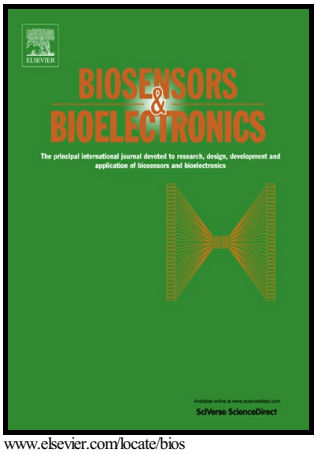

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Interferometric nanoimmunosensor for label-free and real-time monitoring of Irgarol 1051 in seawater

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Abstract

An interferometric nanobiosensor for the specific and label-free detection of the pollutant Irgarol 1051 directly in seawater has been settled. Due to the low molecular weight of Irgarol pollutant and its expected low concentration in seawater, the sensor is based on a competitive inhibition immunoassay. Parameters as surface biofunctionalization, concentration of the selective antibody and regeneration conditions have been carefully evaluated. The optimized immunosensor shows a limit of detection of only 3 ng/L, well below the 16 ng/L set by the EU as the maximum allowable concentration in seawater. It can properly operate during 30 assay-regeneration cycles using the same sensor biosurface and with a time-to-result of only 20 min for each cycle. Moreover, the interferometric nanosensor is able to directly detect low concentrations of Irgarol 1051 in seawater without requiring sample pre-treatments and without showing any background signal due to sea matrix effect.

Keywords

Bimodal waveguide biosensor; competitive immunoassay; Environmental monitoring; Irgarol 1051;

1. Introduction

Every year large quantities of waste and pollutants are dumped in the oceans, derived mainly from anthropogenic activities related to industrial, tourism and urban activities (Albaladejo et al., 2010). The discharges of these contaminants together with the intensive exploitation of the marine resources have caused the continuous degradation of our oceans. Most of the harmful contaminants are present in extremely low concentrations and, therefore, their monitoring is a crucial step towards sustainability of the ocean water quality and the use of the marine ecosystems (Bakker, 2012).

Conventional analytical methodologies are very sensitive and selective tools to control the sea quality but they only operate at laboratory settings, with the associate problems of samples transportation without degradation. Bringing the monitoring tools directly to the contaminated resource can result in cost and time savings, allowing tracing water pollutants evolution in real time and dramatically reducing the response time in case of pollution peaks episodes. Analytical tools which are portable, robust, low-cost and highly reliable are highly demanded. Biosensors emerged some years ago as devices capable to carry out fast,

reproducible and highly sensitive and specific analysis of a target analyte directly in the sample matrix, avoiding sample transportation and pre-treatment (Chocarro-Ruiz et al., 2017).

The bimodal waveguide (BiMW) interferometric biosensor is a new type of photonic sensor offering an unprecedented sensitivity while operating in a label-free scheme. It is also prone to miniaturization and multiplexing as it is fabricated with standard silicon photonics technology (Estevez et al., 2012). Moreover, the simplicity of the BiMW design, based on a common path waveguide, makes the BiMW sensor attractive for mass production since there is no need to use light splitters. This novel device has been successfully applied recently for cancer diagnosis by the direct analysis in urine of a microRNA biomarker related to bladder cancer (Huertas et al., 2016) and for the direct detection of very few bacteria in non-treated ascitic fluid from cirrhotic patients (Maldonado et al., 2016).

