

Sensitive Electrochemical Detection of Microcystin-LR in Water Samples Via Target-Induced Displacement of Aptamer Associated $[\text{Ru}(\text{NH}_3)_6]^{3+}$

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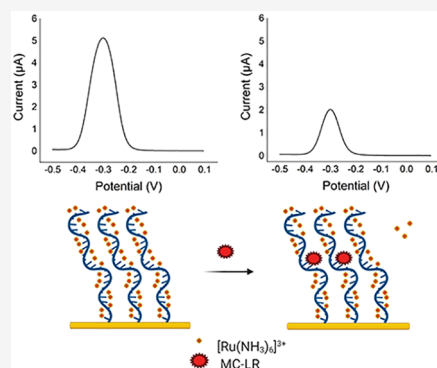


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ABSTRACT: In this study, we demonstrate the successful development of an electrochemical aptamer-based sensor for point-of-use detection and quantification of the highly potent microcystin-LR (MC-LR) in water. The sensor uses hexaammineruthenium(III) chloride ($[\text{Ru}(\text{NH}_3)_6]^{3+}$) as redox mediator, because of the ability of the positively charged (3+) molecule to associate with the phosphate backbone of the nucleic acids. We quantitatively measure the target-induced displacement of aptamer associated, or surface confined, $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in the presence of MC-LR. Upon the addition of MC-LR in the water, surface-confined $[\text{Ru}(\text{NH}_3)_6]^{3+}$ dissociates, resulting in less faradaic current from the reduction of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ to $[\text{Ru}(\text{NH}_3)_6]^{2+}$. Sensing surfaces of highly packed immobilized aptamers were capable of recording decreasing square wave voltammetry (SWV) signals after the addition of MC-LR in buffer. As a result, SWV recorded substantial signal suppression within 15 min of target incubation. The sensor showed a calculated limit of detection (LOD) of 9.2 pM in buffer. The effects of interferences were minimal, except when high concentrations of natural organic matter (NOM) were present. Also, the sensor performed well in drinking water samples. These results indicate a sensor with potential for fast and specific quantitative determination of MC-LR in drinking water samples. A common challenge when developing electrochemical, aptamer-based sensors is the need to optimize the nucleic acid aptamer in order to achieve sensitive signaling. This is particularly important when an aptamer experiences only a small or localized conformational change that provides only a limited electrochemical signal change. This study suggests a strategy to overcome that challenge through the use of a nucleic acid-associated redox label.



KEYWORDS: microcystin-LR, aptamer, sensor, cyanotoxins, electrochemical biosensor

INTRODUCTION

Microcystins are a group of cyclic heptapeptide hepatotoxins produced by several genera of cyanobacteria. They are common in sources of drinking water worldwide posing a significant threat to human and animal health.^{1–3} Their primary mechanism of toxicity is the inhibition of serine/threonine phosphatases, which is crucial in the regulation and control of cellular processes. Although the liver is believed to be the main target organ, other effects have been observed in other organs and tissues.^{4,5} The toxicity of microcystins may lead to major health effects, which include gastroenteritis, liver damage, and tumor promotion.⁶

Microcystin-LR (MC-LR) is the most common, well studied, and toxic variant of the microcystins.⁷ MC-LR includes the variable amino acid leucine (L) and arginine (R) in positions 2 and 4 of the toxin. In most studies, MC-LR is considered the standard variant of microcystin; the levels and activities are expressed as MC-LR equivalents. Human exposure to MC-LR occurs mainly through drinking water

consumption.⁸ Microcystins are not easily removed from the water using conventional treatment processes. For example, Lake Erie experiences recurring problems with harmful blooms of cyanobacteria (Cyano-HABs). In 2014, in the city of Toledo, Ohio, microcystin cyanotoxin was detected in finished drinking water in a drinking water treatment system that obtains its water from Lake Erie; a “do not drink/do not boil” order was announced affecting about 500,000 customers.⁹ The detection of microcystin in the city of Toledo, Ohio, finished water revealed the susceptibilities of drinking water treatment plants, leading to the implementation of steps to better manage Cyano-HABs.

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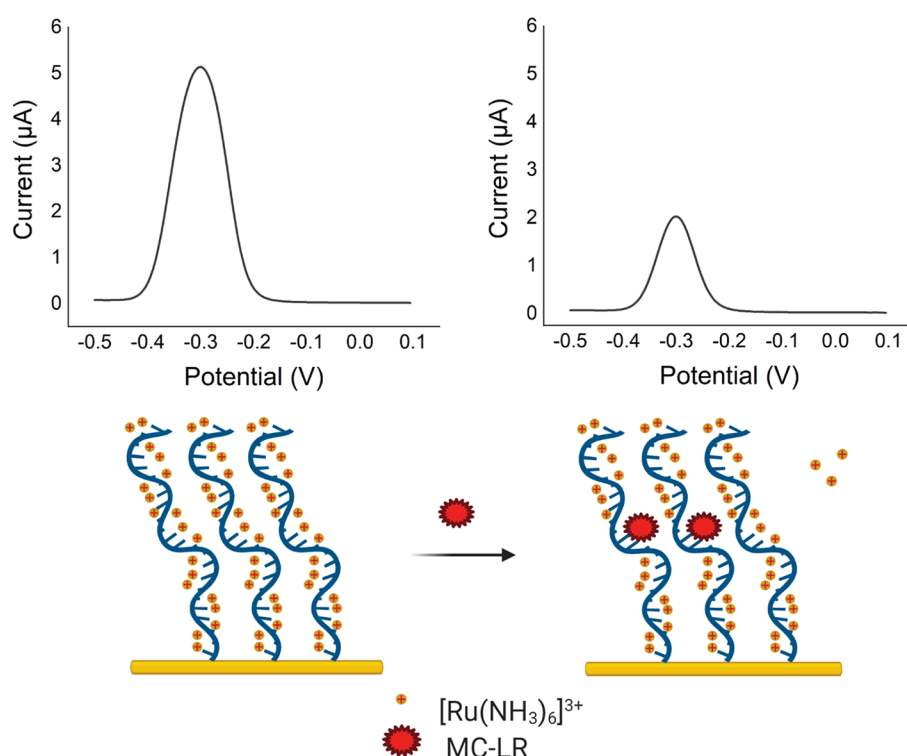
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Scheme 1. Working Principle of the Aptamer-Based Sensor via Target-Induced Displacement of Surface-Confining $[\text{Ru}(\text{NH}_3)_6]^{3+}$ ^a



