross-linked polymers followed by removal of the template molecules to form the recognition cavities of the templates. Molecularly imprinted polymers (MIPs) provides comparable affinity and specificity to the recognition of target molecules, thus MIPs has been widely applied for the development of biosensors [28–30] and other areas [31,32].

So far, two major approaches have been utilized to integrate MIPs with transducers. One of the common methods includes the immobilization of pre-synthesized MIPs particles on a transducer by physical entrapment [14,18], which forms rather a thick film thus reduces the sensitivity of the biosensor. The in situ self-assembly method, however, is more favorable in the development of the sensor surface [33]. The in situ self-assembly method could obtain an extremely thin film with controllable thickness and good homogeneity. In addition, this method performed better sensitivity than physical entrapment [34]. To the best of our knowledge, no research has been reported on the development of MIPs based on the in situ self-assembly method for highly sensitive detection of microcystins.

The detection of MC-LR using normal AT-cut 10 MHz crystal and physical entrapment method was previously reported [18, 35].

In Chianella's work, the author synthesized MIPs using the traditional physical entrapment method. The MIPs were used as a material for solid-phase extraction (SPE) and as a sensing element used to coat the QCM sensor. With the help of SPE, which provides up to 1000-fold pre-concentration, the limit of detection (LOD) of his work was 0.35 nM. Without the use of MIPs-SPE, the sensor may not achieve the detection limit for MC-LR of 1 nM indicated in China's drinking water safety guideline. With this step added, the measurement of MC-LR was still time-consuming and complex. In reality, there is an urgent need for sensor to detect MC-LR of 1 nM directly without any pre-operation.

The higher frequency QCM coated with in situ self-assembled MIPs has the potential to detect MC-LR of 1 nM directly without any pre-operation. However, there is no report on the combination of high frequency QCM and in situ self-assembled MIPs in one biosensor. In this study, a 20 MHz QCM sensor coated with in situ self-assembled MIPs was firstly studied for the detection of MC-LR in water. The sensitivity of the sensor system are investigated and compared with other reported results. The control experiments on the investigation of the sensor response to analogs indicate a good specificity and reproducibility of the sensor system.

2. Material and methods

2.1. Reagents

MC-LR, microcystin-RR (MC-RR), microcystin-YR (MC-YR) and nodularin were purchased from Taiwan Algal Science Inc. (Taiwan, China). Methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA) and 11-mercaptoundecanoic acid (MUA) were purchased from Sigma-Aldrich (Schnelldorf, Germany). 2,2'-Azobisiobutyronitrile (AIBN) and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). All other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China).

2.2. QCM system

The QCM system (Fig. 1) was composed of a homemade detection cell, a network analyzer (E5061B, Agilent, USA) and a PC. AT-cut quartz crystals (12.5 mm diameter, Chenjing Electronic Co. Ltd., Beijing, China) resonating at 10 MHz with Au electrodes

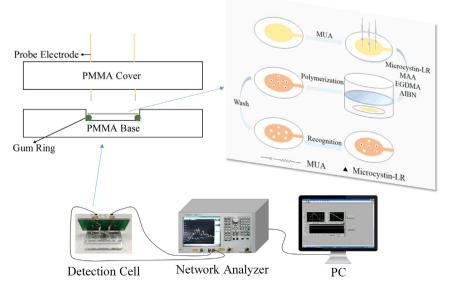


Fig. 1. The MC-LR detection system and the synthesis progress of MIPs on the chip.

(6 mm diameter) on each side and AT-cut quartz crystals (6.5 mm diameter, Chenjing Electronic Co. Ltd., Beijing, China) resonating at 20 MHz with Au electrodes (3.5 mm diameter) on each side were used. The Sauerbrey equation was established for the AT-cut shear mode QCM [19], which relates the mass change per unit area at the crystal surface to the observed change in oscillation frequency of the crystal

$$\Delta f = -2.26 \times 10^{-6} f^2 \Delta m/A \tag{1}$$

where Δf is the frequency change in Hz; f is the resonant frequency of the crystal in Hz; Δm is the mass change on the surface of the crystal in g; and A is the piezoelectric active crystal area in cm². For the 10 MHz and 20 MHz quartz crystals used, Eq. (1) shows that a mass increase of 1 ng/cm on the electrode resulted in a frequency change of 0.226 Hz and 0.904 Hz respectively.

