Determination of Microcystin-LR in waters in the subnanomolar range by sol–gel imprinted polymers on solid contact electrodes

Raquel B. Queirós,ab João P. Noronha,c Paulo V. S. Marques,b João S. Fernandesd and M. Goreti F. Salesa

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The present work reports new sensors for the direct determination of Microcystin-LR (MC-LR) in environmental waters. Both selective membrane and solid contact were optimized to ensure suitable analytical features in potentiometric transduction. The sensing layer consisted of Imprinted Sol–Gel (ISG) materials capable of establishing surface interactions with MC-LR. Non-Imprinted Sol–Gel (NISG) membranes were used as negative control. The effects of an ionic lipophilic additive, time of sol–gel polymerization, time of extraction of MC-LR from the sensitive layer, and pH were also studied. The solid contact was made of carbon, aluminium, titanium, copper or nickel/chromium alloys (80:20 or 90:10). The best ISG sensor had a carbon solid contact and displayed average slopes of 211.3 mV per decade, with detection limits of 7.3 × 10⁻¹⁰ M, corresponding to 0.75 μg L⁻¹. It showed linear responses in the range of 7.7 × 10⁻⁹ to 1.9 × 10⁻⁸ M of MC-LR (corresponding to 0.77–2.00 μg L⁻¹), thus including the limiting value for MC-LR in waters (1.0 μg L⁻¹). The potentiometric-selectivity coefficients were assessed by the matched potential method for ionic species regularly found in waters up to their limiting levels. Chloride (Cl⁻) showed limited interference while aluminium (Al³⁺), ammonium (NH₄⁺), magnesium (Mg²⁺), manganese (Mn²⁺), sodium (Na⁺), and sulfate (SO₄²⁻) were unable to cause the required potential change. Spiked solutions were tested with the proposed sensor. The relative errors and standard deviation obtained confirmed the accuracy and precision of the method. It also offered the advantages of low cost, portability, easy operation and suitability for adaptation to flow methods.

1. Introduction

The quality of freshwaters is a worldwide concern. Many pollutants may endanger human health and there are no wastewater treatment facilities capable of handling specific contaminants with a generalist approach.¹,² When these pollutants outcome from a natural phenomenon, the risk is especially high and uncontrolled. This is the case of bacterial and algae contamination of rivers, fluvial beaches and other water sources for human consumption.

Contamination of waters by cyanobacteria is very common in the summer season.³–⁵ Cyanobacteria, also known as blue-green algae, are Gram-negative photosynthetic prokaryotes found in different habitats.⁶ The occurrence of cyanobacteria blooms is favoured by light intensity, high nutrients concentration, especially nitrogen, phosphorus, and warm temperature.⁷,⁸ These blooms cause drastic reduction of oxygen concentration leading to death of many aquatic organisms and changes in the colour and odour of the water.⁹ They may also be potential disseminators of hazardous cyanotoxins.⁷,⁹,¹⁰

Microcystins (MCs) are a relevant group of toxicants among cyanotoxins.⁶,¹¹–¹⁴ At least 80 MCs have been identified, although only a few of them are regularly found in high concentrations.¹⁵ MCs are a group of cyclic-heptapeptides¹ including two variable amino acids and an unusual aromatic amino acid, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (ADDA) containing a substituted phenyldecadienoic acid.¹⁶

Microcystin-LR (MC-LR) is one of the most frequent and toxic MC congeners containing leucine and arginine in variable sites. It has been implicated in incidents of animal⁵,⁷,¹⁷,¹⁸ and human toxicity,¹⁹ being correlated to the increase of primary liver cancer in China²⁰ and gastroenteritis and stop breathing episodes,²¹ through consumption of contaminated drinking water or recreational activities such as swimming. On account of this, some international organizations, like WHO, recommended for cyanotoxin MC-LR a limiting value of 1 μg L⁻¹ in drinking waters.⁹ EPA-USA included MC-LR in the Contaminant
Candidate List 3 (CCL3), and EU recommended a regular monitoring of this contaminant in the Water Frame Directive.

Classic methods for detection and quantification of MC-LR include biochemical techniques, such as enzyme linked immunoassorbent (ELISA) and phosphatase assays.8,22 These methods are highly sensitive and specific, but turn out expensive and require special conditions and operators for the manipulation of biological materials. In addition, they take 1–3 days to give a result, which is too long to implement effective/corrective measures. Most recent methods rely on chromatographic techniques. They discriminate different MCs but they are unable to carry out on-site analysis, which is fundamental to ensure public health.