^aIncubation of the sensor in solution with higher target toxin concentrations gives a lower signal response caused by the toxin replacing $[\text{Ru}(\text{NH}_3)_6]^{3+}$ on the binding sites of MC-LR on the aptamer. Created with [BioRender.com](https://www.biorender.com).

Several methods have been developed to detect microcystins in various water matrices. Reliable analytical techniques include chromatographic methods (e.g., LC/MS/MS)^{10–12} and enzyme-linked immunosorbent assay (ELISA).^{13,14} LC/MS/MS detects and quantifies precisely very low microcystin levels, and it can differentiate between microcystin congeners. However, the method is limited by a lack of standards and requires highly technical skills. ELISA is a routine methodology to analyze water samples for microcystins. ELISA is inexpensive and easy to perform in the field or laboratory and does not require highly skilled personnel. Both techniques detect various cyanotoxins in water samples; however, the main drawbacks are the lack of instrument portability and the inability for real-time detection of MC-LR in real water samples.¹⁵ There is an urgent need for the development of a sensor that is portable, sensitive, and capable for point-of-use detection of microcystins. Such sensors would alert the presence of cyanotoxins in water in real-time, which is important considering that the prevention of toxins from entering a drinking water treatment plant or appropriate adjustment of chemical treatment are critical to provide safe drinking water.

Biosensors are powerful analytical tools, known for their ability to detect various targets in environmental and medical applications. Specifically, electrochemical sensors with aptamer-based recognition systems have gained popularity due to their specificity, sensitivity, quick response, low cost, and portability compared to conventional methods.^{15–17} Aptamers are short fragments of nucleic acid (DNA or RNA) selected and amplified by a process called Systematic Evolution of Ligands by Exponential Enrichment (SELEX) developed in

1990.^{18,19} Target analyte is bound to randomly generated single-stranded sequences (about 20–80 nucleotides in length) with constant 5' and 3' ends that serve as primers. Bound sequences are amplified by polymerase chain reaction for DNA sequences or reverse transcription polymerase chain reaction for RNA sequences.²⁰ The single-stranded nucleic acid molecules fold around the target analyte yielding a three-dimensional stable structure, offering an excellent affinity bioreceptor for biosensing.^{15,19}

Recent research showed promising results using electrochemical aptamer-based sensors as a tool for the detection of MC-LR.^{15,21} Electrochemical biosensors using glassy carbon-modified electrodes modified with cobalt(II) metallodendrimer and Ag nanoparticles performed well with a detection limit of 40 pM for the detection of MC-LR.²² Lately, an indirect detection of MC-LR using nanostructured electrode support (AuNP molybdenum disulfide covered TiO_2 nanobeads) considerably increased the surface area of the electrode. Additionally, the incorporation of a complementary strand amplified the signal and detected MC-LR with a limit of detection (LOD) of 2 pM.²³ Moreover, another innovative approach was the hybridization of complementary strands that formed an infinity-shaped DNA structure using terminal deoxynucleotidyl transferase with a detection limit of 15 pM.²⁴ These studies demonstrated sensitive detection for MC-LR; however, the complexity of the sensing surfaces may cause challenges for the development of highly reproducible and applicable sensors. In this regard, label-free and direct detection methodologies may be advantageous in providing a controlled electrode surface area with a stable packing density of aptamer. A simple sensing approach has been shown by Lin

et al.²⁵ using an aptamer-based impedimetric biosensor with a LOD of 18 pM for MC-LR in water. Eissa et al.²⁶ used graphene printed electrodes to develop a label-free sensor for the detection of MC-LR. The aptamers were initially adsorbed onto the graphene surface, and the presence of MC-LR could cause the aptamers to dissociate, resulting in a lower signal. They reported an excellent LOD of 1.9 pM (calculated by $3\sigma/\text{slope}$) in binding buffer. However, the adsorption of aptamer on the graphene raises a question on interassay reproducibility of these sensors. Ng et al.²⁷ selected several congener-specific microcystin aptamers through SELEX; three sequences demonstrated high affinity to microcystin congeners MC-LR, MC-YR, and MC-LA.²⁷ Specifically, one aptamer sequence indicated the highest specificity to MC-LR. The latter aptamer provided a 40% signal change in square wave voltammetry (SWV) response upon MC-LR incubation, and the LOD approached the low concentration of 11.8 pM in the presence of 5 μM hexaammineruthenium(III) chloride ($[\text{Ru}(\text{NH}_3)_6]^{3+}$) in electrolyte solution.²⁷ This approach appears promising for the facile development of such sensors and sensitive detection of MC-LR.

