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Sensors for the Detection and Quantification of Bacterial Contamination in Water for Human Use**

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The deterioration of water quality by Cyanobacteria cause outbreaks and epidemics associated with harmful diseases in Humans and animals because of the toxins that they release. Microcystin-LR is one of the hepatotoxins most widely studied and the World Health Organization, recommend a maximum value of $1 \mu\text{g L}^{-1}$ in drinking water. Highly specific recognition molecules, such as molecular imprinted polymers are developed to quantify microcystins in waters for human use and shown to be of great potential in the analysis of these kinds of samples. The obtained results were auspicious, the detection limit found, $1.5 \mu\text{g L}^{-1}$, being of the same order of magnitude as the guideline limit recommended by the WHO. This technology is very promising because the sensors are stable and specific, and the technology is inexpensive and allows for rapid on-site monitoring.

Today, there is worldwide concern about water quality.^[1] Every summer, waters from rivers, fluvial beaches and other water sources for human consumption suffer from bacterial and algae contamination. Bacteria belonging to the Cyanobacteria family are very common in such contamination.^[2] The deterioration of water quality by Cyanobacteria causes outbreaks and epidemics associated with harmful diseases in humans and animals.^[3–5] These diseases are due to bacterial toxins,^[2,6] such as neurotoxins, hepatotoxins and skin irritants. Hepatotoxins are the most frequent toxin encountered having implications in incidents of animals toxicity.^[6,9]

Microcystin-LR (MCYST) is the hepatotoxin most widely studied.^[2] Some international organizations, like the World Health Organization (WHO), recommend a maximum value of $1 \mu\text{g L}^{-1}$ of MCYST in drinking water.^[7,8] Classical methods for toxin detection and quantification are based on the application of samples in a nutritive medium that promotes growth of the microorganisms. These methods take 1–3 days to yield results.^[10] For this reason, most of the time the answer as to whether toxin levels are of concern comes too late to apply effective response measures. In fact, many efforts are being made towards the establishment of rapid tests that allow an individual identification of the microorganisms present in a sample.^[8,11] Molecular imprinting (MI) is a technique for the production of specific binding sites that works as artificial receptors for the target molecule. An MI membrane can act as a sensor because the target analyte modifies the charges in the vicinity of the electrode, altering its potential.^[10] The potential membrane sample-phase boundary emerges as a consequence of a local charge separation of cations and anions at the membrane surface.^[12–14] Molecularly imprinted polymers (MIPs) are prepared by reaction of appropriate monomers in the presence of the template (target) molecule. This pre-complex template/monomer is polymerized in a three-dimensional network.

After polymerization, the template is removed leaving cavities with the ability to recognize the target molecule.^[15] When exposed to a source of the target molecule, monitoring of the dielectric changes in the nanostructure of the polymer is possible; these changes come from the fulfilment of the cavities of the molecular imprinted polymer (MIP) when specifically bound to the target analyte. The main advantages of MI technology include high sensibility and specificity,

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low-cost, robustness, easy preparation and preservation. These advantages have justified the development of several sensing MI devices.^[10]

For these reasons, this work addresses the preparation of polymeric membranes sensitive to MCYST.

Experimental

MIPs for MCYST were prepared from a reagent mixture obtained by mixing 10 µL of MCYST, 3 mL of (3-aminopropyl) trimethoxysilane (APTES), both from Fluka, 3 mL of diphenylmethoxysilane (DPH), from ABCR and 4 mL of methanol (MeOH) from Aldrich with stirring at 60 °C for 30 min. The resulting solution was then hydrolyzed slowly with 1 mL of tetraethoxysilane (TEOS) from ABCR, 500 µL of hydrochloric acid (HCl) 0.1 M from Merck and 500 µL of deionised water at 80 °C, under stirring, until gel formation. The mixture was manually deposited on top of carbon-paste electrodes and then polymerized at 80 °C for 4 h. The template was removed by washing the MIP in deionised water for 4 h. Non-imprinted polymers (NIPs) were also synthesized following exactly the same procedure, but excluding the template MCYST from the formulation.

Results and Discussion

Scanning Electron Microscopy (SEM)

Figure 1 shows representative SEM images of the MIP and NIP thin films deposited in transparent glass. Despite the blurred images, mainly due to the non-conductivity of the samples, it is possible to observe nanostructures with variable dimensions (around 40 to 80 nm). The thickness of the sensing layer, the dimensions of the agglomerates and the size of the channels are very important for the following reasons. Thin sensing layers mean short distances between the recognition sites and the electrodes, so that just a few target molecules can cause a noticeable perturbation on the electrode. Besides, in small agglomerates, only a few sensing cavities can exist in the neighbourhood of the electrode; this means a higher sensing capability. On the other hand, very large channels allow the diffusion of molecules of sizes greater than that of the molecule of interest, diminishing the selectivity of the sensor. In the case of the MIP, the grain size is smaller than the grain size of the NIP. The most probable reason is some sort of tensoactivity of the MCYST, which promotes more controlled hydrolysis.