Presently, many efforts are undertaken to detect MC-LR by means of rapid tests.7,24,25 Still, a simple, low cost, portable and selective method is necessary for MC-LR determination in loco. Ion-selective electrodes (ISEs) with suitable membranes and potentiometric transduction may suit this purpose. They offer high precision and rapidity, low cost of analysis, and enhanced selectivity over a wide range of concentrations.26 Their selectivity may be enhanced by designing sensory materials targeted for a specific compound by means of Molecular Imprint (MI). Different routes may be taken for this purpose. Considering that MC-LR is a complex biological structure, it should be imprinted in mild conditions such as those from sol–gel techniques.

Sol–gel materials with MI can be prepared as indicated in Fig. 1. An organic solution containing the functional sol–gel precursors and the template molecule (MC-LR in this case) is allowed to stand for molecular pre-organization according to in situ electrostatic interactions. As the precursors follow hydrolysis and polycondensation reactions, an interconnected 3-D porous network is formed around the template. Template removal leaves behind free sites able to rebind MC-LR molecules.

This concept of coupling sol–gel MI to ISEs has been proven feasible on a preliminary work but not yet effective for in situ and direct determinations of MC.27 Thus, the present work describes the selection of a suitable (i) sol–gel MI composition for MC (effects of ionic lipophilic additive, time of sol–gel polymerization, ratio of water/alkoxide and solvent/alkoxide, polymerization temperature and pH are studied) and (ii) solid-contact used for potentiometric transduction (carbon, metal or alloy). Only electrodes of solid contact are considered because they are easily adapted to chip-based devices (highly desired in local monitoring operations) by coating a solid conductive layer with the selective membrane.28,29 All prepared sensors are evaluated, characterized and applied.

2. Materials and methods

2.1 Apparatus

All potential measurements were made by a Crison pH-meter GLP 21 (+0.1 mV sensitivity), at room temperature, and under constant stirring. The signal was connected to six ISEs by a homemade switchbox. The assembly of the potentiometric cell was as follows: conductive graphite or metal | MC-LR selective membrane | buffered sample solution | electrolyte solution, KCl | AgCl(s) | Ag. The reference electrode was a Crison 5240 Ag/AgCl double-junction. The carbon-based selective electrodes had an epoxy–graphite mixture as the solid contact. The metal-based electrodes were constructed with 5 different metals: aluminium, titanium, copper, and two alloys of nickel/chromium (90 : 10) and (80 : 20). The temperature curing condition was annealed for titanium, nickel/chromium (90 : 10), aluminium and copper and was hard for nickel/chromium (80 : 20).

Voltammetric and electrochemical impedance spectroscopy measurements were carried out with a Potentiostat Autolab PGSTAT128N with FRA module. Reference and auxiliary electrodes were of Ag/AgCl (Crison 5240) and platinum (Basi MW-1032 mm, 7.5 cm length). N2 of 6.0 quality grade (≥99.9999%, Air Liquide) was used for degassing.

2.2 Reagents

Deionized water was used throughout. All reagents were pro-analysis grade and used without further purification. MC-LR, potassium tetrakis(4-chlorophenyl)borate (TKCIPB) and (3-aminopropyl)triethoxysilane (APTES) were purchased from Fluka. Diphenyldimethoxysilane (DPDMS) and tetraethoxysilane (TEOS) were purchased from ABCR. Hydrochloric acid (HCl) and methanol (MeOH) were from Merck. Ethanol (EtOH) was purchased from Panreac.

Buffer solutions were 1 × 10−3 M piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES) pH 6.6, 1 × 10−1 M phosphate buffered saline (PBS) pH 7.4 and 0.01 M sodium borate (Borax) pH 9.4. These were prepared in deionized water. The pH values were adjusted by adding the appropriate volumes of either concentrated HCl or orthophosphoric acid, or saturated sodium hydroxide (NaOH) solution, freshly prepared.

