Optical Cavity Fibre Sensor for Detection of Microcystin-LR in Water

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ABSTRACT

The deterioration of water quality by Cyanobacteria causes outbreaks and epidemics associated with harmful diseases in Humans and animals because of the released toxins. Microcystin-LR (*mcyst*) is one of the most widely studied hepatotoxin and World Health Organization recommends a maximum value of 1 μg L^{-1} of *mcyst* in drinking-water. Therefore, there is a great demand for remote, real-time sensing techniques to detect and quantify the presence of *mcyst*. In this work a Fabry-Perot sensing probe based on a fibre tip coated with a *mcyst* sensitive thin film is presented. Highly specific recognition membranes, using sol-gel based Molecular Imprinted Polymers (MIPs), were developed to quantify microcystins in water, showing great potential in the analysis of this kind of samples. The fibre Fabry-Pérot MIP sensor shows a linear response to *mcyst* concentration with a sensitivity of -13.2 ± 0.4 nm L μg^{-1}.

Keywords: Microcystin-LR, Molecular Imprinting, Fabry-Pérot Interferometer.

1. INTRODUCTION

The presence of microcystins in water bodies has led to fatalities in wild and domestic animals worldwide and the toxins have also been associated with episodes of human illness [1,2]. Human beings are most likely to be exposed to microcystins through consumption of contaminated drinking water or recreational activities such as swimming [3-5]. Incidences of animal and human fatalities caused by microcystins have led to the introduction of guideline values for drinking water by the World Health Organization (WHO), with a recommended limit of 1 μg L^{-1} [2].

The traditional methods, such as Enzimed-Linked Immunosorbent Assay (ELISA), High-Performance Liquid Chromatography (HPLC) and Mass Spectroscopy (MS) present some difficulties with regard to routine pollution monitoring. Although some of these methods are highly sensitive they are also time consuming and require expensive instrumentation and special reagents [6].

The search for alternative methods for real-time bacteria control requires the development of methods that combine sensitivity and selective recognition capability of low quantities of the analyte, such as Molecular Imprinting (MI) [7], and sensor systems with rapid response, such as the optical fibre Fabry-Pérot interferometer (FFPI) [8,9]. MI is a system based on chemical or biological interaction that creates receptor structures on a polymer surface that can selectively bind to molecules of interest. The principle of MI involves the formation of cavities in a polymer with the right shape and functionality to specifically bind the target analyte. This is achieved by polymerization, activated by an initiator of a pre-organized complex, between the functional monomers, and the target molecule by extensive washing, leaving behind cavities specific for the target analyte [10]. The dielectric changes of nanostructures sensors based in MIPs will recognize the characteristic toxins produced by these microorganisms. The combination of these nanostructures polymers with FFPI will provide a reversible response allowing its application to a continuous control. The main advantages of these optical sensors include high sensitivity and specificity, low-cost, robustness, easy preparation and preservation.

In this work it is presented a FFPI sensing device for detection of *mcyst* based on the deposition of a MIP membrane (prepared by sol-gel technique) at the cleaved end of an optical fibre. All the results were compared with the Non-Imprinted Polymer (NIP), which is a reference polymer, obtained by the same procedure but without the recognition biding sites.

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2. SOL-GEL FABRICATION AND CHARACTERIZATION

2.1 Fabrication

The *mcyst* MIP was prepared by the sol-gel technique from a reagent mixture obtained by mixing 10 µL of *mcyst*, 3mL of (3-aminopropyl)trimethoxysilane (APTES), both from Fluka, 3 mL of diphenylmethoxysilane (DPH), from ABCR and 10 mL of methanol (MeOH) from Aldrich. This mixture was stirred at 60ºC during 30 minutes. Then, the resulting solution was hydrolyzed slowly with 1 mL of tetraethoxysilane (TEOS) from ABCR, 500 µL of hydrochloric acid 0.1 M, and 1.5 mL of deionised water also at 60ºC, under stirring until gel formation. The fibre tip was dip coated on the mixture and polymerized at 60ºC during 8 h. The template was removed by washing the MIP in deionised water for 4 h. In Figure 1 it is represented the MI process where firstly occurs the complexation between the template and the monomers, then the complex template/monomer polymerize in a three dimensional network and finally, when the template is removed from the polymer, the previously occupied space is left as a cavity.