2.3. MIPs film synthesis

For the preparation of pre-polymerization solution, 1 mg of MC-LR and 0.86 mg of functional monomer MAA were dissolved in 500 μ L of DMSO and kept at room temperature for 3 h. Afterward, 1.98 mg of cross-linker EGDMA and 0.2 mg of AIBN as initiator were added into the solution.

Prior to use the chip was cleaned in the Oxygen Plasma Cleaner for 3 min, followed by rinsing with absolute ethanol and deionized water sequentially. The chip was then dried using nitrogen. The freshly cleaned chip was dipped into 10 mL of 50 mM MUAethanol solution, and kept at room temperature for 12 h. The chip was washed with absolute ethanol and deionized water, dried by nitrogen, and a self-assembled monolayer (SAM) was formed on the chip. The chip was then dipped into 10 mL of 200 mM AIBNethanol solution, and kept at room temperature for 3 h and dried by nitrogen; the chip was finally dipped into the prepolymerization solution. The pre-polymerization solution was purged with nitrogen for 10 min, and the container was covered. Polymerization was carried out at 60 °C for 15 h in a hot-air oven. After the polymerization process, the chip was rinsed by an ethanol-acetic acid solution (9/1, v/v) to remove the template and other possible residual chemicals. Finally, the chip was washed by deionized water and dried by nitrogen. In addition, a non-imprinted polymers (NIPs) film was prepared without the presence of template molecules to be used as a control sample for the investigation on sensor specificity.

2.4. QCM measurement

First, the MIPs film coated chip was placed into the homemade detection cell and stabilized for several minutes. This step was

repeated three times and the average frequency was recorded. Secondly, the chip was taken out and dipped into different concentrations of standard solution of MC-LR. The chip was subsequently washed with deionized water and dried by nitrogen. Finally, the chip was returned into the cell and stabilized for several minutes. This step was also repeated three times and the average frequency was recorded. The frequency change for each solution was calculated and recorded. After each measurement, the chip was rinsed with an ethanol–acetic acid solution (9/1, v/v), followed by deionized water, and then dried using nitrogen.

Detections were taken in air and detection conditions were maintained constantly throughout the experiment.

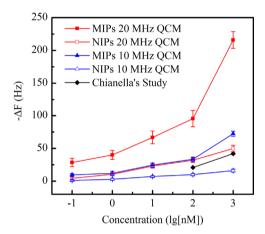


Fig. 3. Sensor responses of MIPs and NIP film coated chips at resonant frequency of 10 MHz and 20 MHz, respectively, for the detection of different concentration of MC-LR (n=3), in comparison with Chianella's study [18].

Table 1

Sensor response of MIPs film coated sensor chip (20 MHz) to different concentrations of MC-LR.

Concentration (nM)	Signal (Hz)	Noise (Hz)	S/N	S/3N
0.1	28.61 40.15	6.88 6.90	4.16 5.82	1.39 1.94
10	66.85 95.63	9.70	6.89 9.17	2.30
100 1000	215.88	10.43 17.75	12.16	3.06 4.05

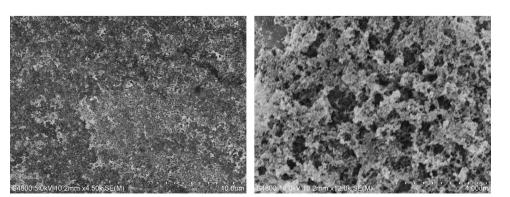


Fig. 2. SEM image of the MIPs film on the chip with a 10 μ m scale (left) and a 4 μ m scale (right).

2.5. Sample preparation

Tap water was collected from the Gaoxin District, Suzhou, China. The water samples were analyzed by HPLC (Agilent

1260LC), and the concentration of the original MC-LR was less than 0.01 nM. The tap water was first filtered using a 0.22 μ m filter. 10 mL of tap water was placed into a 20 mL tube, and then spiked with different concentrations of MC-LR and other analogs.