![Fig. 1 Process of sol–gel MI.](image)

Fig. 1 Process of sol–gel MI. (a) Electrostatic arrangement of template molecule and (functional) alkoxide precursors; (b) polymerization with the solution becoming increasingly thick; (c) MC-LR extraction, leaving behind the recognition cavities with specific sites capable of binding the target molecule.
A stock solution of 2.00 μg L⁻¹ MC-LR was prepared in buffer by rigorous dilution of the commercial solution (10.025 μg mL⁻¹ in ethanol). Less concentrated solutions were prepared by rigorous dilution of this one in buffer.

2.3 Preparation of sensing membrane

The MC-LR ISG membrane was prepared from a reagent mixture of 10 μL of the MC-LR commercial solution, 3 mL of APTES, 3 mL of DPDMS, 10 mL of MeOH and 2.78 to 11.1 μL of TKClPB 3.63 × 10⁻⁴ M. This mixture was stirred for 30 minutes at 60 °C. Then, the resulting solution was hydrolyzed slowly with 1 mL TEOS; 500 μL HCl 0.1 M; 5 mL EtOH; and 1.5 mL deionised water for 30–240 minutes, also at 60 °C. The mixture was deposited on the solid contact by drop (carbon-based electrodes) or dip-coating (metal-based electrodes) and then cured at 60 °C for 12 h in a moisture atmosphere. The template was removed by washing the polymer in deionised water for 3 h (2 × 1.5 h). The NISG was also synthesized similarly, replacing the template MC-LR by the same volume in ethanol.

2.4 Potentiometric measurements

All potentiometric measurements were carried out at room temperature. Electromotive force (emf) values of each electrode were measured in solutions of fixed pH and ionic strength. Calibrations were made by decreasing the initial concentration of MC-LR because the total reagent spent in each procedure is much less than for increasing concentrations. This was done by dipping the electrodes in 5 mL of a 2.0 μL⁻¹ MC-LR solution and adding consecutively increasing amounts of buffer (from 1 to 5 mL). The obtained MC-LR concentrations ranged from 0.37 to 0.5 μL⁻¹. Potential readings were recorded after 2 min and emf was plotted as a function of log MC-LR molar concentration.

2.5 Procedures for selectivity assays

The potentiometric selectivity coefficients (K_str) were assessed by the Matched-Potential Method (MPM). For this purpose, the change in emf caused by the principal ion was measured for a MC-LR rise in concentration from 1 to 5 μL⁻¹. Similarly, the emf of a 1 μL⁻¹ MC-LR solution was measured and a solution of interfering species added until the previous potential change was reached. Solutions of 200 μL⁻¹, 500 μg L⁻¹, 250 mg L⁻¹, 50 μg L⁻¹, 50 mg L⁻¹, 200 mg L⁻¹ or 250 mg L⁻¹ in Al³⁺, NH₄⁺, Cl⁻, Mg²⁺, Mn²⁺, Na⁺, and SO₄²⁻, respectively, were tested for this purpose. All these were prepared in buffer.

2.6 Electrochemical measurements

Cyclic voltammetry (CV) and Electrochemical Impedance Spectroscopy (EIS) assays were carried out at room temperature. The reference and counter electrodes were Ag/AgCl (3 M in KCl) and platinum, respectively. The measuring solution was a 1 × 10⁻³ M PIPES buffer (pH 6.5) with 5 mM of [Fe(CN)₆]₃⁻/₄⁻, degassed by bubbling for ~30 seconds with N₂. CV measurements were done between −1.5 and 1.5 V, with 0.10 V s⁻¹ scan-rate, 0.0025 V step and 10 μA current range. EIS frequency ranges were single sine between 0.1 and 10 000 Hz with an amplitude of 0.005 V. The standard potential (indicated by CV assays) was −0.0979 V, 0.176 V, 0.795 V, 0.410 V, and 0.400 V for carbon, titanium, aluminium, copper, nickel/chromium (90 : 10) and nickel/chromium (80 : 20) alloys.

2.7 Segment sandwich membrane method

The segmented sandwich membrane method is based on direct potential measurements arising from ligand–ion complexation. The electrode was prepared by coating the carbon conductive support with the NISG material. An additional layer of ISG material was deposited on top of it after drying. The electrode was tested in PIPES buffer at pH 6.5 with a MC-LR concentration of 1 μg L⁻¹. Emf was measured each 5 min, along 155 min (the period after which it remained constant).

