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Label-free Detection of Microcystin-LR in Waters Using Real-Time Potentiometric Biosensors Based on Single-Walled Carbon Nanotubes Imprinted Polymers

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Abstract

Microcystin-LR (MC-LR) is a dangerous toxin found in environmental waters, quantified by high performance liquid chromatography and/or enzyme-linked immunosorbent assays. Quick, low cost and on-site analysis is thus required to ensure human safety and wide screening programs. This work proposes label-free potentiometric sensors made of solid-contact electrodes coated with a surface imprinted polymer on the surface of Multi-Walled Carbon NanoTubes (CNTs) incorporated in a polyvinyl chloride membrane. The imprinting effect was checked by using non-imprinted materials. The MC-LR sensitive sensors were evaluated, characterized and applied successfully in spiked environmental waters. The presented method offered the advantages of low cost, portability, easy operation and suitability for adaptation to flow methods.

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Keywords: Biosensors, MC-LR, CNTs, Potentiometric.

1. Introduction

The contamination of freshwaters by cyanobacteria is very common and these are potential disseminators of hazardous toxins, such as MicroCystins (MCs) [1]. Classic methods for routine detection and quantification of MC-LR (the only MC mentioned in national and European legislation) are expensive and require special conditions and operators for the manipulation of biological materials. In addition, they

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take 1-3 days to give a result, which is too long to implement effective/corrective measures. More recent methods use mostly chromatographic techniques [2], being unsuitable for on-site analysis. Presently, many efforts are undertaken to detect MC-LR by means of rapid, simple, low cost and portable methods.

Biosensors constructed at the molecular level are promising devices for MC-LR screening. Good selectivity may be targeted by designing plastic antibodies with Molecular Imprinting (MI) techniques, tailored on nanomaterials of special electrical properties, such as Carbon NanoTubes (CNTs) [3]. Thus, new biomimetic materials made by surface imprinting on CNTs for MC-LR fast-screening are presented. These were used as ionophores in plasticized Poly(Vinyl Chloride) (PVC) and the resulting membranes coated on solid-contact electrodes for subsequent potentiometric transduction.

Nomenclature

CCD	Charge-Coupled Device
CNTs	Carbon NanoTubes
EDS	Energy Dispersive Spectroscopy
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
MC-LR	Microcystin-LR
MCs	MicroCystins
MI-CNTs	Molecular Imprinted Carbon NanoTubes
NI-CNTs	Non-Imprinted Carbon NanoTubes
PVC	Polyvinyl Chloride
TCE	Trichloroethylene
TKpCPB	Tetrakis(4-chlorophenyl)borate
WHO	World Health Organization

2. Materials and Methods

2.1 Equipments and reagents

All potential measurements were made by a Crison pH meter, GLP 21 (± 0.1 mV sensitivity) at room temperature and under constant stirring. The reference electrode was Ag|AgCl||KCl saturated reference electrode from Crison Instrument, S.A.. Scanning electron microscopic images were collected by a FEI Quanta 400FEG ESEM/EDAX Genesis X4M microscope. The Raman experiments were performed using a JOBIN-YVON LABRAM spectrometer, and a He-Ne laser. All chemicals were of analytical grade and double-deionized RNase free water $0.03 \mu\text{S}$ at 25°C from ATS (Portugal) was used in the preparation of all solutions. The buffer used was 10 mM HEPES, 150 mM NaCl with a pH of 6.6. CNTs were $>7.5\%$ CNT basis O.D. \times L 7-15 nm \times 0.5-10 μm .

2.2. Synthesis of host-tailored polymers and preparation of sensory membranes

MI materials were produced by adding the template, MC-LR (3.5 mmol), to CNTs (2 mg) for 4h under continuous stirring. Then, the functional monomers (14 mmol of sodium 4-vinylbenzenesulfonate, 7

mmol (vinylbenzyl)trimethylammonium chloride, vinylbenzoate) were added to the mixture for 4h. The solid was isolated and added of tris(2-methoxyethoxy)vinylsilane, 100 μL . The radical initiator, (benzoyl peroxide, 6 mg), monomer (styrene, 50 μL) and crosslinker (divinylbenzene, 50 μL) were added after for 2h. The obtained solid was isolated and let dry in a desiccator. Non-imprinted materials (NI-CNTs) were prepared similarly, without template. The modified CNTs (MI or NI) were further incorporated in PVC with *O*-nitrophenyl octyl ether (oNFOE) and with/without a charged lipophilic additive (potassium tetrakis(4-chlorophenyl)borate, TKpCPB). PVC/oNFOE and PVC/oNFOE/TKpCPB membranes were prepared as control. These membranes were casted on carbon conductive materials.

3. Results

3.1 SEM and Raman Analysis

SEM images of the CNTs showed that the tubes had an average thickness of 8–20 nm, and the corresponding EDS analysis confirmed the presence of the only C in the CNTs. The subsequent surface modification in MI and NI materials increased the thickness of the carbon rods up to 18–40 nm, thus confirming the existence of a polymer layer covering the nanotubes.

