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Tuning MIP-QCM selectivity for zinc ions *via* cross-linker/monomer ratio

Jialing Song,[a] Charles Jordi Windle,[b] Haoxing Lai,[c] Melvin Zhi Yuan Low,[d] Fun Man Fung, *[e] Xuanhao Lin *[f] and Sam Fong Yau Li *[f,g]

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A highly selective and sensitive molecularly imprinted polymer (MIP) sensor on a quartz crystal microbalance (QCM) was developed for detecting zinc ions, Zn^{2+}, in water. This work provides key insights into the critical role of the cross-linker/monomer ratio in optimizing MIP selectivity and sensitivity. Computational screening identified optimal functional monomers, leading to a stable MIP architecture with high Zn^{2+} binding affinity. The optimized MIP-QCM sensor exhibited remarkable performance: an exceptional selectivity of 99.8% against competing metal ions, a linear range from 50 ppb to 2 ppm (R^2 = 0.9985) and a sensitivity of 51.9 ppb Hz^{-1}. The lower limit of quantification (LLOQ) was 38.1 ppb, meeting regulatory thresholds. A cross-linker/monomer ratio of 1.43 was found to be optimal, as lower ratios caused cavity collapse and higher ratios hindered template removal. Compared to conventional methods, this MIP-QCM sensor is low-cost, rapid, and user-friendly, showing significant potential for commercialization.

Introduction

Heavy metal pollution in aquatic systems represents a severe and growing threat to both ecological stability and human health (Fig. S1 and S2).[1] Among these contaminants, zinc is particularly pervasive, originating from widespread industrial activities such as electroplating, metallurgy, and chemical engineering. The unregulated discharge of zinc-containing wastewater leads to its accumulation in water bodies, contaminating vital water resources.[2,3] The urgency of this issue is twofold. Ecologically, even trace concentrations of zinc exhibit chronic toxicity to aquatic life, impairing physiological function, growth, and reproduction.[4] This threat is underscored by stringent environmental standards, such as Japan's 0.03 mg L^{-1} limit to protect aquatic organisms. Critically, however, the detection limit of conventional methods employed by many wastewater facilities is as high as 0.50 mg L^{-1}.[5] This analytical gap means that ecologically damaging zinc pollution often goes undetected and unmanaged. For human health, zinc bioaccumulates through the food chain, and excessive intake can lead to adverse effects ranging from acute gastric distress to chronic damage of the hematopoietic, endocrine, and reproductive systems.[6,7] Excessive intake of zinc can trigger a series of acute adverse reactions, such as nausea and gastric cramps. Prolonged exposure to zinc pollution may severely damage the human hematopoietic system, endocrine system, and reproductive system, leading to serious consequences such as anaemia, pancreatic and kidney damage, and infertility. In natural water bodies, the zinc content is usually at a relatively low level, generally less than 40 mg L^{-1}. However, in some areas severely polluted by industries, the zinc content in well-water can be as high as 24 mg L^{-1}.[4] For countries in the Global South with relatively low economic development levels and insufficient investment in technology research and development, there is a lack of advanced and cost-effective detection technologies. As a result, the extent of zinc pollution in these areas is often difficult to detect and effectively manage in a timely manner.

To address the challenges of detecting trace heavy metals in water, researchers have been constantly exploring and innovating and have developed a series of detection techniques. Among them, atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), and electrochemical methods

[a]International Joint Laboratory Catalyst Chemistry, Department of Chemistry, College of Science, Shanghai University, Shanghai 200444, China
[b]Yusuf Hamied Department of Chemistry, University of Cambridge, Lensfield Rd, Cambridge CB2 1EW, UK
[c]Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore
[d]School of Computing, NUS School of Computing, COM1, 13, Computing Dr, Singapore 117417, Singapore
[e]School of Chemistry, University College Dublin, Belfield, Dublin 4, D04 C1P1, Ireland. E-mail: funman.fung@ucd.ie
[f]Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore. E-mail: chmlifys@nus.edu.sg, xuanhao-lin@nus.edu.sg; Fax: +65 6779 1691; Tel: +65 6515 2681
[g]NUS Environmental Research Institute (NERI), #02-01, T-Lab Building (TL), 5A Engineering Drive 1, Singapore 117411, Singapore