$[\text{Ru}(\text{NH}_3)_6]^{3+}$ is an especially effective redox mediator in nucleic acid-based sensing because of the ability of the redox active cation to associate with the phosphate backbone of the nucleic acids. Interrogation of aptamer-modified surfaces with adsorbed electroactive $[\text{Ru}(\text{NH}_3)_6]^{3+}$ using chronocoulometric²⁸ or cyclic voltammetric²⁹ techniques is a routine laboratory procedure to estimate probe packing density of ssDNA (aptamer)-modified surfaces.³⁰ The fundamental principle is that one molecule of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ interacts electrostatically with three phosphate residues on the negatively charged DNA backbone (Scheme 1). Several studies indicated the usefulness of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ for DNA applications and particularly the detection of hybridization.^{31–33} Remarkably, Cheng et al.³⁴ utilized the $[\text{Ru}(\text{NH}_3)_6]^{3+}$ electrostatic principle and developed an aptamer-based biosensor for the detection of lysozyme. They demonstrated the quantitative dependence of decreasing charge (in coulombs) on increasing concentration of lysozyme in solution.³⁴

This study proposes an innovative methodology for the detection of targets by transforming a non- or low-conformational change aptamer (low signal change) to a signaling aptamer (readable signal change). In an effort to detect microcystin-LR, we explored hexaammineruthenium(III) chloride ($[\text{Ru}(\text{NH}_3)_6]^{3+}$) as a redox mediator coupled with aptamer-modified electrodes. The negatively charged backbone of the ssDNA probes is known to electrostatically attract ruthenium(III) hexamine ($[\text{Ru}(\text{NH}_3)_6]^{3+}$) ions available in solution. The aim of this work is to develop a sensor with low limits of detection for MC-LR in water using $[\text{Ru}(\text{NH}_3)_6]^{3+}$ as the redox mediator. The key principle of our approach is the easy distinction of signal responses between surface-adsorbed $[\text{Ru}(\text{NH}_3)_6]^{3+}$ species and diffusion-limited $[\text{Ru}(\text{NH}_3)_6]^{3+}$ species. In higher concentrations of toxin in the solution, the interaction between aptamer and toxin forces $[\text{Ru}(\text{NH}_3)_6]^{3+}$ molecules to unwind and this event lowers signal response. This is due to toxin molecules binding to more ssDNA probes with the subsequent loss of surface confined $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in a redox-free buffer (Scheme 1). The hypothesis is that, when toxin molecules bind to the specific aptamer, less surface-confined $[\text{Ru}(\text{NH}_3)_6]^{3+}$ molecules will remain bound to the negatively charged backbone, and this phenomenon will lead to a signal suppression in SWV. SWV provides attractive

aspects, such as the achievement of low limits of detection and the application of rapid scan rates.

MATERIALS AND METHODS

All chemicals and solvents used in this study were analytical grade or better. All solutions used autoclaved ultrapure water using a Biopak Polisher (18.2 $\text{M}\Omega\text{-cm}$, Millipore, Billerica, MA). Initially, experiments with electrochemical impedance spectroscopy (EIS) (using ferricyanide) and SWV (methylene blue as redox mediator) were conducted in binding buffer of 50 mM Tris, 150 mM NaCl, and 5 mM MgCl_2 from Sigma-Aldrich. The remaining experiments were in a buffer of 10 mM Tris-base, pH 7.4 (Sigma-Aldrich). Microcystin-LR analytical standards were obtained from Enzo Biochem, Inc. (Farmingdale, NY, USA). The nucleic acids with and without methylene blue dye were synthesized by Biosearch Technologies (CA, USA) and Invitrogen (ThermoFisher Scientific Inc.), respectively. The ssDNA probe (LR1) used to develop the sensor was specific to MC-LR with a sequence of 5'-(CH₂)-6SS-GGCGCCAAACAGGACCACCATGACAA-TTACCCATACCACCTCATTATGCCCCATCTCCGC-3'.²⁷ The complementary strand (cDNA) used had a sequence of 5'-GCG GAG ATG GGG CAT AAT GAG GTG GTA TGG GTA ATT GTC ATG GTG GTC CTG TTT GGC GCC-3'. For the control experiment, a nonspecific to MC-LR ssDNA probe (CYN2) with sequence 5'-(CH₂)-6SS-GGCATCAGGCAACAACCGATGGTCCG-GCCACCCTAACAACCAGCCCACCCACCACCC-CGCCG-3' was used.⁴⁴ The ssDNA stocks were dissolved in Tris-EDTA, aliquoted, and stored at -24°C . Tris-2-carboxyethyl-phosphine (TCEP), mercaptohexanol (MCH), ruthenium(III) hexamine, and magnesium chloride solution (2 M) in H_2O (MgCl_2) were purchased from Sigma-Aldrich.

Preparation of Aptamer Monolayers. Prior to modification, all electrodes were mechanically polished, then thoroughly rinsed, and electrochemically cleaned using sodium hydroxide and sulfuric acid as reported elsewhere.³⁵ Calculations of the microscopic (real) surface area and the roughness of each electrode were conducted as previously described.³⁶

Immobilization of the mixed monolayer of aptamers and MCH onto the gold disk electrode consisted of several steps. Initially, the reduction of disulfide bonds was accomplished with the addition of 3 μL of 200 mM TCEP in 2 μL of 200 μM ssDNA probe aliquot for 1 h. Furthermore, the mixture was diluted to a 1 μM concentration with autoclaved 50 mM Tris, 150 mM NaCl, and varying MgCl_2 (1–50 mM) to achieve the desired packing density (pH 7.4). Freshly cleaned electrodes were incubated for 1 h with the reduced and diluted ssDNA solution. Then, the electrodes were passivated with 3 mM MCH for 1 h. In between all steps, rinsing for 20 s with Milli-Q water was necessary to remove any residual ssDNA or MCH. All steps were conducted at room temperature ($\sim 22^\circ\text{C}$). Finally, freshly prepared modified electrodes were incubated for 10 min in 50 μM to saturate the monolayer, rinsed thoroughly, and equilibrated for 40 min in 10 mM Tris-base before use. The incubation time with MC-LR was 15 min.

Instrumentation and Electrochemical Measurements.