Raman spectra of the CNTs materials showed three main peaks at 1324, 1574 and 2657 cm^{-1} , assigned to the D, G and 2D bands. These bands altered in terms of relative intensity and/or wavelength location in MI and NI-based materials, so confirming the existence of chemical change over the CNTs. Slight structural differences between MI-CNTs and NI-CNTs materials were also evident for the same changes in the Raman spectra.

3.2 Potentiometric measurements

The potentiometric response of the membranes is shown in Figure 1. The results pointed out that MI and NI-CNTs presented opposite behaviors. Sensors of MI-CNTs and MI-CNTs/additive, showed good linear behaviors (0.998 and 0.996), with negative slopes of -62 and -53 mV decade^{-1} , ranging 7.75×10^{-10} – 1.32×10^{-9} and 6.72×10^{-10} – 1.21×10^{-9} M, respectively, while NI-CNTs sensors displayed a positive slope, of 33 mV decade^{-1} , from 7.24×10^{-10} – 1.28×10^{-9} M. Control membranes with only PVC/additive were unable to promote potentials changes and PVC membranes had a similar behavior to NI-CNTs. In general, the MI sensors did not need to presence of an additive, displaying good reproducibility, fast response (≈ 20 s), Nernstian behaviour, and limits of detection below the guideline value (1 nM).

3.3 Selectivity and application

The selectivity was tested against the most relevant ions controlled in waters for human consumption are Fe^{3+} , NH_4^+ , Cl^- , Na^+ , SO_4^{2-} , TCE and chloroform [4], by the Matched Potential Method [5] and expressed in $\text{Log}(K^{\text{Pot}})$. The initial concentration of MC-LR was 1.0×10^{-6} M, rising to 5.0×10^{-6} M. The interfering species were used in the following concentrations: 0.5 mg L^{-1} NH_4^+ , 250 mg L^{-1} Cl^- , 250 mg L^{-1} SO_4^{2-} , 200 $\mu\text{g L}^{-1}$ Fe^{3+} , 200 mg L^{-1} Na^+ , 100 $\mu\text{g L}^{-1}$ chloroform and 10 $\mu\text{g L}^{-1}$ of TCE. Only chloride and sodium were able to match the potential, with $\text{Log}(K^{\text{Pot}})$ of -3.33 and -3.16, respectively. Thus, the electrodes offered very good selectivity properties for MC-LR, indicating that these are suitable for the analysis of environmental waters.

In order to investigate the application of the present method to the analysis of environmental waters, spiked waters from an artesian well were tested and the corresponding relative errors calculated. The average recoveries of two spiked solutions were $102.4 \pm 1.7\%$, corresponding to average relative errors of -

2.4% and thus confirming the accuracy of the analytical data ($n= 2$ for two sensors). The results were precise, with relative standard deviation $< 6\%$.

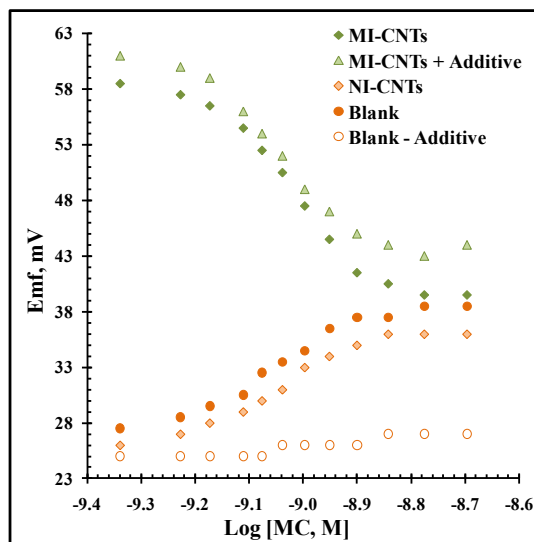


Fig. 1. : Calibrations of CNT MC-LR selective membranes (emf was recorded at room temperature after 20 s for MC-LR standards, ranging from 2.01×10^{-9} to 4.58×10^{-10} M, in 10 mM HEPES, 150 mM NaCl buffer, pH 6.6).

4. Conclusions

The obtained results showed that imprinted polymers were selective to MC-LR. The electrodes displayed an average slope of $-62 \text{ mV decade}^{-1}$, a detection limit of 6.92×10^{-10} M and a linear response in the range of 1.26×10^{-9} – 75×10^{-10} M of MC-LR. The non-imprinted sensor did not present a linear response showing an inverse potential shift. Spiked solutions were tested with the proposed sensor and the relative errors obtained confirmed the accuracy and precision of the method. Overall, the proposed method is simple, precise, accurate and inexpensive regarding reagent consumption and equipment involved.

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