We have applied the BiMW interferometric biosensor for the detection of the pollutant Irgarol 1051 in seawater. Irgarol 1051, also known as cybutryne, is a common booster biocide added to antifouling paints to prevent adhesion and growth of algae (Thomas and Brooks, 2010). This herbicide, belonging to the s-triazines group, is a photosynthetic inhibitor interfering on the electron transport of chloroplasts. The aforementioned, together with its persistence in surface waters and aquatic ecosystems, causes major concerns in the damaging of the marine biodiversity. Because of its spread use is the most frequently detected antifouling biocide worldwide (Konstantinou and Albanis, 2004), being found in both aquatic ecosystems and coastal waters (Okamura et al., 2000; Tolosa et al., 1996). Depending on the analytical method employed, the reported concentrations varies from non-detectable up to low part per billion (Konstantinou and Albanis, 2004). High frequency of Irgarol 1051 can be found in ports, marinas fishery harbours and usually higher levels are reported in early summer, probably related to the seasonal boating activity (Dahl and Blanck, 1996). Because of that, the European Water Framework Directive (WFD) 2013/39/EC included Irgarol 1051 as a priority pollutant in the field of water policy, setting 16 ng/L as the environmental quality standard (EQS) expressed as maximum allowable concentration (The European Parlament and the Council of the European Union, 2013).

There are very few described biosensors for Irgarol 1051 detection. For example, a marine green microalgae was genetically modified for luminescence monitoring, with a 50% effective concentration (EC_{50}), after 2 days, of 760 ng/L (Sanchez-Ferandin et al., 2013). Another recent example is an amperometric immunosensor based on an indirect competitive assay (Salvador and Marco, 2016). The chosen detection format requires a secondary conjugated antibody with the consequent need to employ more reagents and an extra assay step as compared to a label-free scheme. The limit of detection (LOD) of 38 ng/L achieved was low enough for some reported levels in seawater (Konstantinou and Albanis, 2004), but no as low as the one achieved by standard chromatographic methods (Munaron et al., 2012; Readman et al., 1993) or the maximum allowable concentration (16 ng/L) set by the EU. We expect to offer the required high sensitive detection by using our BiMW technology in combination with an appropriate immunoassay. In order to be able to perform label-free biosensing evaluation of the low molecular weight Irgarol 1051 pollutant, a direct competitive immunoassay format was selected. In a competitive immunoassay, the sensor surface is first functionalised with a receptor derivative; after the sample with the target analyte is mixed with a fixed antibody concentration and then injected onto the sensor surface in such a way that the analyte and the receptors compete for the binding sites of the antibodies. The sensor signal will be inversely proportional to the concentration of Irgarol 1051 in the sample because just the remaining free antibodies will interact with the sensor surface. Later, the antibody-analyte interaction can be dissociated by a regeneration solution and then the biosurface can be reused for different cycles.

This assay format is expected to show better signal-to-noise ratio than a non-competitive direct assay format as the detected molecule is the high molecular weight antibodies (~150 kDa) in comparison with the pollutant Irgarol 1051 (253 Da). The chosen immunoassay is not only more appropriate in terms of sensitivity but it also provides a higher stability and higher reusability. Surfaces functionalized with target molecule derivatives as receptor are more suitable to resist many regeneration cycles than those functionalized with antibodies, which easily denatures and lose their biological activity. The chosen antibody was previously developed and proved to be effective for Irgarol 1051 detection in seawater samples with an enzyme-linked immunosorbent assays (ELISAs) (Ballesteros et al., 1998; Sanvicens et al., 2012). In these previous ELISA immunoassays it was also corroborated the inherent affinity and selectivity of the polyclonal antibodies produced against Irgarol 1051. Special attention has been focused on the selection of the optimal surface functionalization strategy for the sensor surface. Additionally, several parameters affecting the biosensor performance have been optimized. Finally, the developed immunosensor has been successfully applied for the sensitive detection of Irgarol 1051 directly in seawater samples.

2. Experimental

Optical bimodal waveguide sensor and experimental set-up

BiMW devices were fabricated as previously described (Zinoviev et al., 2011). The working principle of the BiMW sensor relies in the creation of an interference pattern (Fig. 1), generated by the superposition of two light modes (fundamental and first modes) travelling in a straight waveguide. Light is coupled in a straight single-mode rib waveguide and after passing through a step-junction, two transversal modes with the same polarization are excited. For sensing, the waveguide surface includes a sensing window. Since the fundamental and the first order modes have different evanescent field profiles, any change in the refractive index of the sensing area affects differently both modes. This modification causes a phase variation on the interference pattern, which produces a variation of the output intensity distribution. The sensor response can be determined quantifying the change on this distribution.