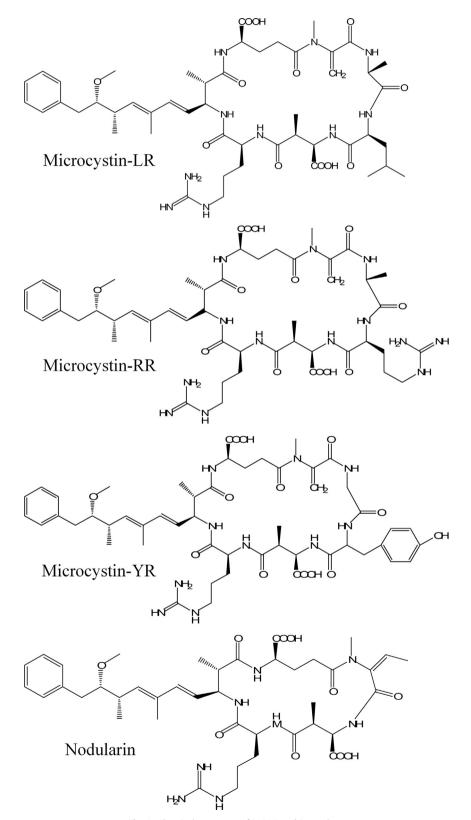


Fig. 4. Chemical structures of MC-LR and its analogs.

3. Results and discussion

3.1. QCM sensor

A layer with porous structure was observed after forming the MIPs on the sensor chip, as indicated in the SEM imaging (Fig. 2). After the deposition of MIPs, the 10 MHz chip had a resonant frequency drop of 967 Hz. According to the Sauerbrey equation, the mass per unit area of the MIPs was 4.3 μ g cm⁻². Assuming that the density of the film is 1.5 g cm⁻³, the thickness of the MIPs film was estimated to be approximately 29 nm.

The responses of the MIPs and NIPs film coated sensor chips at resonant frequency of 10 MHz and 20 MHz, respectively, in the sample solution with different concentrations of MC-LR from 0.1 to 1000 nM were showed in Fig. 3. We compared the results of our study with other work in which the QCM sensor chip was modified with MIPs by physical entrapment [18]. With the same QCM working frequency (10 MHz), our sensor coated with the in situ self-assembled MIPs shows about 60% and 70% higher response at a concentration of 100 and 1000 nM of MC-LR, respectively.

In the traditional physical entrapment method used by Chianella, polymer was first synthesized and then ground into particles. The particles were then added into polyvinyl chloride polymer (PVC)-tetrahydrofuran solution and spread onto QCM chips. After the complete evaporation of solvent, the polymer film was immobilized on the QCM chips. The grind step is complex and inevitably destroys some recognition sites. The size of the obtained particles is highly dispersed. Additionally, some recognition sites embedded in the particles are inaccessible. Using the physical entrapment method, the film is thick and inhomogenous because of the highly dispersed particles. The thickness of the film is more than hundreds of nanometers. And PVC may block some recognition sites. The thick film and the limited availability of the recognition sites reduced the efficiency of the MIPs film [36]. Using the in situ self-assembly method, we synthesized the MIPs film on the chip directly. This can avoid some disadvantage encountered when using the physical entrapment method. And the synthesized film is ultrathin [37]. The thickness of our film was about 29 nm, which is thinner than the film synthesized using the physical entrapment method. Given that the thickness is one of the most important factors that influence the sensitivity of the MIPs film coated sensor [34], the response of our sensor was much better.

Based on Eq. (1), the sensor with 20 MHz should have a quadruple signal compared with the sensor with 10 MHz. At concentrations ranging from 0.1 nM to 1000 nM MC-LR, the sensor signal on 20 MHz QCM was three to four times higher than the sensor with 10 MHz.

The MIPs coated sensor working at the resonant frequency of 20 MHz showed a high response of 40.1 Hz frequency shift (S/N=5.8), at a concentration of 1 nM. To determine the LOD of the sensor, we linearly fitted the signal to triple noise ratio (S/3N) with the concentration of MC-LR (Table 1)

$$S/3N = 5.773 + 0.6453 \, \lg(c)$$
 (2)

where c is the concentration of MC-LR in nM. The LOD of 0.04 nM was deduced as the concentration of MC-LR at which the signal response is three times of the noise.