Fourier Transform Infrared – Attenuated Total Reflectance (FTIR-ATR) Spectroscopy

Figure 2 shows the IR spectra of the MIP and NIP films, in which the absorption bands of OH and NH groups at 3200–3400 cm⁻¹ are observed. At the same time the C_{sp3}-H and C_{sp2}-H bands are observed at 2840–2940 cm⁻¹ and 2940–3030 cm⁻¹, respectively. Silicon-oxygen bands at 690 and 990–1050 cm⁻¹ were also observed. These groups are characteristic of the polymer developed, according to the silica reagents employed and the sol-gel technique. The MIP and

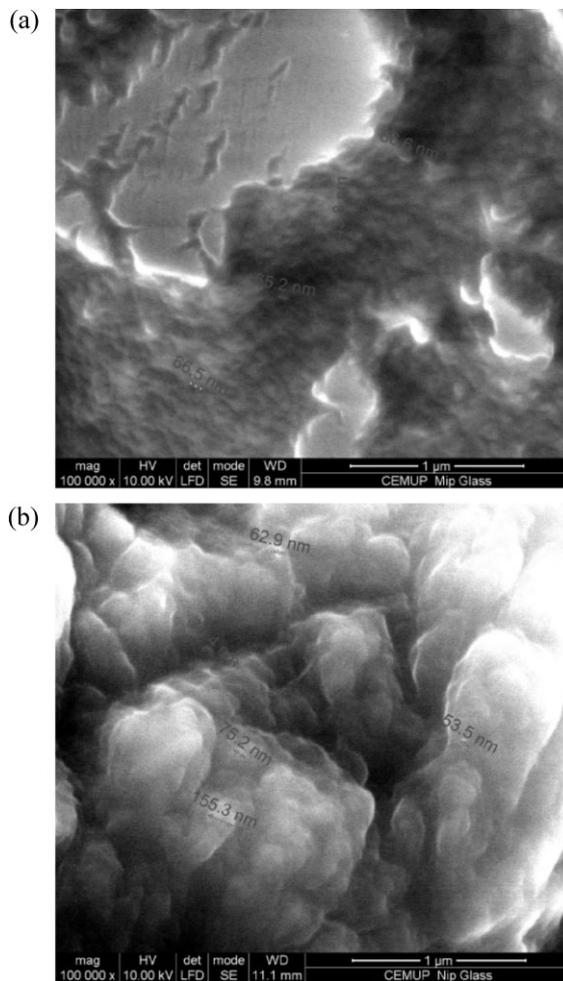


Fig. 1. SEM images of a) MIP, and, b) NIP.

NIP have the same chemical structure and therefore the same functional groups are present.

UV Spectroscopy

The UV spectra of the NIPs and MIPs are transparent in wavelengths above 290 nm. Both spectra are equal for the same reason as described for the FTIR analysis. The absorption band at 277 nm (Figure 3) accounts for the phenyl groups present in one of the reagents (DPH). The presence of MCYST

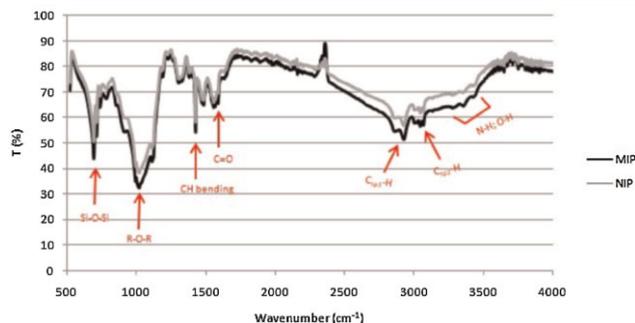


Fig. 2. FTIR analysis of the MIP and NIP films, deposited on glass.

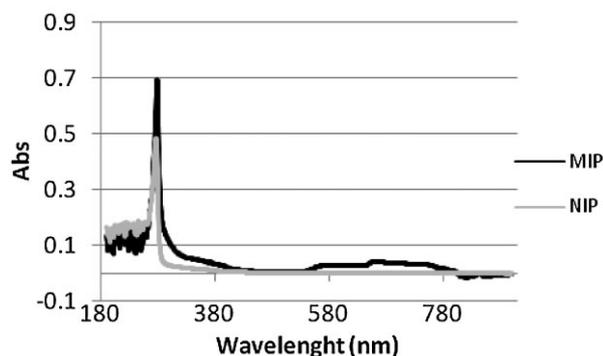


Fig. 3. UV spectra of the MIP and NIP films, deposited on glass.

