On–Off–On Fluorescent Chemosensors Based on N/P-Codoped Carbon Dots for Detection of Microcystin-LR

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ABSTRACT: The sensing of microcystin-LR (MC-LR) as a nonfluorescent species is still challenging since it has a negligible influence on the emission of fluorescent nanomaterials. Herein, a well-defined “on–off–on” indirect chemosensing strategy is developed by means of nitrogen/phosphorus-codoped carbon dots (N/P CDs) for highly sensitive and selective MC-LR determination. The nanoscale N/P CDs were conveniently synthesized by one-step hydrothermal carbonization of adenosine 5′-triphosphate (ATP) in aqueous media. The fluorescence intensity of the N/P CDs was quenched in the presence of Fe^{3+} due to the formation of Fe–O–P bonds between Fe^{3+} and the phosphate groups of the N/P CDs. The resulting N/P CD- Fe^{3+} system was sensitive to MC-LR, and its fluorescence intensity recovered dramatically and quickly since the MC-LR cavity competitively coordinated with Fe^{3+} . Under optimized conditions, the proposed chemosensor exhibited a wide linear response for MC-LR in the range of 0.05–3 $\mu\text{g/L}$, with a detection limit down to 17.1 ng/L. Moreover, the chemosensor was successfully applied to accurately detect MC-LR in real water samples and achieved high recoveries in the range of 95.0–109.3%, with relative standard deviations within 1.8–4.7%. By relying on the benefits of fluorescent chemosensors, such as simple operation, low production cost, fast response time, selectivity, and sensitivity, the proposed sensing platform possesses promising applicability for MC-LR determination in complex environmental water samples.

KEYWORDS: carbon dots, microcystin-LR, fluorescent chemosensors, “on–off–on” mode, indirect chemosensing

INTRODUCTION

Recently, owing to unsustainable industrial development, the concentrations of water pollutants have increased in ecosystems. Water pollutants stimulate the formation of cyanobacterial blooms, which can release a variety of algae toxins, including microcystins (MCs), nodularins, aphatoxins, etc., and seriously threaten human health.¹ MCs, a kind of heptapeptide monocyclic hepatotoxin with a strong carcinogenic effect, have the strongest toxicity among many cyanobacterial toxins. Hence, water pollution stemming from MCs has become a global concern. Among more than 80 variants of known MCs, microcystin-LR (MC-LR) is the most extensively investigated, and it has demonstrated high toxicity even at low doses.² MC-LR is thermally stable; nonvolatile; resistant to hydrolysis, oxidation, and pH changes; and freely soluble in water. Additionally, although MC-LR is a polypeptide substance, it cannot be degraded by general protein or polypeptide hydrolase.³ Because of the high physical and chemical stability of MC-LR, its natural degradation process in water occurs slowly; thus, this toxin seriously threatens animal and human health. It has been suggested that MC-LR inhibits the activity of protein phosphatase in hepatocytes, induces the hyperphosphorylation of cyto-

and results in liver failure in animals.^{4,5} Moreover, it can cause serious damage to embryos, kidneys, gonads, lymphocytes, and the nervous system and possesses potential carcinogenic, teratogenic, and mutagenic effects.⁶ Accordingly, the World Health Organization (WHO) determined the threshold limit value of 1 $\mu\text{g/L}$ for MC-LR in drinking water.⁷ Hence, the selective recognition and detection of MC-LR at trace/ultratrace levels is of great significance and has a close relationship to public health and animal protection.

The current MC-LR analytical techniques mainly include the following: (i) chromatographic-based methods,^{8,9} such as high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS) and capillary electrophoresis coupled with mass spectrometry, although these methods often require separation, purification, dilution, and concentration steps, which are sophisticated, time-consuming, and laborious and

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Scheme 1. Schematic Illustration of the Proposed “On–Off–On” Mode Fluorescent Chemosensor Binding Approach for MC-LR Detection

(ii) alternative approaches^{10–13} such as enzyme-linked immunosorbent assays, immune sensors, enzymatic sensors, and aptasensors. Although the implementation of these methods is simpler than the chromatography-based approaches, utilizing natural receptors as the recognition element is essential. Natural receptors suffer from several impediments, such as high cost, sensitivity to environmental variables, easy denaturation, and insufficient reproducibility. Therefore, designing a novel sensing strategy with high throughput for sensitive MC-LR detection is in high demand.

More recently, most researchers have focused on developing new sensing approaches to promote the recognition selectivity and detection sensitivity of environmental pollution.¹⁴ Among various analytical methods, fluorescence-based chemosensors have received great attention owing to their high specificity, convenience of operation, portability, and stability. However, it is still difficult to construct a fluorescence sensor for MC-LR because it has poor interactions with common fluorescent nanomaterials, including quantum dots, carbon dots (CDs), etc. Therefore, the quantity of MC-LR cannot be reflected by the change in the fluorescence emission of fluorescent nanomaterials. Because of this crucial problem, to date, a few nonaptamer fluorescence-based chemosensors have been designed for the detection of MCs.¹⁵ Therefore, developing an indirect chemosensing strategy for the MC-LR assay is necessary and promising.