3. Results and discussion

3.1 MC-LR sol–gel membrane on carbon contacts

MC-LR ISG selective membranes on a carbon solid contact prepared with APTES, DPDMS and TEOS presented mostly super-Nernstian responses and positive slopes, although a few negative slopes were observed. This behaviour was attributed to the polyionic nature of MC-LR. It has three ionisable groups out of the cyclic peptide structure (Fig. 1). Following Nernst equation, equilibrium partitioning between the membrane and aqueous phases would result in very small potential changes. However, a non-equilibrium ion-exchange process between a polyelectrolyte in the aqueous phase and a counter ion of the ion-exchanger sites in the membrane phase may cause a large potential change, resulting in a non-equilibrium super-Nernstian response.

This possible change of sign was controlled by including in the membranes an anionic lipophilic additive, TKClPB, thus forcing the electrode to respond preferentially to positively charged species of MC-LR. In addition, these anionic sites improve the permselectivity of the membranes for cations in carrier-free membranes and enhance the performance of solid contact ISEs.

The time given for hydrolysis/polycondensation and template leaching of the sol–gel matrix was also optimized.

3.1.1 Effect of additive. Membranes with 8, 30 and 120 μmol of TKClPB in a sol–gel matrix were prepared and evaluated with regard to their potentiometric performance. The corresponding sensors displayed different sensitivities and detection limits. They showed linear responses in the concentration ranges 0.77–2.00, 0.77–2.00, and 0.77–1.67 μg L⁻¹, with slopes of 169.5, 180.7, and 126.0 mV per decade and detection limits of 0.77, 0.77 and 0.67 μg L⁻¹, respectively. These results pointed out that a minimal amount of TKClPB was enough to control the net electric charge sensed by the membrane. Thus, the amount of additive was set to 8 μmol.

3.1.2 Hydrolysis/polycondensation time. The time given for sol–gel hydrolysis/polycondensation affects the overall physical properties of the membrane, including hardness, porosity and integrity. These, in turn, affect with significance the
potentiometric response. For a suitable transduction signal, the outer membrane must hydrate, keep its integrity and have the suitable porosity to enable (re)binding of MC-LR on the sensing layer. Furthermore, a good adhesion between the conductive support and the sol–gel membrane must be ensured to avoid water permeation into this internal layer.

The time required to obtain a suitable sol–gel matrix was selected by letting the reaction take place for 30–240 minutes. The sensors prepared with 30 and 60 minutes presented cracked membranes that could be easily detached from the solid-contact. The membrane obtained by 120 minutes did not crack but dissolved in water while the template was being extracted, indicating that the hydrolysis was incomplete. After 240 minutes the membrane glazed and coating the electrode was impossible. Only membranes prepared after 180 minutes were without cracks and water resistant. The corresponding ISE sensors showed linear responses from 0.67 to 1.67 μL−1 of MC-LR, slopes of 140.5 mV per decade and detection limits of 0.67 μL−1. Thus, this time was considered as optimal for preparing MC-LR sol–gel membranes.

3.1.3 Template extraction. ISG membranes carry template molecules (MC-LR) that should be leached before conducting the potentiometric assays. This procedure ensures that the sites to which MC-LR species in the sample should bond are vacant. MC-LR presents enough solubility in water to be extracted by it from the sol–gel matrix. This is an inexpensive and innocuous material while the use of another solvent could lead to an irreversible damage/alteration of the sensing layer.

Different washout times were tested aiming to extract the maximum amount of MC-LR while ensuring the integrity of the membrane. The extraction of MC-LR from the ISG outer layer was studied for 3 or 6 consecutive hours. The water phase was changed each 1.5 hours to avoid its saturation with MC-LR and enhance the extraction efficiency.

After washing out MC-LR for 3 hours, the corresponding ISEs displayed linear responses in the range of 0.77–1.67 μL−1 of MC-LR, average slopes of 180.0 mV per decade, and detection limits of 0.77 μL−1. When these exact electrodes were subjected to an additional 3 hours washout period, the membranes became opaque (previously transparent) and the electrodes useless. The same result was observed for a 6 hours template extraction period and it became unsuitable to conduct further potentiometric analysis. Thus, the extraction time was set to 3 hours.