3.2. Specificity of the sensor

To investigate the specificity of the sensor, analogs of MC-LR such as MC-RR and MC-YR and a potential interferent such as nodularin (Fig. 4 shows the structure of them) were used to evaluate the specificity of the MIPs film.

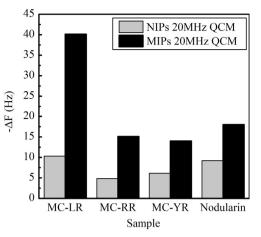


Fig. 5. Sensor response of MIPs and NIPs coated sensors chip (20 MHz) to MC-LR and its analogs at a concentration of 1 nM.

Table 2

Sensor response of MIPs film coated sensor chip (20 MHz) to standard samples and real samples of MC-LR.

Samples	Signal (Hz)			
	1 nM	10 nM	100 nM	
Standard sample ^a	44.08	63.21	101.75	
Lake water ^b	40.12	69.56	110.35	
50% Diluted lake water ^c	46.08	70.97	100.89	

Signal is reported as mean of three experimental results.

 $^{\rm a}$ MC-LR standard sample with different concentrations (1 nM, 10 nM and 100 nM).

 $^{\rm b}$ Lake water sample spiked with MC-LR to form final concentrations of 1 nM, 10 nM and 100 nM.

 $^{\rm c}$ Lake water sample was diluted 50%, with deionized water and then spiked with MC-LR to form final concentrations of 1 nM, 10 nM and 100 nM.

Results shown in Fig. 5 indicate that the sensor was not sensitive to these analogs at a concentration of 1 nM. In contrast to the MIPs film, the NIP film showed similar response to all the compounds indicating non-specific response. All the results strongly demonstrated that the sensor in our study had high selectivity for MC-LR.

3.3. Reproducibility of the sensor

To evaluate the reproducibility of the MIPs film, the sensor chip was incubated with the same concentration of MC-LR solution for several times under the same conditions to record the sensor responses. After each measurement, the chip was regenerated by an ethanol–acetic acid solution (9/1, v/v) and deionized water sequentially, and then dried using nitrogen. After the regeneration, the signal variation was less than 2.58%. Using SAM between the electrode and the film, the film was grafted to the electrode by covalent bonding and did not fall off easily. Therefore, the sensor had good reproducibility.

3.4. Lake water sample analysis

To show the possibility of the MIP–QCM detection for practical use, we analyzed environment water spiked with MC-LR. The real sample was collected from the Taihu Lake near the Suzhou National New & Hi-tech Industrial Development Zone, China. There was no obvious water bloom in this area and the water from this area of the lake was clear. The sample was filtered using a 0.22 µm filter. By using HPLC, the concentration of MC-LR in this

sample was estimated to be 0.49 nM. 10 mL of sample was placed into a 20 mL tube, and then spiked with MC-LR to form final concentrations of 1, 10 and 100 nM, respectively. All the samples were analyzed with our 20 MHz QCM–MIP sensor system. Results in Table 2 showed that there was no significant variation between the standard sample and real sample responses. This indicated that our sensor was possible for practical use.

4. Conclusion

In combination of a higher frequency QCM and the in situ selfassembled MIPs, a MC-LR biosensor has been developed with a low LOD of 0.04 nM. This result demonstrates the feasibility of direct MC-LR detection in water. With the quartz crystals working at the same resonant frequency of 10 MHz, the in situ selfassembled MIPs showed 60% higher response than the traditional physical entrapped MIPs. The sensor with 20 MHz chip shows three to four times higher response than the 10 MHz sensor chip, which is comparable with the theoretical results. The low sensor responses to the analogous indicate good specificity to MC-LR. The sensor is stable, and the signal variation is less than 2.58% after regeneration. The lake water sample analysis shows the possibility of the sensor for practical use. The combination of the high frequency QCM with in situ self-assembled MIPs provides a good candidate for the detection of other molecules.

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