CDs are cost-effective, low-toxicity, biocompatible, and high-fluorescence-performance nanomaterials that have gained outstanding attention in the sensing and imaging fields.^{16,17} Compared with traditional semiconductor quantum dots and organic fluorescent dyes, CDs can be prepared from different kinds of green and eco-friendly carbon sources through relatively simple and controllable methods.^{18,19} According to CD precursors and synthesis routes used, various types of CDs with different functionalities, heteroatom dopants, and fluorescence quantum yields (QYs) can be obtained.^{20,21}

MC-LR has a special chemical structure in terms of functional groups and stereochemistry. The carboxylic groups of MC-LR and its cavity have been shown to interact with cationic species electrostatically or through complexation in the cavity.¹⁵ Herein, by virtue of the benefits of CDs and the

unique chemical structure of MC-LR, we rationally designed an indirect sensing strategy for MC-LR. As shown in Scheme 1, nitrogen/phosphorus-codoped CDs (N/P CDs) were prepared in aqueous media via a one-step hydrothermal method by using adenosine 5'-triphosphate (ATP) as a green carbon source. After the addition of Fe^{3+} , the blue fluorescence emission of the N/P CDs was quenched, which is ascribed to the formation of Fe–O–P bonds between Fe^{3+} and the phosphate groups of the N/P CDs. In the presence of MC-LR, on the basis of MC cavity competitive coordination with Fe^{3+} , the fluorescence of the N/P CDs recovered. According to the mentioned mechanism, an “on–off–on” fluorescent chemosensor can be readily designed for indirect analysis of MC-LR in different environmental samples.

EXPERIMENTAL SECTION

Synthesis of N/P CDs. The N/P CDs were prepared through a green and one-step procedure according to a previous study.²² The details of the N/P CD synthesis method are presented in the Supporting Information.

Detection of MC-LR. MC-LR measurements were performed simply in aqueous media and at room temperature. First, 50 μL of the as-prepared N/P CD solution was diluted into 30 mL of ultrapure water for sensor construction. For a typical assay, 100 μL of the above diluted N/P CD solution and 10 μL of Fe^{3+} solution (50 mmol/L) were mixed in 880 μL of water solution at pH 5. The concentration of N/P CDs was 5.38 mg/L for detection. Subsequently, 10 μL of different concentrations of MC-LR solutions was added separately to the above system. Finally, the fluorescence emission spectra were recorded under excitation at 320 nm, and both of the excitation and emission slit widths were 9 nm.

Analysis of Water Samples. The practical applicability of the fluorescent chemosensor for MC-LR detection in complex water samples was examined. Tap water samples were collected from the Yantai Institute of Coastal Zone Research after flowing for 5 min. Seawater samples were randomly collected from the surface seawater of the Yellow Sea, and lake water samples were acquired from San Yuan Lake, located in the coastal zone region of Yantai City (Shandong, China). To remove suspended particles, all of the water samples were filtered through a 0.22 μm microfiltration membrane before analysis. The tap water and lake water samples were diluted 100-fold, and seawater samples were diluted 1000-fold for spiking detection with different concentrations of MC-LR. Additionally, the MC-LR concentrations in the aforementioned samples were tested by

Figure 1. (A) XPS survey spectrum, (B) C 1s XPS spectrum, (C) N 1s XPS spectrum, and (D) P 2p XPS spectrum of the as-prepared N/P CDs.

HPLC–MS. The details of the HPLC–MS determination conditions are presented in the [Supporting Information](#).

RESULTS AND DISCUSSION

Preparation and Characterization of the N/P CDs.

ATP is a bioorganic compound and supplies energy for cellular reactions in living cells. The criterion for the selection of ATP is the abundant nitrogen- and phosphorus-containing functional groups in its structure, which remain after hydrothermal

Figure 2. UV–vis absorption spectra of the N/P CDs (black line), MC-LR (green line), Fe^{3+} (purple line), N/P CD- Fe^{3+} system (red line), and N/P CDs- Fe^{3+} -MC-LR (blue line).

Table 1. Zeta Potentials of the N/P CDs, Fe^{3+} , MC-LR, and Their Mixtures

	CDs	Fe^{3+}	MC-LR	CDs- Fe^{3+}	Fe^{3+} -MC-LR	CDs- Fe^{3+} -MC-LR
zeta potential (mV)	−15.4	30.8	−0.169	5.97	41.3	12.5

Figure 3. Fluorescence spectra of the N/P CD- Fe^{3+} system with the addition of different concentrations of MC-LR (0.05–3 $\mu\text{g/L}$). The inset graph indicates the Stern–Volmer plots for MC-LR detection, $\lambda_{\text{ex}} = 320 \text{ nm}$.

carbonization. These active functional groups can be used as the basis for the subsequent construction of the fluorescent

Figure 4. Fluorescence enhancing efficiency of the fluorescent chemosensor for recognition of MC-LR and other different toxins, amino acids, and their chemical structures (below): (A) the concentrations of MC-LR and other interferents were 1 µg/L; (B) the concentration of MC-LR was 1 µg/L, and the concentrations of other interferents were 100 µg/L.