Experimentally, a TE polarised light of a λ=660 nm and P=120 mW diode laser (ML101J27, Mitsubishi) is coupled into the BiMW sensor, by end-fire coupling method, using a lenses system composed by collimated lens (C240TME-D, Thorlabs), a polarization-dependent isolator (IO-3D-660-VLP, Thorlabs) and a coupling objective 40x (Achro, Leica). A four quadrants photodetector (S4349, Hamamatsu) is employed for collecting the light at the end of the device. Signal acquisition is performed in real time using a home-made LabVIEW software (National Instruments, USA). A five-channel PDMS flow cell (channel of 18.5 mm long, 1.25 mm wide and 0.5 mm high) is interfaced with the BiMW sensor to confine the liquid samples through the sensing window during experiments. A constant flow is delivered to the fluidic channels by using a syringe pump (NE300, New Era) and an injection valve (150 µL sample loop, V-451, Idex) which allows the injection of different solutions (e.g. sample, regeneration buffer) without changing the flow rate. The BiMW sensor holder also incorporates a temperature controller providing temperature stabilization with an accuracy of 0.01 degrees.

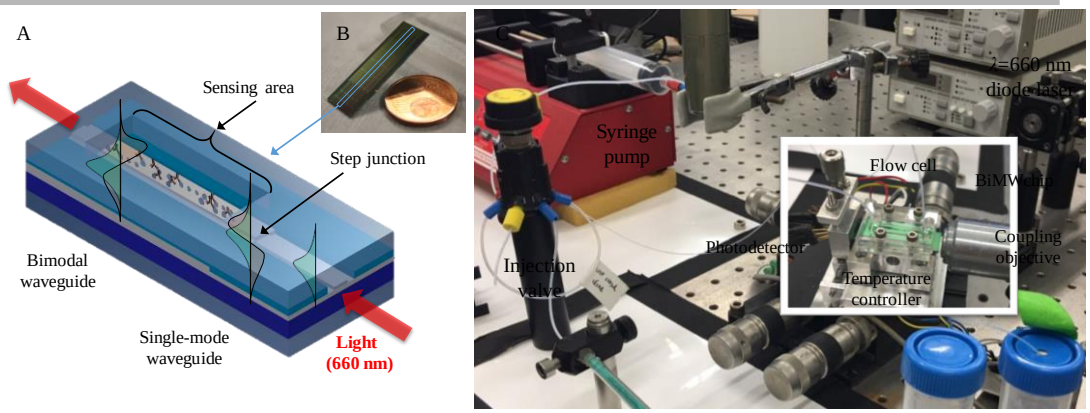

Figure 1. *(A) Scheme of the sensing principle of a BiMW. Light is coupled in the single mode rib waveguide and after a modal splitter; two modes are excited and propagate until the device output. (B) Photograph of the BiMW chip containing 16 sensors. (C) Photograph of the experimental set-up for the evaluation of the BiMW sensors.*

3. Introduction

3.1. Surface biofunctionalization

The development of effective label-free interferometric biosensors relies heavily on the careful control of the density of the bioreceptors immobilized in the sensor surface which will directly affect the analytical performance of the biosensor. In addition, the biofunctionalization protocol should avoid as much as possible the non-specific adsorptions from a complex matrix as the seawater. For that reason, we have evaluated six different functionalization protocols based on silanization and subsequent covalent receptor immobilization.

The chosen immunochemical format is a competitive configuration in which an haptenized protein (competitor bioconjugate) is covalently immobilized on a previously silanized sensor surface. The hapten *4e,* an Irgarol 1051 chemical derivative with a carboxylic group, and the bioconjugate *4e*-conalbumin (*4e*-cona), were employed. To evaluate the most suitable biofunctionalization procedure, several silanization protocols were employed. Figure 2 shows the scheme of the different protocols which have been evaluated. The reagents and materials and biofunctionalization protocols are shown in the supplementary material section 1 and 2, respectively.