A standard three-electrode configuration with a 2 mm diameter gold working electrode, a platinum wire auxiliary electrode, and an Ag/AgCl (3 M NaCl) reference electrode (CH Instruments, Austin, TX) was used to perform electrochemical measurements. SWV and chronocoulometric measurements

were performed using a 1000C multipotentiostat (CH Instruments, Austin, TX). Single step chronocoulometry was conducted for initial surface coverage calculations using $50 \mu\text{M}$ $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in 10 mM Tris-base buffer (pH = 7.4). The potential step was from 0.1 to -0.4 V with a pulse width of 0.5 s, sample interval of 5×10^{-4} s, and sensitivity of 1×10^{-5} C. For SWV measurements, amplitude was 20 mV, and potential was scanned between 0.1 V (initial) and -0.5 V (final); sensitivity was set at 1×10^{-5} A, increment at 5 mV, and the range of frequency was between 10 and 300 Hz (optimal of 40 Hz). EIS measurements were conducted on an SP-150 Biologic instrument using a multi sine mode and a set potential of 0.226 V. Frequency ranged from 100 kHz to 100 mHz with a sinus amplitude of 5 mV. Both SWV and EIS experiments were conducted in Tris binding buffer (50 mM Tris, 150 mM NaCl, and 2 mM MgCl_2 , pH = 7.5).

In a first attempt to develop a sensitive aptamer-based sensor for MC-LR, EIS was used as a method of detection with $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as a redox mediator. Furthermore, the SWV technique was used with aptamer LR1-MB (methylene blue attached on aptamer as redox mediator) and its cDNA. The aptamer LR1-MB ($0.2 \mu\text{M}$) modified electrode was incubated with its fully complementary strand ($0.1 \mu\text{M}$) in a hybridization buffer, followed by incubation of 100 nM MC-LR in Tris buffer. Finally, we tested the response of aptamer-modified electrodes in 5, 0.5, and $0.1 \mu\text{M}$ $[\text{Ru}(\text{NH}_3)_6]^{3+}$.

For the sensor development, the aptamer-modified electrodes were first incubated in a solution of $5 \mu\text{M}$ $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in 10 mM Tris-base with pH of 7.4 for 10 min. Furthermore, the incubated aptamer-modified electrodes were transferred in a redox-free buffer (only 10 mM Tris-base, pH = 7.4). The sensors remained in this buffer until equilibrated (30–40 min). After equilibration, the sensors were incubated in buffered solutions of MC-LR concentrations in the presence of interferences and water samples.

Surface coverage was calculated using two electrochemical methods. Initially, a previously established method was used to determine probe surface densities.²⁸ The negatively charged backbone of ssDNA probes electrostatically attracts the positive $[\text{Ru}(\text{NH}_3)_6]^{3+}$ molecules from bulk solution. Reduction of this surface-bound $[\text{Ru}(\text{NH}_3)_6]^{3+}$ is measured electrochemically as charge in coulombs. In brief, probe surface coverage is calculated from the difference between the acquired chronocoulometric intercepts in 10 mM Tris-base buffer solutions in the presence and absence of $[\text{Ru}(\text{NH}_3)_6]^{3+}$.

Percent signal suppression was calculated using the following equation:

$$\% \text{ signal suppression} = (I_0 - I) / I_0 \times 100$$

where I_0 is the peak current (μA) in the absence of target and I is the final peak current (μA) in the presence of target toxin.

RESULTS AND DISCUSSION

MC-LR binds to aptamer LR1; however, the faradaic current did not exhibit a significant change. This low signal change may be a result of small or localized conformation changes upon toxin binding and not larger conformational or flexibility changes. Tests using EIS showed a change in charge-transfer resistance; however, the readable signal change was minimal for generating a sensor with the desired dynamic range of concentrations (Figure S1). This may indicate an aptamer with localized conformation, which agrees with Eissa et al.'s circular dichroism spectroscopy observations using the LR1 aptamer.²⁶

In the experiments with LR1-MB and cDNA, the hypothesis was that the interaction or binding of MC-LR on the aptamer forces complementary strands to denature from the duplex configuration, and a higher signal change is expected as a response. However, the MC-LR presence did not show a significant signal change in SWV (Figure S1). This may be attributed to a potential MC-LR binding without complete displacement of the complementary strand. If the 60-mer long aptamer has only one binding site for MC-LR, this single binding site may not be adequate to force the displacement of strongly bound complementary strands. The results from EIS and SWV indicated an aptamer that undergoes minimal small or localized conformational changes upon toxin binding, which do not generate a significant detectable electrochemical signal response.²⁶

The redox mediator $[\text{Ru}(\text{NH}_3)_6]^{3+}$ electrostatically associates with electrode-bound DNA, and voltammetry exhibits both diffusion-limited and surface-confined character. More specifically, in a solution of $5 \mu\text{M}$ $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in Tris buffer, we observed two peaks in the square wave voltammogram (Figure 1). When the electrode is transferred in a redox-free

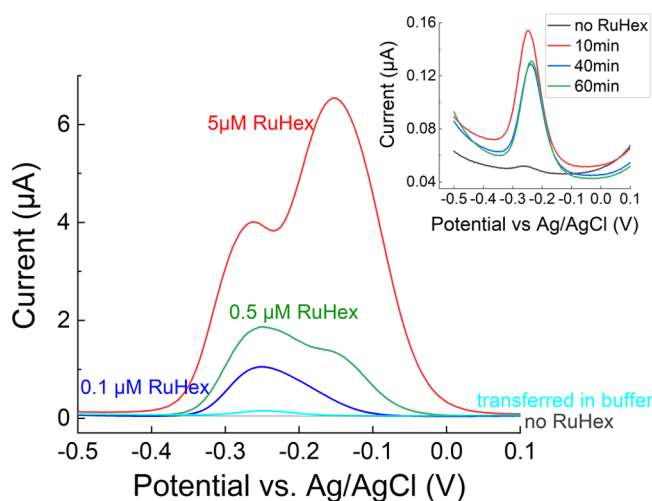


Figure 1. Optimization of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ concentration (5, 0.5, and $0.1 \mu\text{M}$) in Tris buffer and testing of signal response based on surface-confined $[\text{Ru}(\text{NH}_3)_6]^{3+}$ after transferring the sensor into a redox-free Tris-base buffer. Inset shows equilibration time of surface-confined $[\text{Ru}(\text{NH}_3)_6]^{3+}$ peak in redox-free Tris-base buffer. The control line of only Tris-base buffer (no $[\text{Ru}(\text{NH}_3)_6]^{3+}$ incubated electrodes) is depicted in the graph for baseline reasons.