Table 2. Spiked Recoveries and RSDs (% , $n = 3$) for the Determination of MC-LR in Real Water Samples by Using the Proposed Fluorescent Chemosensor and HPLC–MS Method

sample	added (µg/L)	found (µg/L)	recovery ^a ± RSD (%)	HPLC–MS results	
				found (µg/L)	recovery ^a ± RSD (%)
tap water	0.4	0.417	104.4 ± 3.5	0.422	105.5 ± 3.3
	1	1.093	109.3 ± 2.9	1.001	100.1 ± 4.1
	2	1.928	96.4 ± 4.1	1.953	97.7 ± 2.9
lake water	0.4	0.382	95.5 ± 2.2	0.378	94.5 ± 3.8
	1	1.039	103.9 ± 1.8	1.003	100.3 ± 2.5
	2	1.900	95.0 ± 4.7	1.804	90.2 ± 4.7
seawater	0.4	0.396	99.0 ± 3.6	0.401	100.3 ± 1.8
	1	1.053	105.3 ± 3.1	0.998	94.8 ± 3.6
	2	1.908	95.4 ± 2.5	1.976	98.8 ± 3.1

^aAverage value from three individual experiments.

chemosensor. In addition, due to the high solubility of ATP, the carbonization process can be performed in aqueous media without consumption of any hazardous reagent, which is in accordance with green and sustainable chemistry principles. The prepared N/P CDs have excellent fluorescent properties and stability, which provides the fluorescent signal source of the chemosensor.

From the transmission electron microscopy (TEM) image (Figure S1A), the as-prepared N/P CDs have a nearly spherical morphology with an average diameter of approximately 5–6 nm and uniform dispersion. Energy-dispersive X-ray spectroscopy (EDXS) analysis was carried out to investigate the elemental composition of the N/P CDs, and the results are indicated in Figure S1B. The N/P CDs are

composed of C, O, N, and P. The high contents of nitrogen and phosphorus, with atomic compositions of 15.42% and 13.26%, respectively, fully confirm that N and P were codoped in the N/P CDs. The X-ray photoelectron spectroscopy (XPS) measurement results are given in Figure 1. The C 1s XPS spectrum (Figure 1B) can be decomposed into three peaks at 284.4 eV (C–C/C=C), 285.7 eV (C–N), and 287.5 eV (C=O).^{23,24} The peaks in the high-resolution XPS spectrum of N 1s (Figure 1C) at 398.9, 400.1, and 401.3 eV are assigned to N–H, N=C, and N–C=C bonds, respectively.²³ The P 2p XPS spectrum (Figure 1D) reveals the presence of P–O (132.9 eV) and P=O (133.7 eV) groups.²² Fourier transform infrared (FT-IR) spectroscopy was further used to identify the functional groups of the N/P CDs. As shown in Figure S2A,

Table 3. Comparison of Different Reported Fluorescence Methods for MC-LR Detection

system	fluorescence signal	analysis time (min)	linear range ($\mu\text{g/L}$)	LOD ($\mu\text{g/L}$)	ref
a fluorescence enzyme-linked immunoassay based on Si-CDs ^a	Si-CDs	50	0.001–3.2	0.600×10^{-3}	39
a fluorescent immunosensor based on CdTe QD ^b –antibody conjugate-immobilized self-assembled multilayers	CdTe QD	60	0.0001–3	0.044×10^{-3}	40
a fluorescent aptasensor based on SWNTs ^c and dapoxyl	dapoxyl	75	0.398–1194.24	0.137	41
an aptasensor fabricated by a MoS ₂ nanosheet platform grafted with core/shell UCNPs ^d	NaYF ₄ :Yb,Tm@NaYF ₄ :Yb CS-UCNPs	30	0.01–50	0.002	42
using donor (UCNPs) and acceptor (BHQ ₄) couples fabricated for aptasensors	NaYF ₄ : Yb, Ho UCNPs	80	0.1–50	0.025	43
using hpDNA-CuNCs ^e as a fluorescent probe	CuNCs	10	0.005–1200	0.003	44
an all-solid-state optical sensor based on DCHQ-Ph ^f for indirect detection	DCHQ-Ph	15	0.10–9.95	0.05	15
an “on–off–on” mode fluorescent chemosensor based on N/P CDs and Fe ³⁺	N/P CDs	8	0.05–3	0.0171	this work

^aSilane-doped carbon dots (Si-CDs). ^bQuantum dots. ^cSingle-walled carbon nanotubes. ^dUpconversion nanoparticles. ^eHairpin DNA-templated copper nanoclusters. ^fPhenyl-substituted diaza-18-crown-6 hydroxyquinoline.

the characteristic bands at approximately 1404, 1631, and 1747 cm^{-1} are assigned to the stretching vibrations of C–N, C=C, and C=O, respectively.²⁵ Moreover, the characteristic peaks of P–O and P=O are located at 899, 951, and 1060 cm^{-1} ,^{22,26} revealing that N/P CDs contain abundant active sites, which can interact with Fe³⁺.

The optical characteristics of the N/P CDs were further explored by UV–vis absorption and fluorescence spectroscopy analyses. As indicated in Figure 2, the UV–vis absorption spectra of the N/P CDs contain intense absorption peaks at 255 nm corresponding to the π – π^* transition of C=C, C=N, or N=P,²⁷ and the absorption at 314 nm is attributed to the n – π^* transition of C=O.²⁸ The prepared N/P CDs exhibited excitation-dependent emission behavior as the excitation wavelength increased from 310 to 370 nm and showed a maximum emission located at 413 nm at an excitation wavelength of 320 nm (Figure S3). The QY of the N/P CDs was measured by a fluorescence QY meter, and an absolute fluorescence yield of 9.8% was obtained. Moreover, the stability of the N/P CDs was systematically investigated, as presented in Figure S4A. The fluorescence intensity was strong and remained approximately unchanged under 320 nm excitation for 1 h, which confirmed the high photostability of the prepared N/P CDs. Moreover, within the temperature range of 20–70 °C, there was almost an inconspicuous change in the fluorescence intensity of the N/P CDs (Figure S4B). On the other hand, the intensity of the N/P CDs also changed slightly, even at a high ionic strength of 5.0 mol/L (Figure S4C), demonstrating the substantial stability of the N/P CDs over a wide range of temperatures and ionic strengths. Therefore, it can be concluded that the prepared N/P CDs possess excellent fluorescence stability and great potential for the development of high-throughput chemosensors.