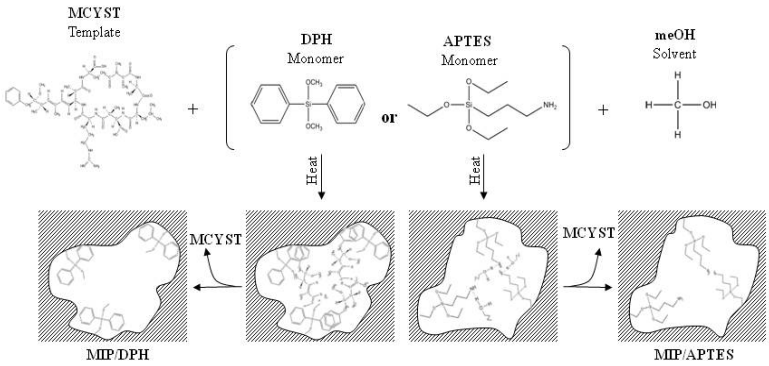

Figure 1. Scheme of MI process.

The NIP was synthesized following exactly the same procedure, but excluding the template from the formulation. The result is a similar membrane but with no cavities in its formation. Not only the MIP but also the NIP can adsorb *mcyst* from aqueous solutions. However, the adsorption capacities of MIP are larger than the NIP because it has higher specific and complementary sites for the toxin.

2.2 Characterization

To characterize the interferometer a simple setup was used, as shown in Figure 2. It consists of a broadband source in the 1550 nm spectral range with a bandwidth of 100 nm, and an optical circulator to obtain the reflected channelled spectrum of the sensing head. The reflected Fabry–Pérot signal was observed using an optical spectrum analyser (OSA) with a maximum wavelength resolution of 10 pm. The detail shows the developed sensing head which is based on a MIP of *mcyst* membrane prepared by sol-gel technique and coated on a fibre tip.

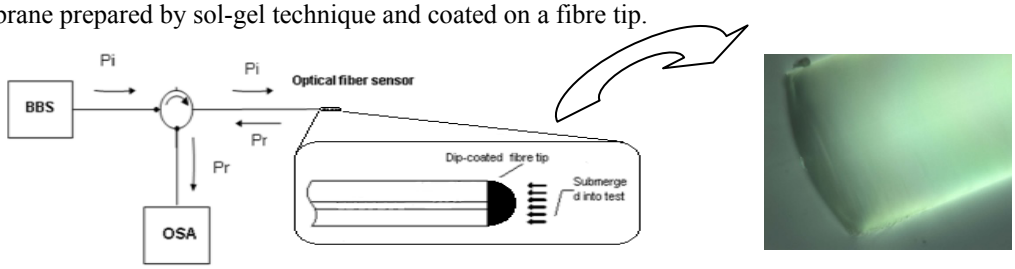

Figure 2. Experimental setup with the detail of the sensing head.

The fibre Fabry-Pérot cavity is then obtained by two interfering waves, one from the Fresnel reflection at the distal end of the fibre probe and the other from the reflected light at the end of the sensitive membrane deposited in the fibre tip. Figure 3 shows the optical spectrum of the two FFPI with the MIP and the NIP membranes.

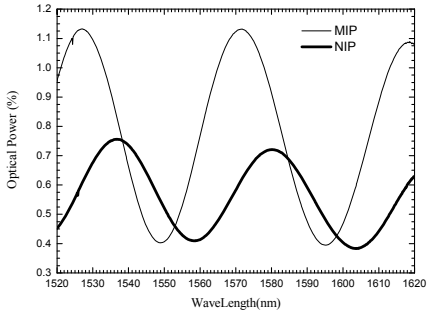

Figure 3. Optical spectrum of the fibre Fabry-Pérot interferometer with MIP and NIP membranes .