3.2 Effect of pH

The effect of pH in a sol–gel potentiometric sensor is a crucial aspect to consider. It affects both the sensing sol–gel layer and the MC-LR ionic structure. Furthermore, the dominant MC-LR species in highly acidic media is cationic while in alkaline media is anionic. This study was carried out by testing three different buffer solutions with pHs ranging from 6.5 to 9.4, the pH ranges in environmental waters. Different buffers were selected for this purpose, meaning that the performance of the electrodes was connected to both pH and nature of the buffer. The obtained analytical features were indicated in Table 1.

The best analytical features were found for pH 6.5 (PIPES buffer). As may be seen in Fig. 2, a slight pH change to 7.4 increased the lower limit of linear range and the limit of detection (LOD) but extended the linear behaviour. Further increase to pH 9.4 gave no emf changes (Fig. 2). The predominant form of MC-LR in this pH is anionic, thus hindering its interaction with a membrane that carried an anionic lipophilic additive (also playing as an anionic excluder).

The similarity between ISG and NISG membranes obtained in pH 7.4 indicated, however, that the recognition of the template was made by means of general electrostatic interactions instead of stereo-chemical effects. Increased specific detection of MC-LR was achieved by decreasing the pH down to 6.5. Under this condition, the average slope of the ISG biosensors was ~20% higher than that of the NISG. The limit of detection was also enhanced, decreasing in 20%. For this reason, the pH 6.5 was used in subsequent experiments.

The overall differences between ISG and NISG were small and attributed to the stereochemical recognition of the analyte by the imprinted sites. The major response of the electrode resulted from non-specific electrostatic interactions between the sol–gel matrix and the target analyte. Overall, these materials were found suitable for potentiometric ionophores, with the

| Table 1 Characteristics of membrane/solid contact tested for MC-LR selective readings in solutions of different pHs, along with the corresponding analytical features (n = 2) |
|-----------------|--------------|----------------|-----------------|--------|------|
| ISE | Membrane | Solid contact | Buffer, pH | Linear range/μL−1 | Slope/mV per decade | LOD/μL−1 | r² |
|---|---|---|---|---|---|---|
| I | ISG | Carbon | PIPES, 6.5 | 0.77–1.67 | 211.3 | 0.73 | 0.993 |
| II | NISG | Carbon | PIPES, 6.5 | 0.77–1.67 | 179.7 | 0.75 | 0.992 |
| III | ISG | Carbon | PBS, 7.4 | 0.91–2.00 | 58.2 | 0.91 | 0.988 |
| IV | NISG | Carbon | PBS, 7.4 | 0.91–2.00 | 59.3 | 0.91 | 0.995 |
| V | ISG | Carbon | Borax, 9.4 | — | — | — | — |
| VI | NISG | Carbon | Borax, 9.4 | — | — | — | — |
| VII | ISG | Aluminium | PIPES, 6.5 | 0.77–1.00 | 594.3 | 0.77 | 0.983 |
| VII | NISG | Aluminium | PIPES, 6.5 | 0.77–0.91 | 740.8 | 0.77 | 0.963 |
| IX | ISG | Titanium | PIPES, 6.5 | 0.91–1.67 | −103.0 | 0.91 | 0.997 |
| X | NISG | Titanium | PIPES, 6.5 | 1.00–2.00 | 75.7 | 1.00 | 0.985 |
| XI | ISG | Copper | PIPES, 6.5 | 0.77–1.00 | −1019.3 | 0.77 | 0.986 |
| XII | NISG | Copper | PIPES, 6.5 | 0.77–1.25 | −560.2 | 0.77 | 0.971 |
| XIII | ISG | Alloy Ni90Cr10 | PIPES, 6.5 | 0.77–2.00 | 220.6 | 0.75 | 0.991 |
| XIV | NISG | Alloy Ni90Cr10 | PIPES, 6.5 | 0.84–2.00 | 132.0 | 0.77 | 0.993 |
| XV | ISG | Alloy Ni80Cr20 | PIPES, 6.5 | 0.91–1.67 | 173.0 | 0.84 | 0.996 |
| XVI | NISG | Alloy Ni80Cr20 | PIPES, 6.5 | 0.77–1.43 | 142.0 | 0.84 | 0.997 |
imprinted ones displaying higher slopes. Their ability to discriminate selectively MC-LR among other ionic species is evaluated by selectivity studies, addressed later in the manuscript.