solution (Tris buffer only), the negative peak of -285 mV remains; however, the -165 mV disappears. The peak at -165 mV correlates with the reduction of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in the bulk of solution and is diffusion limited. Conversely, the peak at -285 mV is attributed to surface confined $[\text{Ru}(\text{NH}_3)_6]^{3+}$ ions (i.e., electrostatically associated with DNA).^{29,37,38} When the $[\text{Ru}(\text{NH}_3)_6]^{3+}$ concentration was decreased, we observed a decrease in the diffusion-limited peak during SWV measurements (Figure 1). When $0.1 \mu\text{M}$ $[\text{Ru}(\text{NH}_3)_6]^{3+}$ is used, we observed a broadening of the peak, which probably was caused by an overlap between the two peaks and the existence of lateral interactions between adsorbed species.³⁷ After exposure to $5 \mu\text{M}$ $[\text{Ru}(\text{NH}_3)_6]^{3+}$ solution and then a transfer of the electrode to a redox-free buffer solution, the peak at -285 mV dominates. This indicates a surface-confined reaction between $[\text{Ru}(\text{NH}_3)_6]^{3+}$ ions on the negative backbone of ssDNA, which

is in agreement with previous studies.³⁸ Peak currents due to surface-confined $[\text{Ru}(\text{NH}_3)_6]^{3+}$ increase linearly with the square root of scan rate.²⁹ Once the electrode is transferred into $[\text{Ru}(\text{NH}_3)_6]^{3+}$ -free solution, peak current decreases and most electrostatic $[\text{Ru}(\text{NH}_3)_6]^{3+}$ diffuses into bulk solution. However, enough bound $[\text{Ru}(\text{NH}_3)_6]^{3+}$ is left on the negative backbone to provide a considerable signal.³⁸ After immersion of sensors in a redox-free buffer solution, the peak reaches equilibrium (inset, Figure 1). Studies using electrochemical in situ scanning tunnelling microscopy showed that $[\text{Ru}(\text{NH}_3)_6]^{3+}$ is far from static as there is a time dependence relationship on how $[\text{Ru}(\text{NH}_3)_6]^{3+}$ penetrates gradually into the monolayer.³⁹ Our results lead to a similar conclusion. In our experiments, a 10 min incubation of aptamer-modified electrodes in 5 μM $[\text{Ru}(\text{NH}_3)_6]^{3+}$ was enough to allow a possible penetration of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ into the monolayer.

The introduction of MC-LR leads to a quantitative change in the faradaic current measured on the basis of the reduction of $[\text{Ru}(\text{NH}_3)_6]^{3+}$. Binding of MC-LR to the aptamer sequence displaces $[\text{Ru}(\text{NH}_3)_6]^{3+}$ molecules from being bound to the DNA backbone. The magnitude of the observed signal loss (or signal suppression) is a strong function of the voltage switching frequency employed during SWV (Figure 2).^{36,40} At a packing

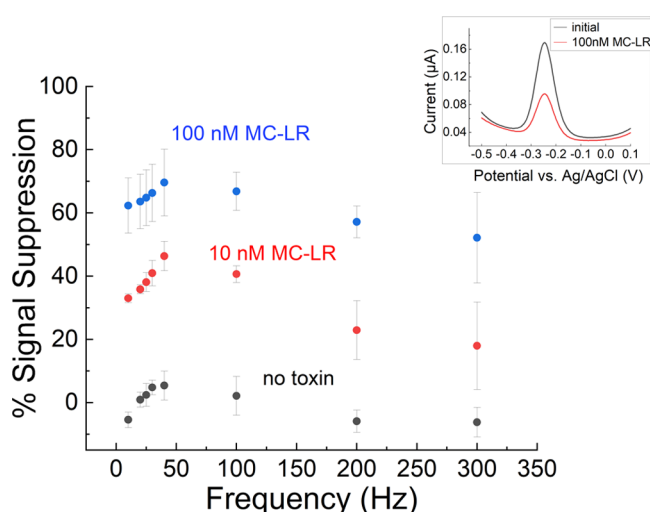


Figure 2. Frequency sweep examination for optimal signal suppression after incubation of equilibrated surface confined $[\text{Ru}(\text{NH}_3)_6]^{3+}$ electrodes with 0, 1, 10, and 100 nM MC-LR. Controls show the baselines. The packing density is at 3.7×10^{12} molecules/cm². Inset shows the highest percent in SWV signal suppression after 100 nM MC-LR incubation was at 40 Hz.

density of 3.7×10^{12} molecules/cm², we tested the response of aptamer modified electrodes in the presence and absence of MC-LR in three different concentrations (1, 10, and 100 nM) over a range of SWV frequencies (10–300 Hz). We find an optimal signaling frequency of 40 Hz at which a high signal suppression of $70 \pm 10\%$ and $13 \pm 3.9\%$ in the presence of 100 and 1 nM MC-LR in the electrolyte, respectively, was observed. Since the maximum yield in percent of signal suppression was at the 40 Hz, this frequency was used for the remaining experimental work of this Article.