Possible Sensing Mechanism. The fluorescence intensity of the N/P CDs decreased sharply with a slight redshift after the addition of Fe³⁺. Accordingly, Fe³⁺ was used as a quenching agent to suppress the fluorescence intensity of N/P CDs by coordinating with the phosphate groups of N/P CDs and forming Fe–O–P bonds. Fluorescence lifetime measurements were implemented to identify the mechanism of quenching. As demonstrated in Figure S5, the average lifetimes of the N/P CDs and N/P CDs in the presence of Fe³⁺ were 5.69 and 5.56 ns, respectively. The approximately constant lifetime revealed that the fluorescence quenching of N/P CDs by Fe³⁺ is probably a static quenching process owing to the generation of

new complexes.²⁹ To further prove the formation of Fe–O–P bonds in the N/P CD-Fe³⁺ system, FT-IR analysis was performed. From Figure S2B, the emergence of two characteristic peaks at 1120 and 1002 cm^{-1} confirmed the formation of Fe–O–P bonds.^{30,31} In addition, different from the UV–vis spectra of individual N/P CDs and Fe³⁺ solution, there was a new weak absorption peak at 271 nm in the N/P CD-Fe³⁺ system (Figure 2), further demonstrating the strong interaction between Fe³⁺ and N/P CDs and the successful formation of Fe–O–P bonds.

After introducing MC-LR into the N/P CD-Fe³⁺ system, the fluorescence intensity recovered gradually with increasing amounts of MC-LR. As illustrated in Scheme 1, MC-LR has a cavity spatial structure containing numerous functional groups such as amide and carboxyl groups, which can act as metal coordination sites.³² Two carboxyl groups of MC-LR become negatively charged by being deprotonated at the desired pH.^{33,34} When MC-LR molecules are exposed to the N/P CD-Fe³⁺ sensor, Fe³⁺ ions electrostatically interact with the negatively charged carboxyl groups of MC-LR and/or complex with the amide groups (electron density donors) in the MC-LR cavities.^{15,35} As a result, the bonds between the N/P CDs and Fe³⁺ are broken, and the fluorescence intensity of the N/P CDs is recovered and recorded as an analytical signal.

To further understand the sensing mechanism, the zeta potential of different systems was investigated. As listed in Table 1, after the addition of Fe³⁺ to the N/P CD solution, the zeta potential of the solution changed from –15.4 to 5.97 mV due to the formation of Fe–O–P bonds. When MC-LR was added to the system, the observed zeta potential of CDs-Fe³⁺-MC-LR was 12.5 mV, which was much lower than that of Fe³⁺-MC-LR (41.3 mV). This phenomenon is attributed to the removal of Fe³⁺ from the N/P CDs via competitive adsorption interactions and the recovery of negatively charged N/P CDs. On the other hand, after the addition of MC-LR, the intensity of the new absorption peak (271 nm) in the N/P CD-Fe³⁺ system decreased obviously owing to complex formation between MC-LR and Fe³⁺, thus collapsing the former N/P CD-Fe³⁺ system (Figure 2). Consequently, an “on–off–on” mode fluorescent chemosensor was successfully constructed and effectively operated according to the aforementioned mechanism.

Fluorescence Properties of N/P CDs toward Cationic Species. Several metal ions with different charges and ionic radii such as Ni²⁺, Pb²⁺, Na⁺, Hg²⁺, Mg²⁺, Cu²⁺, and Fe³⁺ at

identical concentrations of 0.5 mmol/L interacted with the N/P CDs. As displayed in Figure S6A, the fluorescence intensity of the N/P CDs was obviously quenched when exposed to Fe^{3+} , and the other metal ions showed a negligible effect. In addition, when 1 $\mu\text{g/L}$ MC-LR was added to the N/P CD solutions containing individual metal ions (Figure S6B), only the fluorescence intensity of the N/P CD- Fe^{3+} system recovered dramatically. Therefore, Fe^{3+} was selected as a selective quenching agent for the construction of an “on–off–on” mode fluorescent chemosensor for MC-LR detection.

Optimizations of Experimental Conditions. To attain the highest sensitivity, the experimental variables that affect the performance of the sensor were optimized. The fluorescence recovery efficiency of the N/P CD- Fe^{3+} system was investigated at different pH values and defined as $(F/F_0) - 1$.³⁶ As seen in Figure S7A, the recovery efficiency was enhanced by increasing the pH up to a pH value of 5 and then decreased with further increasing alkalinity. By increasing the content of OH^- , Fe^{3+} would be hydrolyzed and fail to efficiently coordinate with MC-LR.³⁷ Hence, the optimal pH in further experiments was chosen as pH 5.

To determine the maximum fluorescence recovery, the effect of Fe^{3+} concentration on the fluorescence intensity of N/P CDs in MC-LR solution (0.5 $\mu\text{g/L}$) was studied. As shown in Figure S7B, the maximum fluorescence recovery was achieved at 0.5 mmol/L Fe^{3+} . At concentrations below 0.5 mmol/L, the fluorescence quenching of the N/P CD- Fe^{3+} system was insufficient. In addition, when the concentration of Fe^{3+} was more than the optimal value, the nonbonded excess Fe^{3+} ions may interact with MC-LR molecules without fluorescence recovery of N/P CDs and thus reduce the sensitivity. Accordingly, for subsequent experiments, 0.5 mmol/L Fe^{3+} was used.