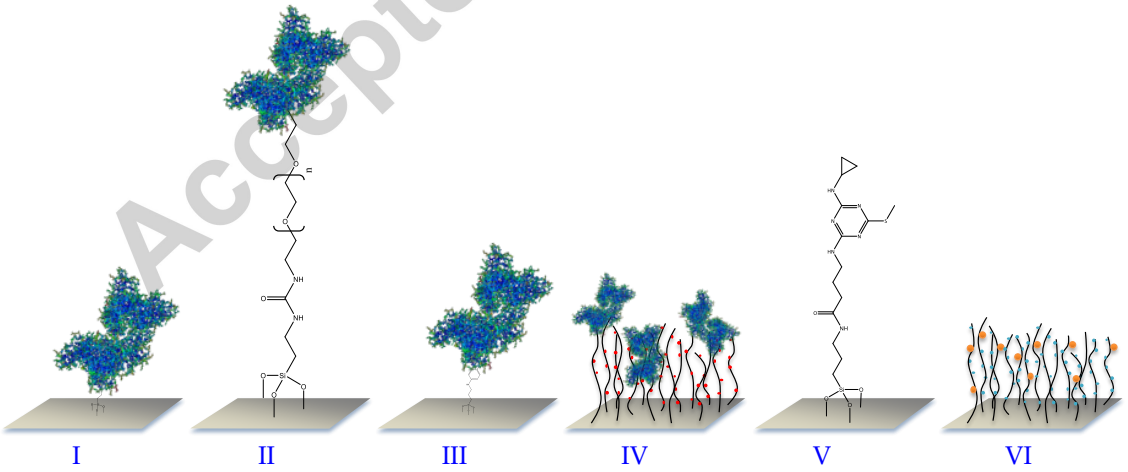

Figure 2. *Evaluated sensor surfaces: (I) Covalent immobilization of 4e-cona via CTES, (II) covalent immobilization of 4e-cona via silane-PEG-COOH, (III) covalent immobilization of 4e-cona via APTES and the cross-linker PDITC, (IV) covalently cross-linked 4e-cona via CM-dextran, (V) covalent immobilization of hapten4e via APTES and (VI) covalently cross-linked hapten 4e via amine-dextran.*

First, the effect of the antibody concentration was evaluated using different antisera dilutions for the six biofunctionalized surfaces prepared on the BiMW sensors. Moreover, the different surface sensors were tested with samples with and without Irgarol 1051 content. Results are shown in Figure 3. The detailed explanation of the biosensors immunoassays evaluation is explained in the section 3 of the supplementary material.