are widely used.[8] However, these existing trace-metal analysis instruments have many limitations in practical applications.[9–12] For example, AAS equipment is expensive, with high maintenance costs, and the detection process is complex, requiring professional technicians to operate. AFS has strict requirements for the detection environment and is easily interfered with. The detection accuracy and selectivity of electrochemical methods need to be further improved.[12–14] In the face of disasters such as earthquakes and emergencies such as major leaks or spills, the limitations of these traditional detection techniques become even more prominent (Table S1).[15] Currently, rapid and accurate detection of heavy-metal concentrations is crucial for assessing the severity of pollution and taking effective countermeasures in a timely manner. When detecting zinc using AAS, the pretreatment alone requires a large amount of time, and the entire detection process takes 3 to 6 h, which clearly cannot meet the requirements for detection timeliness in emergency situations.[16,17]

Molecular imprinting technology, as an emerging and cutting-edge method for detecting heavy metals in water, has received extensive attention and favor in the scientific research community in recent years.[18,19] Molecularly imprinted polymers (MIPs) are polymer materials with unique molecular recognition functions. They contain structures with highly specific recognition sites for the analyte. Thanks to their remarkable stability, MIPs have demonstrated great application potential in many fields such as purification and separation, biosensing, and drug analysis, and have been widely applied and deeply studied.[20–22] However, existing MIP-based Zn^{2+} detection methods still face unresolved limitations that hinder practical application: fluorescence-based MIPs exhibit high limits of detection (LOD = 60–638.3 ppb) and poor anti-interference ability; electrochemical MIPs rely on complex nanomaterial modification (*e.g.*, MOF-derived composites) and lack stability in real wastewater; most reported MIPs neglect critical formulation optimization (*e.g.*, crosslinker/monomer ratio), leading to cavity collapse, incomplete template removal, or weak target binding affinity.[18] A few MIPs have been integrated with a quartz crystal microbalance (QCM) for rapid mass-sensitive detection, and none have achieved a balance of high selectivity, low LOD, rapid response, and compatibility with complex water matrices—key requirements for on-site monitoring.[23]

To fill this research gap, we developed a Zn^{2+}-specific MIP-QCM sensor through two core innovations: (1) density functional theory (DFT)-guided screening of functional monomers (MAA/HEMA/DMPAPS) to ensure strong Zn^{2+} binding *via* synergistic coordination, hydrogen bonding, and ionic chelation; and (2) systematic optimization of the crosslinker/monomer ratio (1.43 as optimal) to avoid cavity collapse and template removal hindrance—two longstanding challenges in MIP design. This study aims to provide a cost-effective, rapid, and highly selective detection method for Zn^{2+} in water, addressing the unmet need for practical on-site monitoring tools that outperform both conventional spectrometry (in speed and cost) and existing MIPs (in selectivity, sensitivity, and real-world applicability). The resulting sensor offers a viable technical solution for environmental monitoring and industrial wastewater compliance, particularly in resource-constrained settings.

Results and discussion

Observation of polymer deposition on the MIP chip surface

The deposition of the polymer on the surface of the MIP chip was observed using an optical microscope. It was successfully confirmed that the film of the final polymer solution was successfully applied to the MIP chip (Fig. 1). This result serves as the foundation for the smooth progress of subsequent experiments, ensuring that the MIP sensor has a complete functional structure. The uniform and stable deposition of the polymer film provides a material basis for the specific recognition and detection of zinc ions by the MIP sensor, guaranteeing that the sensor can effectively interact with the target zinc ions. If there are defects or non-uniformity in the polymer film, it may lead to a decrease in the sensor's ability to adsorb zinc ions, affecting the accuracy and reliability of the detection. In practical applications, uniform film deposition helps to improve the stability and repeatability of the sensor, making the detection results more comparable each time. Studies have shown that if the deviation in the uniformity of the film thickness exceeds a certain range, it will cause significant differences in the sensor's response to different zinc-ion concentrations, thereby affecting the detection accuracy.