The response time of the proposed chemosensor was also optimized within the range of 0–10 min. The fluorescence intensity of the N/P CD- Fe^{3+} system dramatically recovered after adding MC-LR within 3 min and then showed a slight increase over time (Figure S7C). Therefore, the N/P CD- Fe^{3+} system can serve as a fluorescence chemosensor for MC-LR detection with a rapid response. To ensure that the reaction reached equilibrium, 8 min was selected as the optimal response time in further experiments.

The influence of temperature and ionic strengths on the sensitivity of the developed chemosensor for MC-LR determination was investigated. As shown in Figure S8A, the chemosensor exhibited high sensitivity at mild temperatures (between 20 and 40 $^{\circ}\text{C}$). At high temperatures (50 and 60 $^{\circ}\text{C}$), the sensitivity significantly decreased. Hence, the MC-LR determination process can be implemented at ambient temperature by the chemosensor. The effect of ionic strength was studied by using NaCl salt in the concentration range of 0–0.15 mol/L (Figure S8B). A low ionic strength has a negligible influence on the sensitivity of the chemosensor. By increasing the ionic strength, the sensitivity of the chemosensor gradually decreased so that the lowest sensitivity was observed at the highest tested ionic strength. Therefore, further experiments were carried out without the addition of salt.

Sensitivity and Selectivity of the Chemosensor. Under the optimal conditions, the sensing performance of the fluorescent chemosensor was evaluated quantitatively by tracking the recovery of the fluorescence intensity versus MC-LR concentration (Figure 3). The fluorescence enhancement was calculated according to the Stern–Volmer

equation:³⁸ $F/F_0 = 1 + K_{\text{sv}}C$, where F and F_0 are the fluorescence intensities of the N/P CD- Fe^{3+} system in the presence and absence of MC-LR, respectively; K_{sv} is the Stern–Volmer constant; and C is the concentration of MC-LR. As indicated in the inset of Figure 3, the proposed chemosensor exhibited two linear plots over the range of 0.05–1 $\mu\text{g/L}$ ($R = 0.9958$) and 1.5–3 $\mu\text{g/L}$ ($R = 0.9898$). Based on $3\sigma/s$ (σ and s are the standard deviations of the blank measurements and the sensitivity of the calibration plot, respectively), the lowest limit of detection (LOD) was calculated to be 17.1 ng/L for MC-LR, which is much lower than the value set by the WHO for drinking water (1 $\mu\text{g/L}$).⁷ Consequently, the developed fluorescent chemosensor can be applied as a promising tool for rapid and sensitive detection of MC-LR.

To examine the selectivity of the proposed chemosensor, the fluorescence response of the N/P CD- Fe^{3+} system toward several toxins such as microcystin-RR (MC-RR), acetamiprid, niclosamide, and dinotefuran was analyzed. In addition, since the chemical structure of MC-LR contains leucine and arginine, several amino acids, including L-threonine, L-alanine, L-arginine, L-leucine, and L-histidine, were tested. As demonstrated in Figure 4A, the fluorescence enhancement efficiency of the chemosensor was the highest for MC-LR, followed by MC-RR, the values of which were much larger than those for other toxins and amino acids. Since the chemical structures of MC-RR and MC-LR are similar, the degree of fluorescence recovery for MC-RR was also high, and this phenomenon further proves that the cavity in the structure of MC-LR plays a key role in the fluorescence recovery process. By contrast, the fluorescence intensity of the other interfering compounds such as toxins and amino acids did not show obvious changes, even at concentrations 100-fold higher than that of MC-LR (Figure 4B). These results attested to the remarkable selectivity of the as-prepared fluorescent chemosensor for MC-LR detection.

Analysis of Real Water Samples. The practical applicability of the sensor was investigated by detecting MC-LR in different water samples, including tap water, lake water, and seawater. The conventional HPLC–MS method was also utilized for comparison. Accuracy was determined through evaluation of the recovery obtained at three different concentrations (0.4, 1, and 2 $\mu\text{g/L}$) of MC-LR using a standard addition method, as listed in Table 2. Satisfactory recoveries in the range of 95.0–109.3% with relative standard deviations (RSDs) of 1.8–4.7% were achieved for all tested samples. These results are in good agreement with the values obtained through the HPLC–MS method. Therefore, the proposed chemosensor can be employed for accurate and reliable MC-LR detection in real water samples.

Comparison of Method Performance. Several fluorescence detection methods have been used for MC-LR detection, and most of the reported methods were based on immunoassays or aptasensors (Table 3). The previously reported fluorescence enzyme-linked immunoassays were shown to be precise and sensitive;^{39,40} however, their operation processes were complicated and required costly enzymatic reagents as well as strict experimental conditions such as temperature adjustment and long response time. Although the aptasensors had excellent specific recognition performance, they suffered from a long incubation time and specific aptamer design in the fluorescent system.^{41–44} Lvova et al.¹⁵ developed an optical sensor by using a phenyl-substituted diaza-18-

crown-6 hydroxyquinoline (DCHQ-Ph) ligand for MC-LR detection. However, the DCHQ-Ph ligand requires a complex preparation procedure. To our knowledge, there are a few methods based on the direct usage of fluorescent nanomaterials, especially low-toxicity CDs, as fluorescent chemosensors for MC-LR detection. Without any complicated functionalization, the “on–off–on” mode fluorescent chemosensor was creatively constructed for sensitive and selective MC-LR assays.