The sensitivity of the $[\text{Ru}(\text{NH}_3)_6]^{3+}$ displacement assay depends on the surface packing density of the aptamers. We observed optimal signaling at a relatively high packing density. Optimization of surface packing density is necessary to achieve

detection of MC-LR within a dynamic range of concentrations in environmental samples. The alteration of the packing density of surface-bound aptamers has shown tremendous variations in signal change responses in previous studies.^{35,41,42} We altered the packing density by varying the concentration of MgCl_2 in the binding buffer during the fabrication of the sensor. Divalent cations of Mg^{2+} have been shown to contribute to the thermal stability and folding kinetics of ssDNA.⁴³ Increased concentrations of MgCl_2 assisted with the immobilization of more probes on the electrode during fabrication. The variations of the concentration of MgCl_2 from 1 to 50 mM produced sensing surfaces with higher packing densities (ranging between 3.7 and 8.6×10^{12} molecules/cm², Figure S3). The peak potential (V) indicates an increasing trend with increasing aptamer packing density. When the surface-confined $[\text{Ru}(\text{NH}_3)_6]^{3+}$ is reduced to $[\text{Ru}(\text{NH}_3)_6]^{2+}$, this creates a local deficit of positive charge on the negative ssDNAs. Electroneutrality is maintained by the temporary equilibration of charge with cations available in solution (e.g., Tris). The inflow of cation from solution toward the monolayer creates a negative displacement in potential. Higher packing density corresponds to larger deficit, which shifts the peak potential to negative values (Figure 3A).²⁹ The

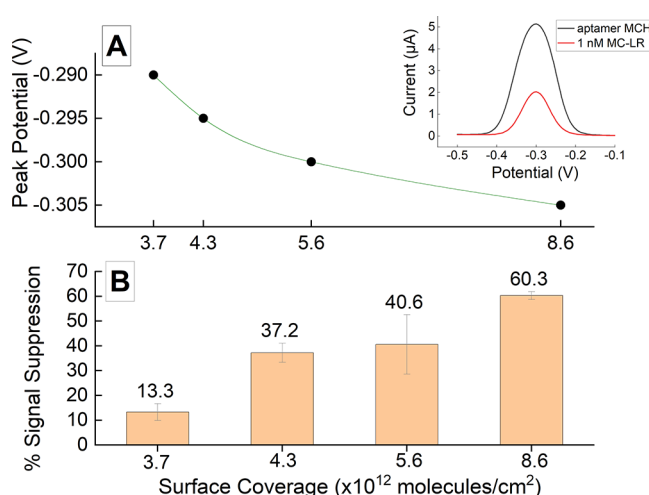


Figure 3. (A) Variation of surface coverage vs signal suppression and peak potential after incubation with 1 nM MC-LR in Tris-base buffer; (B) SWV signal suppression after 1 nM MC-LR incubation for sensors with packing densities of 3.7 , 4.3 , 5.6 , and 8.6×10^{12} molecules/cm² in Tris-base buffer. Inset shows the SWV signal change after 1 nM MC-LR incubation for the high packing density of 8.6×10^{12} molecules/cm².

sensors with packing densities of 4.3 and 5.6×10^{12} molecules/cm² responded similarly to the presence of 1 nM MC-LR with $37 \pm 3.9\%$ and $41 \pm 12.0\%$, respectively. The packing density of 8.6×10^{12} molecules/cm² showed a high signal change of $60 \pm 1.6\%$ for 1 nM MC-LR. Additionally, the 8.6×10^{12} molecules/cm² packing density showed larger peak current and better intrastudy reproducibility (Figure S2) compared to lower probe packing densities. The significant signal suppression of 60% is shown in the voltammogram of Figure 3B. A 2.3-fold change in surface packing density (from 3.7 to 8.6×10^{12} molecules/cm²) showed a 2-fold improvement in sensitivity for MC-LR detection (Figure 3). For the remainder of this Article, sensors were fabricated with 1 μM aptamer in

50 mM MgCl_2 (for a packing density of 8.6×10^{12} molecules/ cm^2).

Calibration Curve. The MC-LR aptamer-based sensor responds quantitatively to the presence of MC-LR in Tris-base buffer (Figure 4). The sensor was incubated with different