Optimization of functional monomers

To determine the optimal functional monomers for the zinc-specific MIP, computational simulations were carried out to explore the binding affinities of different types of MIP functional monomers for Zn^{2+} ions. The density functional theory (DFT) method was applied on the ORCA program to screen for the best functional monomer that is able to most strongly interact with the zinc ions, which would allow for the formation of the most defined and specific zinc recognition site. From the ORCA output script (Fig. S5 and S6), the binding affinities of our template–monomer complex were calculated using the following equation[24] for commonly-used ion-imprinted MIP functional monomers:

$$\Delta E_{\text{bind}} = E_{\text{complex}} - (E_{\text{monomer}} + E_{\text{template}})$$

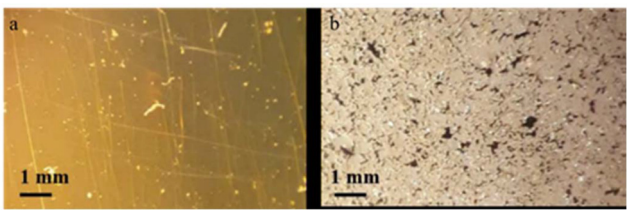

Fig. 1 QCM gold chips viewed under a microscope, 100× magnification. (a) Clean QCM chip without polymers. (b) QCM chip with MIP solution prepared for analysis of Zn^{2+} ions.

The magnitude of ΔE_{bind} for MAA and the zinc template was found to be very strong at 2533.237 Hartree, which is comparable with previous literature.[25] DMPAPS showed the highest binding affinity with the zinc template, while acrylamide had the least (Table S2). The simulation results indicated that MAA, HEMA, and DMPAPS exhibited the highest binding affinities for Zn^{2+} ions (Fig. S7). The mixed monomers (MAA/HEMA/DMPAPS, 1 : 1 : 1) achieved 99.8% selectivity for Zn^{2+} and a high sensitivity of 51.9 ppb Hz^{-1}, which are far superior to the performance of single-monomer systems implied by the binding affinity results (Table S3). MAA contains a carboxyl group that can form hydrogen bonds and coordination bonds with zinc ions, enhancing its binding ability to zinc ions. The hydroxyl group of HEMA can participate in the formation of hydrogen bonds. Additionally, its molecular structure helps to regulate the hydrophilicity and flexibility of the polymer, making it easier for the MIP to come into contact with and bind to zinc ions. DMPAPS contains special functional groups that can effectively chelate zinc ions through ionic interactions, further improving the specific recognition ability of the MIP for zinc ions. The synergistic effect of these three functional monomers enables the MIP to have a highly specific binding to zinc ions, laying a molecular foundation for the high selectivity and sensitivity of the sensor. Compared with other functional monomers that do not show high binding affinities, MAA, HEMA, and DMPAPS can more precisely construct specific recognition sites around the zinc-ion template during the formation of the MIP structure, greatly improving the sensor's recognition efficiency for zinc ions. Some studies have compared the performance of MIP sensors with different functional-monomer combinations and found that the absence of any one of these three key monomers leads to a significant decline in the sensor's selectivity and sensitivity to zinc ions.

Selectivity analysis

Experimental results demonstrated that our MIP exhibited an extremely high selectivity for Zn^{2+}, reaching 99.8% (calculated based on the theoretical frequency change at a given analyte concentration according to the Sauerbrey equation) (Fig. 2). Even in the presence of other metal ions such as Cu^{2+}, Ni^{2+}, Co^{2+}, and Mg^{2+}, which are similar to Zn^{2+} in terms of charge, atomic number, and ionic radius, the MIP still showed excellent selectivity for Zn^{2+}. As can be seen in Fig. 5, when exposed to other cations, the change in the frequency signal was significantly lower, with an average selectivity of only 11.8%. Under the control of the non-imprinted polymer (NIP), the selectivity for all ions was almost negligible. This fully demonstrates that the unique molecular structure of the MIP can accurately recognize Zn^{2+} ions and effectively exclude the interference of other ions. The specific recognition sites inside the MIP are highly complementary to Zn^{2+} ions in terms of shape, size, and charge distribution. Only Zn^{2+} ions can specifically bind to these sites, resulting in a significant change in the frequency signal and enabling highly selective detection of Zn^{2+} ions. In practical waste-sample detection, this high selectivity is of great importance. For example, in the detection of industrial wastewater, the wastewater usually contains multiple metal ions. Traditional detection methods are easily interfered with, while our MIP sensor can accurately measure the zinc-ion concentration, providing precise data support for wastewater treatment.