CONCLUSIONS

In summary, an “on–off–on” mode indirect fluorescent chemosensing strategy based on N/P-codoped CDs was successfully developed for the sensitive and selective determination of MC-LR. The N/P CDs were simply synthesized from biosources, and specificity was quenched by Fe^{3+} through the formation of $\text{Fe}-\text{O}-\text{P}$ bonds. By exposing the N/P CD- Fe^{3+} system to a trace amount of MC-LR, the fluorescence intensity of the N/P CDs recovered effectively since the MC-LR cavity competitively coordinated with Fe^{3+} . The impressive merits of the proposed chemosensor, namely, its simple synthesis route, low cost, fast and facile operation, excellent practicability, and high sensitivity, distinguished it from other available sensors. We envision that the presented novel analytical sensing strategy could provide great potential for the development of MC-LR detection in environmental samples.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsanm.1c00921>.

Experimental materials and instrumentations; synthesis of N/P CDs; method of HPLC–MS determination; TEM, EDXS, FT-IR, and emission spectra of N/P CDs; stability of fluorescence intensity of the N/P CDs; fluorescence lifetime of the Fe^{3+} on the N/P CDs; effects of various metal ions, pH values, concentrations of Fe^{3+} , response times, and ionic strengths of experimental conditions (PDF)

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Author Contributions

X.W. and L.C. developed the concepts and designed the experiments; X.W., J.Y., and W.J. performed the experiments and processed the experimental data; X.W., J.Y., and M.A. analyzed the results and drafted the manuscript. X.W., J.Y., L.C., B.L., and L.F. contributed to the writing of the final version of the manuscript. All authors read and approved the final manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

- (1) Melanie, K.; Neilan, B. A. Ecological and Molecular Investigations of Cyanotoxin Production. *FEMS Microbiol. Ecol.* **2001**, *35*, 1–9.
- (2) Carmichael, W. W.; Boyer, G. L. Health Impacts from Cyanobacteria Harmful Algae Blooms: Implications for the North American Great Lakes. *Harmful Algae* **2016**, *54*, 194–212.