The periodicity of the channelled spectrums are ~ 44.5 nm and 43.4 nm which means that in the central region the optical thickness of the membranes are ~ 190 nm and 194 nm, respectively. The different structures of the MIP and NIP membranes will cause different effective indices which in turn will be responsible by the different fringe amplitudes in the two cases. It is expectable that the cavities in the sol-gel polymer decrease the effective index of the MIP membrane, therefore causing fringes with higher amplitude when compared to the fringes of the NIP membrane due to a higher mismatch with the fibre core refractive index.

3. EXPERIMENTAL RESULTS

The interaction of the analyte with the sol–gel *mcyst* membrane strongly influenced the phase of the FFPI. The change in phase was proven by the wavelength shift variation with the concentration of *mcyst* which can be seen in Figure 4 a) and b) for the MIP and NIP membranes, respectively. The *mcyst* concentration range was changed between 0.3 and 2.5 μg L^{-1} at a constant temperature of 18.3 ± 0.1 ºC.

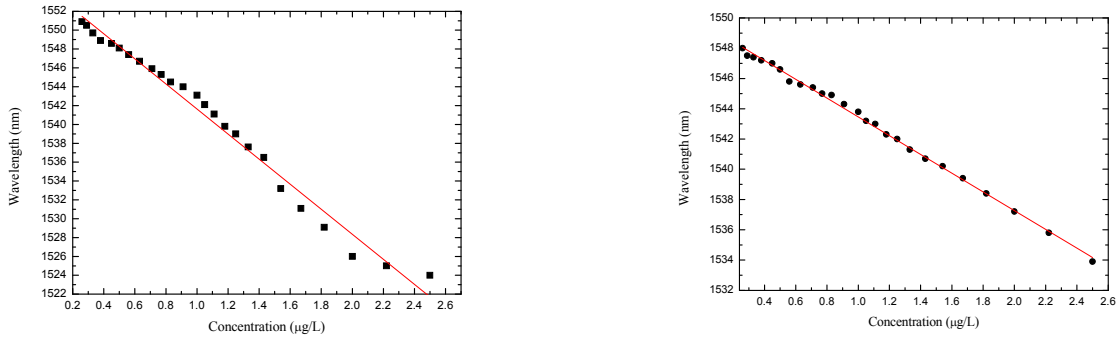

Figure 4. Wavelength shift variation according to the concentration of *mcyst* using an FFPI with a) MIP and b) NIP membranes.

The wavelength variation, for the full concentration range, of the FFPI with MIP membrane was 27 nm, almost the double of NIP (14 nm). Both MIP and NIP showed a fairly linear response and sensitivities of -13.2 ± 0.4 nm L μg^{-1} and -6.2 ± 0.1 nm L μg^{-1}, respectively. These results indicate that the presence of the analyte created a change in the effective index of the sensing membrane, introducing changes in the optical path of the cavity and, consequently, modifying the phase of the interferometric pattern. Confirming that the adsorption capacities of MIP are larger than the NIP, it was observed that MIP binding sites reached a near saturation state for higher *mcyst* concentrations. In particular, it can be observed that, in the recommended limit range of *mcyst* in drinking waters ≤ 1 μg L^{-1}, both FFPIs present good linear behaviour with a wavelength detection range of 8 nm and 4 nm for the MIP and NIP polymers, respectively.

For the characterization of thermal cross-sensitivity, the sensing head was immersed in distilled water. The temperature was increased from the room temperature to 60 ºC with steps of 5 ºC. The response of the FFPIs to temperature variation is shown in Figure 5.