Sensitivity analysis and linear calibration

Our MIP-QCM was able to clearly distinguish between zinc-ion concentrations of 50 ppb, 200 ppb, 500 ppb, 1 ppm, and 2 ppm. Each data point plotted was obtained as the average of at least three readings, ensuring the reliability and accuracy of the data. A significant linear relationship was observed between the concentration and the frequency change, with an R^2 value as high as 0.9985 (Fig. 3). Compared with calibration using the Sauerbrey equation (R^2 value of 1), the fitting was nearly perfect. The slope of the calibration curve is a crucial indicator for measuring sensitivity. In this experiment, a frequency change of 1 Hz corresponded to a change of 51.9 ppb of Zn^{2+} ions, indicating that the sensor had an extremely high response sensitivity to minute changes in zinc-ion concentration. High sensitivity means that in practical detection, it can accurately detect extremely low-concentration zinc ions, meeting the increasingly stringent requirements for water-quality detection. The good linear relationship allows for the accurate calculation of the zinc-ion concentration within different concentration ranges through a simple mathematical model, greatly improving the detection efficiency and accuracy and providing great convenience for practical applications. Besides, using existing QCM frequency response data (50 ppb–

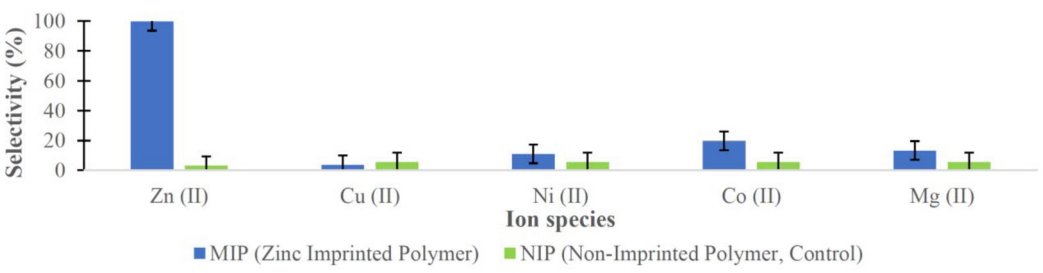

Fig. 2 Selectivity of QCM for various ions, selectivity at 100% based on the Sauerbrey equation. QCM pump flow rate: 150 μL min^{-1}.

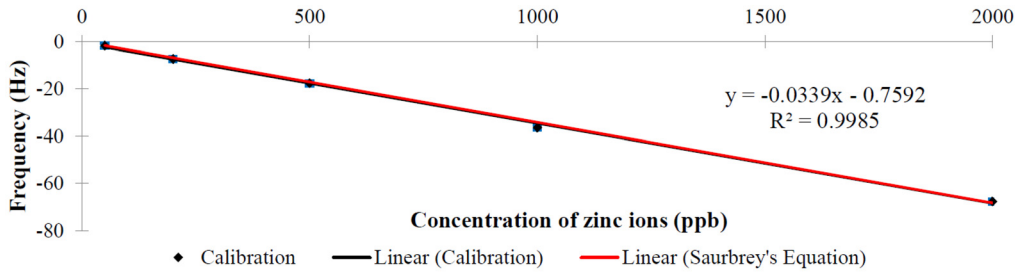

Fig. 3 Calibration line of frequency changes against concentration of Zn^{2+} ions. Frequency changes were measured over 5 min, QCM pump flow rate: 150 μL min^{-1}.