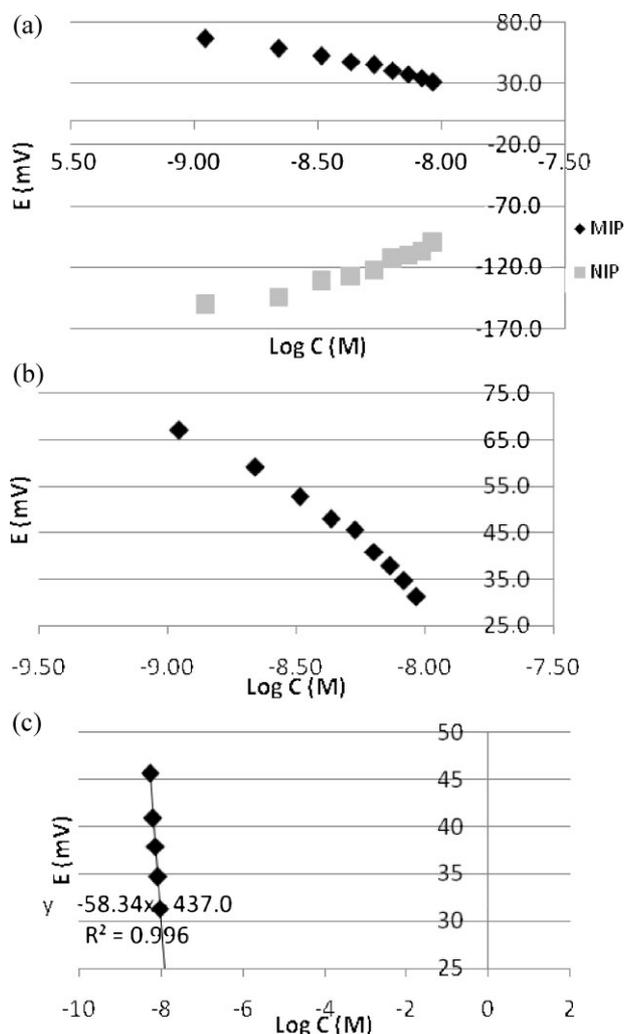


Fig. 4. a) Potentiometric sensor response (PSR) of MIP and NIP to different concentrations of MCYST. b) Enlarged PSR of MIP. c) Extended linear range of the MIP response.

in the prepared MIP is therefore masked by the DPH peak, as peptide peaks normally appear in this same region.

Potentiometric Response

The presence of the analyte changes the dielectric permittivity of the MIP and this result in an observable

potential difference. The potentiometric response of the MCYST/MIP in water is represented in Figure 4a. The detection limit found was $1.5 \mu\text{g L}^{-1}$; this value was calculated according to Ref. [16] and is in the same magnitude as the guideline value established by the WHO. In Figure 4b the linear range of [MCYST] was extended. The slope of the curve, -58.3 mV , is quasi-Nernstian, indicating good behaviour of the sensor, being linear at concentrations higher than $5.3 \mu\text{g L}^{-1}$.

These preliminary results show potential because they allow the same chemical technology to be shared across two different media: carbon electrodes and silica-based optic fibers.

Conclusions

A molecularly imprinted polymer was developed to afford trace quantification of microcystins in water for human use. When incorporated on the surface of normal carbon electrodes, these MIPs have great potential in the analysis of these kinds of sample. The obtained results were very promising, the detection limit found, $1.5 \mu\text{g L}^{-1}$, is of the same order of magnitude as the guideline value for drinking-water recommended by the WHO ($1 \mu\text{g L}^{-1}$). This technology is very advantageous because the sensors are stable and specific, and are also capable of proper functioning at high temperatures, in a variety of solvents and over wide range of pH. In addition, this technology is inexpensive and allows rapid on-site monitoring. The present method also offers advantages when compared to those in Ref. [8,11], which are of high selectivity because they employ biological materials, but are also of high cost and low reproducibility, besides requiring special laboratory safety conditions in some cases.

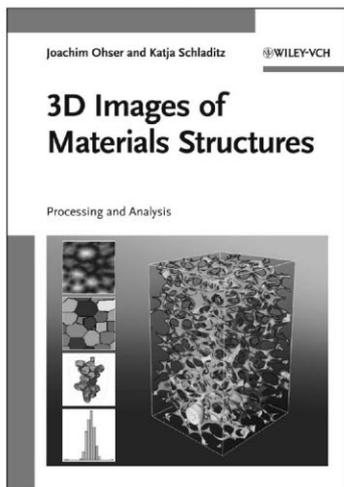
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