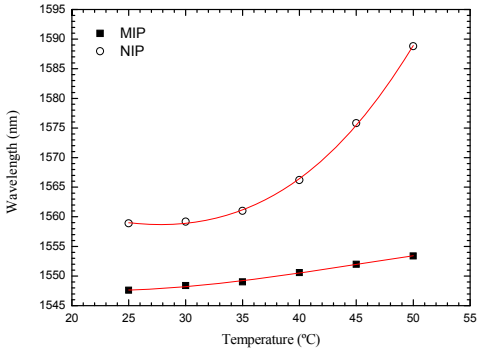

Figure 5. Wavelength shift versus temperature variation for the FFPI with a) MIP and b) NIP membranes.

As it would be expected, the observed non-linear responses are due to the thermo-optic coefficient variation of each sensing polymer. The FFPI with the MIP membrane presents higher stability to temperature, which combined with its recognition biding capability indicates a great potential for *mcyst* quantitative detection. The data presented in Figure 4 was obtained reading with an OSA the shift of the FFPI channelled spectrum. This approach readily permitted to test the viability of this sensing structure, but now it is under development a dedicated interrogation unit to read with high sensitivity the cavity phase, with clear benefits considering the factors measurement resolution and cost.

4. CONCLUSIONS

An interferometric optical fibre sensor for the detection of *mcyst* based on sensitive sol–gel membranes (MIP and NIP) coated on a fibre tip was demonstrated. Both sensing membranes showed linear response to *mcyst* concentrations in the 0.3–2.5 μg L^{-1} range and sensitivities of -13.2 ± 0.4 nm L μg^{-1} and -6.2 ± 0.1 nm L μg^{-1}, respectively. Sensors with MIP membranes have higher sensitivity and lower linear ranges than the corresponding NIP polymers. The developed FFPIs are promising *mcyst* sensing structures for applications in situ and real time monitoring of bacteria and environment control.

REFERENCES

1. Carmichael, W.W., "A review: cyanobacteria secondary metabolites-the cyanotoxins", Journal of applied bacteriology 72, 445-459 (1992).
2. World Health Organization (WHO), [Guidelines for drinking-water quality], Vol. 1, 3rd Ed. (2003).
3. Kuiper-Goodman, T., Falconer, I.R. and Fitzgerald, J., [Human health aspects], In: Chorus, I. and Bartram, J., Editors, E&FN Spon, London, 113–153 (1999).
4. Vivegnis J., El Lioui M., Leclercq A., Lambert B., Decallonne J., Biotechnol. Agron. Soc. Environ. 3, 159–164 (1999).
5. Swanson M.S., Hammer B.K., "Legionella pneumophila pathogesesis: a fateful journey from amoebae to macrophages", Annu. Rev. Microbiol. 54, 567-613 (2000).
6. Wang L., Chen W., Xu D., Shim B.S., Zhu Y., Sun F. Liu L., Peng C., Jin Z., Xu C., Kotov N.A., "Simple, rapid, sensitive, and versatile SWNT-Paper sensor for environmental toxin detection compepitive with ELISA", Nano Letters 9 (12), 4147-4152 (2009).
7. Li T., Wu T.-D., Mazéas L., Toffin L., Guerkin-Kern J.-L., Leblon, G., Bouchez, T., Environmental Microbiology 10, 580–588 (2008).
8. C. Jesus, S.F.O. Silva, M. Castanheira, G. González Aguilar, O Frazão, P.A.S. Jorge and J.M Baptista, "Measurement of acetic acid using a fibre Bragg grating interferometer", Meas. Sci. Tecnol. 20, 125201 (2009).
9. Susana F.O. Silva, O. Frazão, P. Caldas, J. L. Santos, F. M. Araújo, L. A. Ferreira, "Optical fiber refractometer based on a Fabry-Perot interferometer", Optical Engineering 47, 054403 (2008).
10. Mosbach, K., Trends Biochem. Sci. 19, 9–14 (1994).