2 ppm), we conducted Scatchard analysis, yielding a dissociation constant (K_d) of 1.2×10^{-6} M and a linear correlation coefficient (R^2) of 0.992. This strong linearity confirms that the MIP primarily contains a single class of high-affinity binding sites, supporting relative uniformity. In the field of environmental monitoring, for the detection of zinc ions in rivers, lakes, and other water bodies, the high sensitivity and linear relationship of this sensor can promptly detect minute changes in zinc-ion concentration, contributing to the early warning of water-body pollution. Through long-term monitoring of multiple natural water bodies, it has been found that the sensor can capture the seasonal changes in zinc-ion concentration and the concentration fluctuations caused by sudden pollution events, providing a powerful basis for environmental-protection decision-making.

Optimization of the cross-linker/monomer ratio for the MIP

The cross-linker plays a crucial role in the MIP polymer matrix, and its amount directly affects the formation of highly stable and specific recognition sites. When the amount of cross-linker is too low, the cavities formed by the functional monomers cannot be effectively retained, and the structure is prone to collapse, resulting in the loss of the MIP's ability to recognize zinc ions.[26] On the other hand, an excessive amount of cross-linker makes it difficult to remove the zinc template from the polymer chain, reducing the number of recognition sites for Zn^{2+}-ion binding and also leading to low selectivity for Zn^{2+} ions. Different MIP chips were prepared with varying amounts of cross-linker. The experimental frequency changes were compared with the theoretical prediction results according to the Sauerbrey equation, and the selectivity of Zn^{2+} ions was calculated (Table 1). The results showed that when the molar ratio of cross-linker to monomer was 1.43, the selectivity for Zn^{2+} ions was the highest, reaching 76.3%. This optimal ratio ensures that the MIP can form stable and sufficient specific recognition sites, enabling efficient recognition and binding of Zn^{2+} ions.[27] In the actual preparation of MIP sensors, strict control of the cross-linker–monomer ratio can guarantee the stability and reliability of the sensor performance, providing strong support for large-scale production and commercial applications. In the process of large-scale production, precise control of the cross-linker/monomer ratio can improve the product-qualification rate and reduce production costs. Through performance tests on different batches of products, it has been found that MIP sensors prepared strictly according to the 1.43 ratio have the best performance consistency.

Table 1 The effect of crosslinker–monomer ratio on the selectivity of Zn^{2+} ions. The functional monomers comprise a mix of DMPAPS, HEMA and MAA, used at a 1 : 1 : 1 molar ratio. QCM pump flow rate: 270 μL min^{-1}

	MIP1	MIP2	MIP3	MIP4
Crosslinker : monomer (molar ratio)	1.00	1.43	2.50	4.97
Frequency change (Hz)	10.2	18.8	11.0	8.54
Selectivity of Zn^{2+} ions (%)	41.3	76.3	44.8	34.8

Analysis of the lower limit of quantification (LLOQ)

During the oscillation of the QCM sensor, small fluctuations in the resonant frequency are inevitable. To study this fre-

Table 2 Comparison with Zn^{2+} ion detection performance reported in various reports

Materials	Method	Limit of detection (LOD) (ppb)	Detection range (mg L^{-1})	Ref.
PLA/PEO@PAN	Colorimetric	60	15	28
Carbon dots	Fluorescence	78.5	2.5–50	29
Terpyridine derivative	Fluorescence	638.3	9.76–75	30
Lanthanide-doped upconversion nanoparticles	Fluorescence	51.1	15–90	31
Quinoline-based flavanol fluorescent probe	Fluorescence	326.2	—	32
Unmodified glassy carbon electrode	Electrochemical	45.3	—	33
$BiCu_{0.5}$-ANPs@CF/SPCE	Electrochemical	35	0.15–0.6	34
Molecularly imprinted polymer	Quartz crystal microbalance	38.1	—	This work