3.3 Carbon versus metal solid contact

The material of the solid contact is important because it should communicate the events occurring in the membrane. Mostly, they should be inert and offer high conductivity. A good adhesion to the membrane should also be observed, preventing the sample from permeating and contacting the conductive material directly. In this study, 5 different metals were coated with the same ISG or NISG composition (ISEs VII to XVI) and compared to the carbon-contact electrodes (I and II). All these measurements were conducted in PIPES buffer of pH 6.5.

As may be seen in Fig. 3, most of the ISG membranes applied on metals did not show a suitable potentiometric performance. In addition, negative slopes were observed for ISEs IX, XI and XII despite the negative anionic additive in the membrane and the pH of the test solution. The best general behaviour was recorded for carbon and nickel/chromium (90 : 10) (Table 1). All these measurements were conducted in PIPES buffer of pH 6.5.

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mium (90 : 10) and nickel/chromium (80 : 20)), in 10/C0 conductive supports (carbon, aluminium, titanium, copper, nickel/chromium for nickel/chromium (80 : 20) and 0.098 for carbon) for different

75 points; amplitude of 0.005 V; potential was 0.861 for titanium, 0.176 for

(A) CV (potential range between −1.5 V and 1.5 V; step of 0.0025 V and scan rate of 0.10 V s⁻¹) and (B) EIS (frequency range 0.1 to 10 000 Hz; 75 points; amplitude of 0.005 V; potential was 0.861 for titanium, 0.176 for aluminium, 0.795 for copper, 0.410 for nickel/chromium (90 : 10), 0.400 for nickel/chromium (80 : 20) and 0.098 for carbon) for different conductive supports (carbon, aluminium, titanium, copper, nickel/chromium (90 : 10) and nickel/chromium (80 : 20)), in 10⁻³ M of PIPES buffer (pH 6.5) with 5 mM [Fe(CN)₆]³⁻/⁴⁻. The different conductive electrodes were tested against an Ag/AgCl (3 M in KCl) reference electrode and a platinum wire counter electrode. Inset: lower Z range.

In general, nickel/chromium alloys and carbon displayed smaller resistances to charge transfer (Fig. 4B). Only the nickel/chromium alloy (90 : 10) showed measurable resistances in EIS studies, with the other materials having almost linear responses (Fig. 4B). Their conductivity ability followed this order: nickel/chromium (90 : 10) < nickel/chromium (80 : 20) < carbon. This may be easily seen on the CV voltammogram (Fig. 4A). Overall, ISE/carbon electrodes exhibited a much higher sensitivity than those relying on nickel/chromium alloys and offered a much higher potential stability. The potential drift observed for the ISG membranes applied on nickel/chromium alloys seemed to corroborate with the formation of an aqueous layer at the interface of the sensing membrane/alloy. Since the composition of this aqueous layer was not controllable, the emf changed in an unpredicted manner.³²,³³

In general, considering both signal stability and sensitivity data of all electrodes, the electrode of carbon solid contact showed much better operating features for the intended purposes and was selected for the following studies.

3.4 Sensor selectivity

According to 307/2008 Portuguese Law (DL 307/2007) the most relevant ions controlled in waters for human consumption are Al³⁺, NH₄⁺, Cl⁻, Mg²⁺, Mn²⁺, Na⁺, and SO₄²⁻.³⁴ Thus the selectivity of the chemical sensor was tested against these species. Their maximum admitted levels were used as maximum interfering concentrations because their presence in environmental waters above these limits is highly unlikely. These were 200 µg L⁻¹, 500 µg L⁻¹, 250 mg L⁻¹, 50 µg L⁻¹, 50 mg L⁻¹, 200 mg L⁻¹ and 250 mg L⁻¹, respectively.

The potentiometric selectivity coefficients (Kᵢᵢʲ) for a main ion (I) against a foreign one (J) were assessed by the MPM, first reported in ref. 35. This method is applicable to almost any sensor and does not require that a Nernstian response is observed.³⁶ The values of Kᵢᵢʲ were obtained by following eqn (1) where aᵢ is the interfering concentration required to give the same potential jump as the concentration of aᵢ (both added over a common background of I).

\[ K_{ij}^{pot} = \frac{\Delta a_{ij}^{(1)}}{a_{ij}^{(1)}(IJ)^{2}} \]  

Almost none of the tested species was able to provide the potential change of the primary ion solution, indicating that the electrodes displayed a very high selectivity for MC-LR. Only chloride managed to change the potential suitably. The corresponding average log (Kᵢᵢ沄⁻LR,Cl) was −2.57, indicating that the response for MC-LR prevailed (chloride was in much higher concentration than MC-LR when the emf matched that of the main ion).