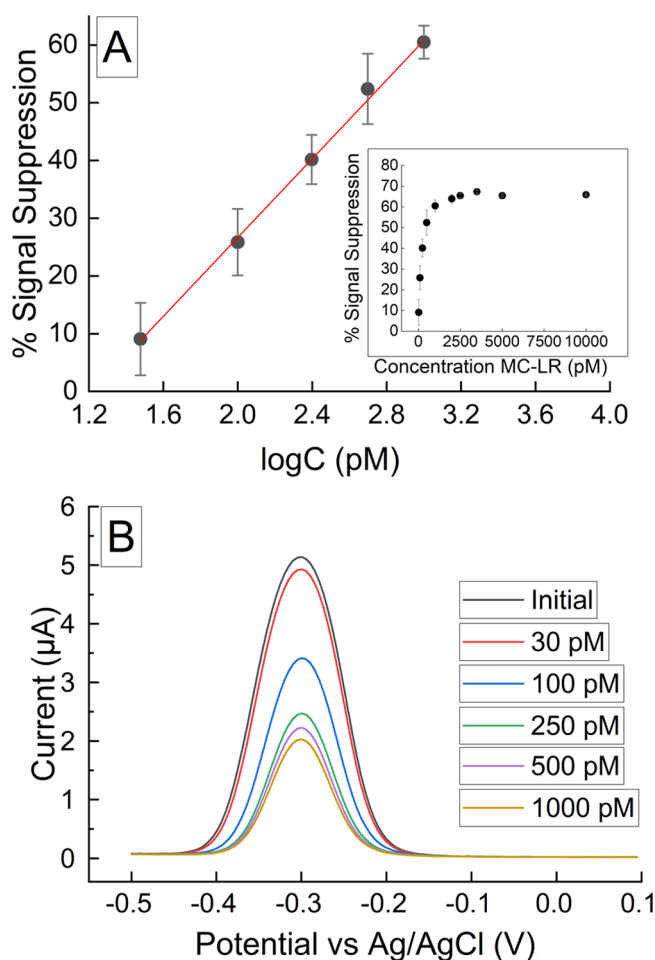


Figure 4. (A, inset) Dose–response curve for concentrations of 30, 100, 250, 500, 1000, 2000, 2500, 3500, 5000, and 10 000 pM MC-LR in Tris-base buffer for 15 min. (A) Linear response between signal suppression% versus the logarithm of MC-LR (in pM) for concentrations between 30 and 1000 pM. (B) SWV responses after incubation of sensor with 0 (initial), 30, 100, 250, 500, and 1000 pM MC-LR in Tris-base buffer.

MC-LR concentrations for 15 min, and SWV was recorded before and after incubation within the range of 30 pM to 10 nM. The dose–response curve yielded a signal suppression change that reaches its optimal plateau at MC-LR concentrations between 1 and 10 nM. In the dynamic range of 30 pM to 1 nM, signal suppression % versus the logarithm of MC-LR concentration (pM) showed a good linear relationship of $(I_0 - I)/I_0\% = 34.11 \times \log C - 41.58$ and a correlation coefficient of 0.99831 ($N = 5$). From this data, the LOD (3σ) of the sensor was determined to be 9.2 pM in 10 mM Tris-base with pH of 7.4.

Specificity, Selectivity, and Water Samples. To determine the specificity of the sensor, control experiments were conducted in the presence of other cyanotoxins in Tris-base buffer (Figure 5). The cyanotoxins tested were similar structured microcystin congeners, nodularin (NOD) and

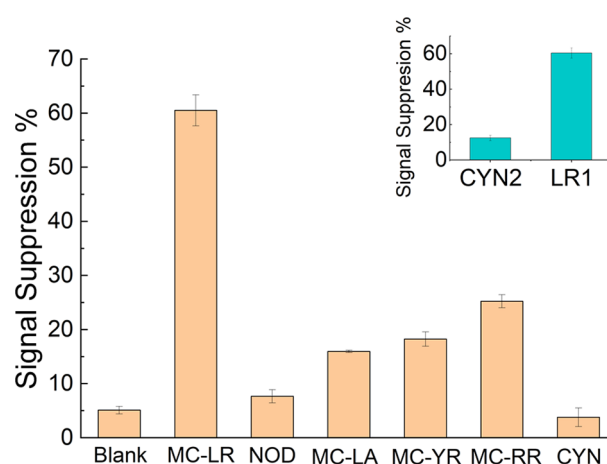


Figure 5. Specificity of the sensor in response to the presence of 1 nM of various cyanotoxins in Tris buffer. Cyanotoxins tested include MC-LR, NOD, MC-LA, MC-YR, MC-RR, and CYN. The inset is a control experiment with the MC-LR specific ssDNA probe (LR1) and a similar length ssDNA probe that is nonspecific to MC-LR (CYN2).

cylindrospermopsin (CYN). The sensor responded with $7.7 \pm 1.2\%$ and $3.8 \pm 1.7\%$ in the presence of NOD and CYN, respectively. Both signal changes are far lower than 60% caused by 1 nM MC-LR. However, the sensor responded to microcystin congeners MC-LA, MC-YR, and MC-RR with signal changes ranging between $16 \pm 0.2\%$ and $25 \pm 1.2\%$. These values of NOD and CYN are significantly different from those of MC congeners, according to a t test and ANOVA test, using $P = 0.05$ (Table S1). The higher value of sensor response in samples with microcystins indicates a relative affinity to these microcystin congeners, although the strongest affinity was for MC-LR. This is attributed to similarities in the chemical structure of these heptapeptides and potential binding of the common ADDA group or L or R amino acid on the specific aptamer. Yet, other studies have shown no interference between MCs and MC-LR using this LR1 aptamer.²⁷ The relative affinity of MC congeners to the aptamer LR1 needs extensive investigation to estimate K_D for each MC congener. Isothermal titration calorimetry and circular dichroism spectroscopy may be used in future investigations to precisely estimate K_D .⁴⁵ Finally, the mechanism of detection was tested with a control aptamer CYN2⁴⁴ (versus the specific aptamer LR1) that showed insignificant signal suppression in the presence of 1 nM MC-LR.

The sensor performance was challenged in different water parameters including commonly found salts and natural organic matter (NOM). Commonly found interferents, such as NaCl, MgCl_2 , CaCl_2 , and humic acids, are critically important to evaluate the possible impact on sensor efficiency. The concentrations used were the average values found in Cincinnati, Ohio, for 2020 based on the monitoring estimations reported in the Water Quality – Raw and Finished Richard Miller Treatment Plant Report.⁴⁶ The sensor performed well in samples with 1 nM MC-LR in the presence of 25 mg/L NaCl, 10 mg/L MgCl_2 , 34 mg/L CaCl_2 , and 0.8 mg/L humic acid. The ANOVA and t tests indicated no significant difference between those samples using $P = 0.05$ (Tables S2–S5). The samples of 10 mg/L MgCl_2 and 0.8 mg/L humic acid showed a higher signal suppression percentage of $67 \pm 8.7\%$ and $66 \pm 4.8\%$, respectively, than our calibrated

estimation ($60 \pm 2.8\%$). MgCl_2 is known to influence the stringency of the interaction between a ssDNA and a target.⁴³ Divalent ions, such as MgCl_2 , are known to affect ssDNA elasticity and the formation of secondary structures, which may affect the interaction of the target with the aptamer.^{47,48} A natural presence of MgCl_2 in real samples may help or worsen the detection, depending on the concentration of MgCl_2 . On the other hand, NOM may cause nonspecific adsorption or biofouling at the electrode interface. In our sample of 0.8 mg/L humic acid + 1 nM MC-LR, there is no statistical difference (Table S5) between these samples; however, a significant overestimation of MC-LR concentrations may occur when surface waters reach high NOM concentrations (Figure S4). Nonetheless, biofouling is a known limitation of electrochemical sensors; increased research efforts examine several antibiofouling strategies.^{49,50}