quency change, deionized water was run at a constant flow rate on the NIP for 30 minutes. A stable signal was observed, with a fluctuation amplitude of 0.50 Hz. According to the Sauerbrey equation, this quantification error was 7.62 ppb. Since the lower limit of quantification (LLOQ) on the calibration curve requires that the frequency-change response of the zinc analyte be at least 5 times greater than that of the blank, under the experimental conditions of this study, the LLOQ of deionized water was 38.1 ppb (Fig. 4). This LLOQ value is close to the allowable Zn^{2+}-ion emission limit in Japan and also within the allowable limit of NEA, indicating that our MIP-QCM sensor has high sensitivity in detecting low-concentration Zn^{2+} ions (Table 2). Although the LLOQ of this sensor is slightly higher than that of methods such as atomic absorption spectrometry (AAS), which is approximately 5 ppb, considering its advantages in terms of cost and ease of operation, it still has high practical value in practical applications. In many practical detection scenarios, there is no need to detect extremely low-concentration zinc ions, and the LLOQ of 38.1 ppb can meet the requirements of most environmental-monitoring and industrial-wastewater-treatment applications. In the wastewater treatment of some small-scale enterprises, this sensor can quickly detect whether the zinc-ion concentration meets the standards without complex operation and high equipment-maintenance costs, greatly reducing the detection costs of enterprises.

Analysis of real water samples

To evaluate the practical applicability of the MIP-QCM sensor, its performance was tested in diverse real water matrices, including tap water, river water, and industrial wastewater. The samples were spiked with known concentrations of Zn^{2+} (100 and 500 ppb). The Zn^{2+} concentrations determined by our sensor were compared with those obtained by inductively coupled plasma mass spectrometry (ICP-MS), the standard reference method. As summarized in Table 3, the recovery rates ranged from 95.4% to 104.2%, with relative standard deviations (RSD) below 5% (n = 3). These results demonstrate the high accuracy and reliability of the sensor in complex environments. The excellent recovery rates, particularly in the wastewater sample, underscore the sensor's strong anti-interference capability and its potential for real-world environmental monitoring and industrial effluent compliance checking.

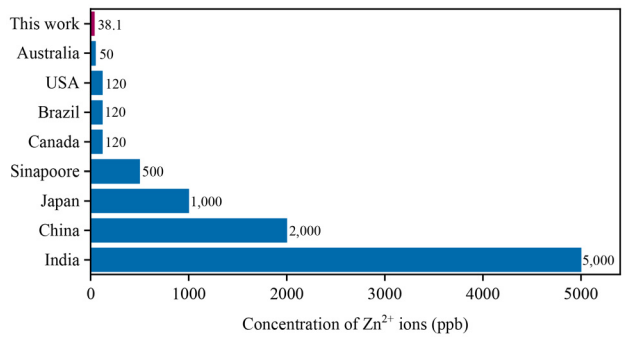

Fig. 4 Allowable limits for effluent discharge of Zn^{2+} ions.

Table 3 Determination of Zn^{2+} in real water samples using the MIP-QCM sensor (n = 3)

Sample type	Added (ppb)	Found (ppb)	Recovery (%)	RSD (%)	ICP-MS (ppb)
Tap water	100	98.5	98.5	3.2	101.1
	500	495.3	99.1	2.8	488.7
River water	100	104.2	104.2	4.1	102.5
	500	512.5	102.5	3.5	505.8
Industrial wastewater	100	95.4	95.4	4.7	97.9
	500	498.7	99.7	3.9	503.2

Sensor reusability and stability

The economic viability and practical utility of a sensor heavily rely on its reusability and operational stability. The reusability of the MIP-QCM sensor was investigated by performing ten consecutive adsorption–desorption cycles using a 500 ppb Zn^{2+} solution. After each measurement, the bound Zn^{2+} was desorbed with 0.01 N HNO_3, followed by rinsing with deionized water until a stable baseline was recovered. As quantified in Fig. 5a, the sensor retained over 92% of its initial frequency response throughout all ten cycles, demonstrating excellent regeneration capability and robustness. The stability was further assessed by storing the sensor at 4 °C and testing its response to 500 ppb Zn^{2+} at weekly intervals. The results, summarized in Fig. 5b, show that the sensor maintained 94% of its original signal after 4 weeks of storage, indicating satisfactory long-term stability for practical applications.