- (3) Bialczyk, J.; Kochanowski, A.; Czaja-Prokop, U.; Chrapusta, E. Removal of Microcystin-LR from Water by Polymers Based on N-vinylformamide Structure. *Water Sci. Technol. Water Supply* **2014**, *14*, 230–237.
- (4) Zhang, M. L.; Yan, H.; Pan, G. Microbial Degradation of Microcystin-LR by *Ralstonia Solanacearum*. *Environ. Technol.* **2011**, *32*, 1779–1787.
- (5) Zhao, Y.; Zheng, F. J.; Ke, W.; Zhang, W.; Shi, L. X.; Liu, H. Gap-tethered Au@Ag Raman Tags for the Ratiometric Selection of MC-LR. *Anal. Chem.* **2019**, *91*, 7162–7172.
- (6) Moreno, I. M.; Molina, R.; Jos, A.; Pico, Y.; Camean, A. M. Determination of Microcystins in Fish by Solvent Extraction and Liquid Chromatography. *J. Chromatogr. A* **2005**, *1080*, 199–203.
- (7) World Health Organization. *Guidelines for Drinking-Water Quality, Addendum to Volume 2: Health Criteria and Other Supporting Information*; World Health Organization: Geneva, Switzerland, 1998; pp 94–110.
- (8) Foss, A. J.; Aubel, M. T. Using the MMPB Technique to Confirm Microcystin 8 Concentrations in Water Measured by ELISA and HPLC (UV, MS, MS/MS). *Toxicon* **2015**, *104*, 91–101.
- (9) Zheng, B. X.; Fu, H. Z.; Berry, J. P.; McCord, B. A Rapid Method for Separation and Identification of Microcystins Using Capillary Electrophoresis and Time-of-flight Mass Spectrometry. *J. Chromatogr. A* **2016**, *1431*, 205–214.
- (10) He, X. X.; Stanford, B. D.; Adams, C.; Rosenfeldt, E. J.; Wert, E. C. Varied Influence of Microcystin Structural Difference on ELISA Cross-reactivity and Chlorination Efficiency of Congener Mixtures. *Water Res.* **2017**, *126*, 515–523.
- (11) Hou, L.; Ding, Y. H.; Zhang, L. L.; Guo, Y.; Li, M. S.; Chen, Z.; Wu, X. P. An Ultrasensitive Competitive Immunosensor for Impedimetric Detection of Microcystin-LR Via Antibody-conjugated Enzymatic Biocatalytic Precipitation. *Sens. Actuators, B* **2016**, *233*, 63–70.
- (12) Wei, J.; Xie, X.; Chang, W. D.; Yang, Z. H.; Liu, Y. J. Ultrasensitive Photoelectrochemical Detection of Microcystin-LR Based on Hybridization Chain Reaction Assisted Exciton-plasmon Interaction and Enzymatic Biocatalytic Precipitation. *Sens. Actuators, B* **2018**, *276*, 180–188.
- (13) Abnous, K.; Danesh, N. M.; Nameghi, M. A.; Ramezani, M.; Alibolandi, M.; Lavaee, P.; Taghdisi, S. M. An Ultrasensitive Electrochemical Sensing Method for Detection of Microcystin-LR Based on Infinity-shaped DNA Structure Using Double Aptamer and Terminal Deoxynucleotidyl Transferase. *Biosens. Bioelectron.* **2019**, *144*, No. 111674.
- (14) Mitra, R.; Saha, A. Reduced Graphene Oxide Based “Turn-On” Fluorescence Sensor for Highly Reproducible and Sensitive Detection of Small Organic Pollutants. *ACS Sustainable Chem. Eng.* **2017**, *5*, 604–615.
- (15) Lvova, L.; Lvova, C. G.; Prodi, L.; Lombardo, M.; Zaccheroni, N.; Viaggiu, E.; Congestri, R.; Guzzella, L.; Pozzoni, F.; Di Natale, C.; Paolesse, R. Non-enzymatic Portable Optical Sensors for Microcystin-LR. *Chem. Commun.* **2018**, *54*, 2747–2750.
- (16) Ji, C. Y.; Zhou, Y. Q.; Leblanc, R. M.; Peng, Z. L. Recent Developments of Carbon Dots in Biosensing: A Review. *ACS Sens.* **2020**, *5*, 2724–2741.
- (17) Li, S. Q.; Zhang, Z. W. Recent Advances in the Construction and Analytical Applications of Carbon Dots-based Optical Nano-assembly. *Talanta* **2021**, *223*, No. 121691.
- (18) Zhang, X. Y.; Jiang, M. Y.; Niu, N.; Chen, Z. J.; Li, S. J.; Liu, S. X.; Li, J. Natural-Product-Derived Carbon Dots: From Natural Products to Functional Materials. *ChemSusChem* **2018**, *11*, 11–24.
- (19) Sharma, V.; Tiwari, P.; Mobin, S. M. Sustainable carbon-dots: Recent Advances in Green Carbon Dots for Sensing and Bioimaging. *J. Mater. Chem. B* **2017**, *5*, 8904–8924.
- (20) Hu, Y. P.; Gao, Z. J. Yellow Emissive Se, N-codoped Carbon Dots Toward Sensitive Fluorescence Assay of Crystal Violet. *J. Hazard. Mater.* **2020**, *388*, No. 122073.
- (21) Long, C. C.; Jiang, Z. X.; Shangguan, J. F.; Qing, T. P.; Zhang, P.; Feng, B. Applications of Carbon Dots in Environmental Pollution Control: A Review. *Chem. Eng. J.* **2021**, *406*, No. 126848.
- (22) Shangguan, J. F.; Huang, J.; He, D. G.; He, X. X.; Wang, K. M.; Ye, R. Z.; Yang, X.; Qing, T. P.; Tang, J. L. Highly Fe³⁺-selective Fluorescent Nanoprobe Based on Ultrabright N/P Codoped Carbon Dots and Its Application in Biological Samples. *Anal. Chem.* **2017**, *89*, 7477–7484.
- (23) Huang, Q. T.; Li, Q.; Chen, Y. F.; Tong, L. L.; Lin, X. F.; Zhu, J. J.; Tong, Q. X. High Quantum Yield Nitrogen-doped Carbon Dots: Green Synthesis and Application as “Off-On” Fluorescent Sensors for the Determination of Fe³⁺ and Adenosine Triphosphate in Biological Samples. *Sens. Actuators, B* **2018**, *276*, 82–88.
- (24) Jiao, Y.; Gao, Y. F.; Meng, Y. T.; Lu, W. J.; Liu, Y.; Han, H.; Shuang, S. M.; Li, L.; Dong, C. One-Step Synthesis of Label-free Ratiometric Fluorescence Carbon Dots for the Detection of Silver Ions and Glutathione and Cellular Imaging Applications. *ACS Appl. Mater. Interfaces* **2019**, *11*, 16822–16829.
- (25) Ananthanarayanan, A.; Wang, Y.; Routh, P.; Sk, M. A.; Than, A.; Lin, M.; Zhang, J.; Chen, J.; Sun, H. D.; Chen, P. Nitrogen and Phosphorus Co-doped Graphene Quantum Dots: Synthesis from Adenosine Triphosphate, Optical Properties, and Cellular Imaging. *Nanoscale* **2015**, *7*, 8159–8165.
- (26) Song, W.; Duan, W. X.; Liu, Y. H.; Ye, Z. J.; Chen, Y. L.; Chen, H. L.; Qi, S. D.; Wu, J.; Liu, D.; Xiao, L. H.; Ren, C. L.; Chen, X. G. Ratiometric Detection of Intracellular Lysine and pH with One-pot Synthesized Dual Emissive Carbon Dots. *Anal. Chem.* **2017**, *89*, 13626–13633.
- (27) Shi, B. F.; Su, Y. B.; Zhang, L. L.; Huang, M. J.; Liu, R. J.; Zhao, S. L. Nitrogen and Phosphorus Co-doped Carbon Nanodots as a Novel Fluorescent Probe for Highly Sensitive Detection of Fe³⁺ in Human Serum and Living Cells. *ACS Appl. Mater. Interfaces* **2016**, *8*, 10717–10725.
- (28) Li, S. H.; Li, Y. C.; Cao, J.; Zhu, J.; Fan, L. Z.; Li, X. H. Sulfur-doped Graphene Quantum Dots as a Novel Fluorescent Probe for Highly Selective and Sensitive Detection of Fe³⁺. *Anal. Chem.* **2014**, *86*, 10201–10207.
- (29) Lu, W. J.; Gao, Y. F.; Jiao, Y.; Shuang, S. M.; Li, C. Z.; Dong, C. Carbon Nano-dots as a Fluorescent and Colorimetric Dual-readout Probe for the Detection of Arginine and Cu²⁺ and Its Logic Gate Operation. *Nanoscale* **2017**, *9*, 11545–11552.
- (30) Tejedor-Tejedor, M. I.; Anderson, M. A. Protonation of Phosphate on the Surface of Goethite as Studied by CIR-FTIR and Electrophoretic Mobility. *Langmuir* **1990**, *6*, 602–611.
- (31) Elzinga, E. J.; Kretzschmar, R. In Situ ATR-FTIR Spectroscopic Analysis of the Co-Adsorption of Orthophosphate and Cd(II) onto Hematite. *Geochim. Cosmochim. Acta* **2013**, *117*, 53–64.
- (32) Gao, M. X.; Deng, C. H.; Fan, Z. Q.; Yao, N.; Xu, X. Q.; Yang, P. Y.; Zhang, X. M. A Simple Pathway to the Synthesis of Magnetic Nanoparticles with Immobilized Metal Ions for the Fast Removal of Microcystins in Water. *Small* **2007**, *3*, 1714–1717.
- (33) De Maagd, P. G. J.; Hendriks, A. J.; Seinen, W.; Sijm, D. T. H. M. pH-Dependent Hydrophobicity of the Cyanobacteria Toxin Microcystin-LR. *Water Res.* **1999**, *33*, 677–680.
- (34) Lee, J.; Walker, H. W. Mechanisms and Factors Influencing the Removal of Microcystin-LR by Ultrafiltration Membranes. *J. Membr. Sci.* **2008**, *320*, 240–247.
- (35) Yan, F.; Ozsoz, M.; Sadik, O. A. Electrochemical and Conformational Studies of Microcystin-LR. *Anal. Chim. Acta* **2000**, *409*, 247–255.
- (36) Wang, X. Y.; Yu, J. L.; Wu, X. Q.; Fu, J. Q.; Kang, Q.; Shen, D. Z.; Li, J. H.; Chen, L. X. A Molecular Imprinting-based Turn-on Ratiometric Fluorescence Sensor for Highly Selective and Sensitive Detection of 2,4-dichlorophenoxyacetic Acid(2,4-D). *Biosens. Bioelectron.* **2016**, *81*, 438–444.
- (37) Zhu, X. W.; Zhang, Z.; Zhang, Z. J.; Huang, C. H.; Shan, Y.; Shan, C.; Qin, X. Y.; Yang, W. S.; Chen, X.; Wang, T. Understanding the Selective Detection of Fe³⁺ Based on Graphene Quantum Dots as