Thus, the electrodes offered very good selectivity properties for MC-LR, indicating that these are suitable for the analysis of environmental waters.

3.5 Response time and lifetime

The time required to achieve a steady potential response (±0.5 mV) using the proposed sensors in 0.37 to 2.00 µg L⁻¹ MC-LR concentration solutions with a rapid increase in concentration was established in 2 minutes. Replicate calibrations for equal electrodes indicated a small potential drift and confirmed their high reproducibility. Detection limits, response times, linear ranges and calibration slopes were reproducible of their original values for at least 4 weeks when sensors were stored out of water and in dry atmosphere. The selected conditions for this have been summarised in Table 2.

3.6 Binding constant

A selective ISG material requires the formation of stable complexes between the template and the polymeric matrix.
The higher the stability of these complexes, the higher the probability of reaching higher levels of complexation between the ISG guest sites and the analyte. This being the molecular-level occurrence sensed by an ISE, the MC-LR/guest site complexation affects both selectivity and sensitivity. Thus, this binding constant was established on the surface of the proposed sensors, having carbon as the solid contact material. The sandwich method earlier proposed in ref. 30, was selected for this purpose. It is based on the transient membrane potential measurements taken on two-layer sandwich membranes. The following equation was used for this purpose:

\[
\beta_{\text{IL}} = \left( \frac{L_T - n R_T}{Z} \right)^{-n} \exp \left( \frac{E_{\text{exp}} Z F}{RT} \right)
\]

(2)

where \( L_T \) is the total concentration of ISG guest sites in the membrane segment, \( R_T \) is the concentration of the lipophilic ionic site, \( n \) is the ion–ionophore complex stoichiometry and \( R \), \( T \) and \( F \) are the gas constant, the absolute temperature and the Faraday constant respectively, for an ion carrying a charge of \( z_I \). The obtained formation constant, \( \beta_{\text{IL}} \), was \( 1.14 \times 10^9 \), thus revealing a high affinity between guest sites and MC-LR under the potentiometric experimental conditions tested.

3.7 Application

In order to investigate the application of the present method to the analysis of environmental waters, spiked solutions were tested and the corresponding relative errors calculated. These assays were conducted in water (pH 6.5) with ISEs calibrated within 0.65–2.00 ppm of MC-LR. The average recoveries of two spiked solutions were 101.2 ± 5.5%, corresponding to average relative errors of 1.18% and thus confirming the accuracy of the analytical data (\( n = 2 \) for two ISEs). The results were precise, with relative standard deviation <6%.

4. Conclusions

Portable sensors were developed for the direct and in situ determination of MC-LR in environmental waters. The selective membrane was of the ISG material. It required an anionic lipophilic additive to control the charged response and a solid-contact made of carbon to produce stable/sensitive and reproducible responses. The sensors displayed high selectivity properties for the most common ions in environmental waters and offered simplicity in designing and analysis, short measurement times, good precision, high accuracy, high analytical throughput, low limit of detection and good selectivity.

Overall, the proposed method was proven suitable for the routine screening of MC-LR, essentially because this method allowed in situ real-time monitoring of MC-LR in several water media and decreased significantly the time between the sampling and the analytical result. Comparing to previous optical-based systems including imprinted sol–gel materials,25 this electrical transduction system turned out much less expensive, presenting the ability to carry out on-site analysis. In both cases, the limiting levels of MC-LR in waters are included in the linear range of the calibration, for which no sample pre-treating steps are required.

Care must be taken prior to local and routine application. A quick screening of the water sample composition should be made first and selectivity assays conducted for any organic compound found later.

Acknowledgements

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References


Table 2 Parametric data for MC-LR selective electrodes

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<th>Parameter</th>
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<tr>
<td>pH</td>
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<td>6.5</td>
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<td>Buffer nature</td>
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<td>Template extraction time/h</td>
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<td>Sol–gel chemical stability/week</td>
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