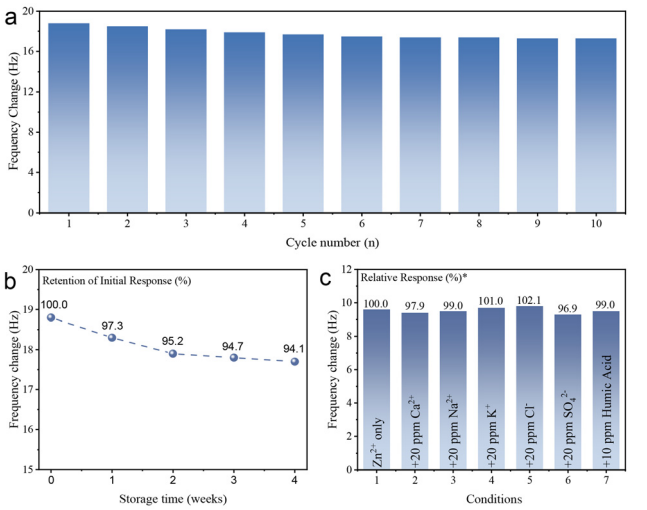

Fig. 5 (a) Reusability of the MIP-QCM sensor over consecutive adsorption–desorption cycles; (b) long-term stability of the MIP-QCM sensor over 4 weeks of storage at 4 °C; (c) interference study: frequency response of the MIP-QCM sensor to 200 ppb Zn^{2+} in the presence of potential interfering substances (*relative response (%) = (frequency change with interferent/frequency change of Zn^{2+} only) × 100%).

Comprehensive interference study

While the sensor exhibited exceptional selectivity against other metal cations, real water samples contain a myriad of potential interferents. To further validate the anti-interference capability, the sensor's response to Zn^{2+} was tested in the presence of high concentrations of common inorganic ions and organic matter. As depicted in Fig. 5c, the frequency change for 200 ppb Zn^{2+} was not significantly affected by the addition of 20 ppm of Ca^{2+}, Na^{+}, K^{+}, Cl^{-}, SO_4^{2-}, or 10 ppm of humic acid. The signal variation was within ±5% of the response from the pure Zn^{2+} solution. This confirms that the specific recognition cavities within the MIP matrix effectively shield the binding sites from non-specific interactions, ensuring accurate Zn^{2+} quantification even in complex water chemistries.

Conclusions

In summary, this study details the development and optimization of a zinc-specific MIP sensor for detecting Zn^{2+} ions in water systems. Observation of the polymer deposition on the MIP chip confirmed the sensor's structural integrity. The selected functional monomers provided the molecular basis for the sensor's selectivity and sensitivity. The sensor's performance in selectivity, sensitivity, and its lower limit of quantification (LLOQ) enabled the detection of zinc ions across a defined concentration range. The cross-linker–monomer ratio was identified as a critical parameter for achieving stable sensor performance. Compared to spectroscopic and electrochemical methods, the MIP-QCM sensor offers the advantages of lower cost, rapid detection, and simpler operation. The sensor demonstrated practical applicability by quantifying Zn^{2+} in diverse real water matrices, showing high recovery rates and resilience against common interferents. Scatchard analysis provided experimental evidence for the presence of high-affinity binding sites within the MIP. Furthermore, the sensor exhibited batch-to-batch reproducibility, reusability over multiple cycles, and long-term stability. These performance metrics indicate the sensor's potential for real-world environmental monitoring and commercial applications.

Author contributions

Jialing Song: validation, formal analysis, investigation, writing – original draft, and writing – review and editing; Charles Jordi Windle: investigation and writing – review and editing; Haoxing Lai: software, validation, formal analysis, and writing – review and editing; Melvin Zhi Yuan Low: software, validation, and formal analysis; Fun Man Fung: supervision, conceptualization, methodology, resources, project administration, funding acquisition, and writing – review and editing; Xuanhao Lin: supervision, conceptualization, methodology, validation, formal analysis, resources, project administration, writing – review and editing, and visualization; and Sam Fong Yau Li: conceptualization, methodology, validation, formal analysis, resources, writing – review and editing, visualization, supervision, project administration, and funding acquisition.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The detailed experimental section, additional figures tables are listed in the Supporting Information (SI). The authors have cited additional references within the Supporting Information. See DOI: https://doi.org/10.1039/d5an01106j.

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