Testing the sensor in real samples showed good performance in drinking water samples rather than lake water samples. To further investigate the applicability of the sensor, lake water and finished drinking water samples were used (Figure 6). The

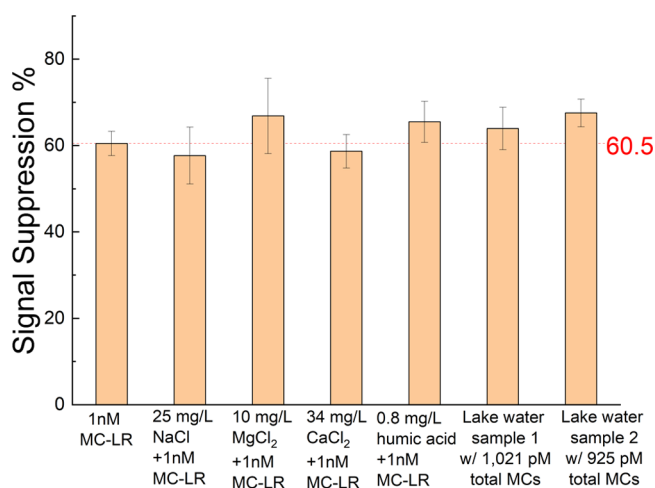


Figure 6. Sensor performance in different water parameters and Lake Erie samples. The sensor was challenged in the presence of 25 mg/L NaCl, 10 mg/L MgCl_2 , 34 mg/L CaCl_2 , and 0.8 mg/L humic acid with the addition of 1 nM MC-LR, and finally in Lake Erie water samples 1 and 2 with the presence of 1021 and 925 pM total MCs, respectively.

lake water samples (from Lake Erie) included several microcystin congeners, including MC-LA, MC-YR, MC-RR, and other congeners (see Tables S8 and S9 and Figures S5 and S6 for more information). The sensor detected total microcystins of 1581 pM in a diluted Lake Erie sample 1 ($\text{pH} = 7.4$). Total MCs in the same sample were 1021 pM based on LC-MS/MS analysis.^{51,52} The ANOVA and *t* tests indicated a significant difference between this sample using $P = 0.05$ (Table S6). For Lake Erie sample 2 ($\text{pH} = 7.4$), the sensor estimated the concentration of microcystins as 1241 pM, where the LC-MS/MS analysis showed a concentration of 925 in a diluted sample. The ANOVA and *t* tests indicated no significant difference between this sample using $P = 0.05$ (Table S7). Overall, the sensor overestimates the concentration of microcystins in lake water samples. Possible causes may be interference of high TOC levels, MC congeners, or both in lake waters. For example, the average TOC in 2020 based on the Water Quality – Raw and Finished Richard

Miller Treatment Plant Report⁴⁶ presents an average of 3.23 mg/L for TOC. To prove this hypothesis, we challenged the sensor in conditions of increasing humic acid concentrations (Figure S4). The developed sensor can withstand up to 0.4 mg/L humic acid, where at concentrations of humic acids greater than 0.8 mg/L probably a nonspecific adsorption or biofouling effect occurs. However, in water samples that were collected from a drinking water treatment plant, Greater Cincinnati Water Works (GCWW), at the end of the treatment (finished water), the sensor responded with a high accuracy to MC-LR. Both tests in finished water showed good recovery rates responding to two distinct concentrations of MC-LR (toxin was spiked in finished water samples). Also, it is worth noticing that the low level of TOC in the finished water samples (0.48 mg/L, $\text{pH} = 7.5$) showed no interference with the detection of the spiked MC-LR. Overall, the developed sensor performed substantially better in finished water samples than for the lake water samples. The sensor may be applicable to detect MC-LR in finished, tap water rather than lake water with complex matrices.

A novel electrochemical sensing approach has been developed for the detection of MC-LR using surface-confined $[\text{Ru}(\text{NH}_3)_6]^{3+}$ as redox mediator. Sensing surfaces of highly packed immobilized aptamers were capable of recording decreasing SWV signals after the addition of MC-LR in redox-free buffer. The dose–response curve was linearly proportional to the logarithm of the MC-LR concentration in a dynamic range of concentrations. Higher concentrations of MC-LR resulted in lower SWV percent signal changes with the highest being 60% and a calculated LOD of 9.2 pM. Sensitivity and selectivity of the sensor were good. The sensor may be applicable to detect MC-LR in drinking water samples rather than lake samples. This study suggests a new strategy for the detection of toxins in water using an aptamer with localized conformational changes that provides limited conformational change and thus small electrochemical signal changes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsestengg.1c00256>.

Figures and tables with information related to a sensor approach using EIS and a complementary strand; reproducibility of the sensor after equilibration; chronoulometric responses of the various packing densities during the optimization step; explanation of the TOC effect on the sensor; details on statistical *t* tests and ANOVA tests for significant or nonsignificant differences between variables; the data of LC/MS-MS analysis of Lake Erie samples; the secondary structures of the aptamer used in this study (PDF)

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Notes

The authors declare no competing financial interest.

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