Fluorescent Probes: The Ksp of a Metal Hydroxide-assisted Mechanism. *Anal. Chem.* **2017**, 89, 12054–12058.

(38) Zong, J.; Yang, X. L.; Trinchì, A.; Hardin, S.; Cole, I.; Zhu, Y. H.; Li, C. Z.; Muster, T.; Wei, G. Carbon Dots as Fluorescent Probes for “Off–On” Detection of Cu²⁺ and L-cysteine in Aqueous Solution. *Biosens. Bioelectron.* **2014**, 5, 330–335.

(39) Xu, Z. L.; Xu, S. L.; Luo, L.; Hua, X.; Lai, J. X.; Cai, X. P.; Liang, Q. W.; Lei, H. T.; Sun, Y. M.; Chen, Y. P.; Shen, X. Fluorescent Enzyme-linked Immunoassay Based on Silane-doped Carbon Dots for Sensitive Detection of Microcystin-LR in Water and Crucian Samples. *Sci. Total Environ.* **2020**, 708, 134614–134623.

(40) Liu, B.; Wang, X. P.; Sun, X. Y.; Yang, C. X.; Li, P. A Sensitive Immunosensor for Microcystin-(leucine–arginine) Based on Fluorescent Self-assembled Multilayers. *Anal. Methods* **2017**, 9, 282–286.

(41) Taghdisi, S. M.; Danesh, N. M.; Ramezani, M.; Ghows, N.; Shaegh, S. A. M.; Abnous, K. A Novel Fluorescent Aptasensor for Ultrasensitive Detection of Microcystin-LR Based on Single-walled Carbon Nanotubes and Dapoxyl. *Talanta* **2017**, 166, 187–192.

(42) Lv, J. J.; Zhao, S.; Wu, S. J.; Wang, Z. P. Upconversion Nanoparticles Grafted Molybdenum Disulfide Nanosheets Platform for Microcystin-LR Sensing. *Biosens. Bioelectron.* **2107**, 90, 203–209.

(43) Wang, S. J.; Duan, N.; Zhang, H.; Wang, Z. P. Simultaneous Detection of Microcystin-LR and Okadaic Acid Using a Dual Fluorescence Resonance Energy Transfer Aptasensor. *Anal. Bioanal. Chem.* **2015**, 407, 1303–1312.

(44) Wang, Y. L.; Zhu, Z. Y.; Teng, X.; Lai, Y. Q.; Pu, S. F.; Pang, P. F.; Wang, H. B.; Wang, C.; Barro, C. J.; Yang, W. R. Enzyme-free Fluorescent Detection of Microcystin-LR Using Hairpin DNA Templated Copper Nanoclusters as Signal Indicator. *Talanta* **2019**, 202, 279–284.