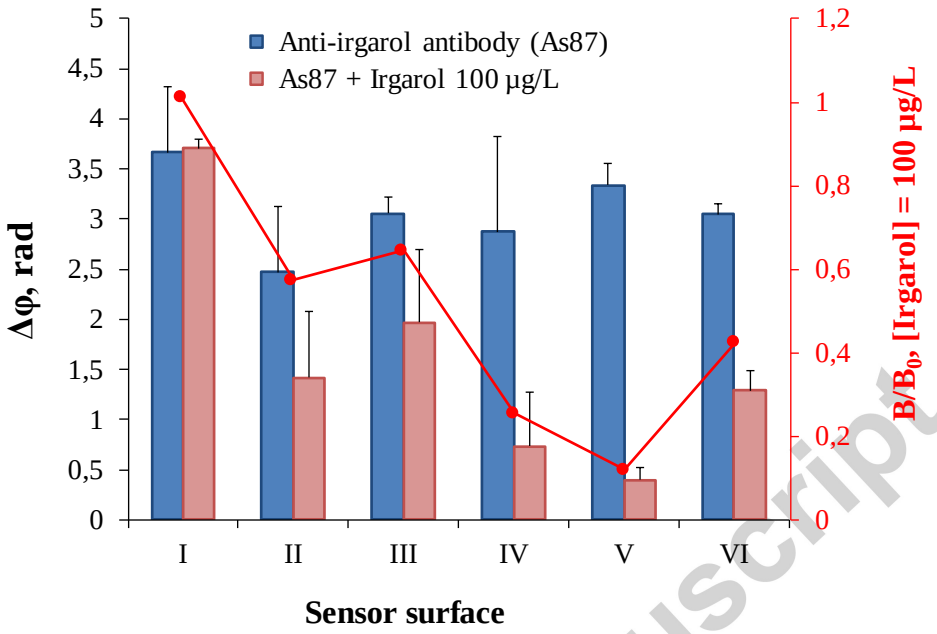

Figure 3. *Comparison of the different sensor surfaces responses in terms of absolute signal (Δϕ) and sensitivity (B/B0 ratio). For sensor surface details, see Figure 2. The employed anti-Irgarol 1051 serum in all the surfaces was 1:1000 (v/v) except for surface V with a dilution factor of 1:2000 (v/v) and surface VI that was 1:1500 (v/v).*

Sensor surface I, resulted from the covalent immobilization of conjugate *4e*-cona on the BiMW chip previously silanized with carboxyethylsilanetriol sodium salt (CTES), produced the highest response in presence of the As87 anti-irgarol serum (dilution 1:1000, v/v) and in absence of Irgarol 1051 (Δφ = 3.7 rad). However, no signal inhibition was observed in the presence of 100 µg/L Irgarol 1051 in the sample. This is probably due to a low functionalization yield leading to a surface with low density of bioreceptors, or to the non-specific binding of other proteins present in the anti-Irgarol 1051 serum.

In order to reduce the possible non-specific binding of serum proteins, the replacement of CTES by silane-PEG-COOH (surface II) or 3-aminopropyltriethoxy silane (APTES) in combination with both *p*-phenylenediisothiocyanate (PDITC) cross-linker (surface III) and carboxymethyl-dextran sodium salt (CM-dextran) (surface IV) was evaluated modifying the sensor surface prior to the covalent immobilization of the conjugate *4e*-cona. PEGylation has been described to greatly suppress non-specific binding of charged molecules to the modified surface (Sharma et al., 2004). The cross-linker PDITC has the potential to form well-organized assemblies driven by π-π stacking, also leading to a reduction of non-specific binding of matrix compounds to the functionalized sensor surface (Gandhiraman et al., 2010). On the other hand, CM-dextran potentially provides a pseudo-3D hydrophilic layer favourable to most antigen-antibody interactions and increases the surface capacity for receptor immobilization in comparison with the flat surface when using PDITC as cross-linked.

The response observed for surfaces II, III and IV in presence of the As87 anti-irgarol antiserum and in absence of Irgarol 1051 was slightly lower than that for surface I. This was attributed to the reduction of the non-specific binding of serum proteins, binding which would falsely increase the sensor response. Additionally, the presence of Irgarol 1051 in the sample led to a

signal inhibition, as expected. The inhibition was relatively small for surfaces II and III, improving the results when using CM-dextran, which is consistent with the formation of a pseudo-3D layer of higher receptor immobilization capacity.

As an alternative to cona-*4e* conjugate, the use of receptor *4e* was also studied. Two different approaches were evaluated for *4e* covalent immobilization: direct covalent immobilization onto an APTES-modified surface (surface V) and the attachment to a CTES-modified surface using cross-linking to an amine-dextran network (surface VI). The best results, both in terms of absolute signal (Δφ) and in terms of sensitivity (B/B_0 ratio) were obtained for surface V, in which 88 % of the signal due to the anti-Irgarol antibody (in absence of Irgarol 1051, B_0) is inhibited by the presence of 100 µg/L of Irgarol 1051 in the sample (B). This was surprising, since a similar sensitivity enhancement observed for surface IV compared to surface I was expected for surface VI compared to surface V. The reason to directly attach the derivative *4e* is that the closer to the sensor surface the receptor is, the more sensitive the response is. The best performance of surface V was attributed to the physical proximity of the receptor to the surface. BiMW transducing mechanism is based on the evanescent wave detection principle, and considering that the intensity of the evanescent field is maximum at the surface and exponentially decreases along the direction perpendicular from the interface, the sensor responds mainly to events happening in close vicinity to the waveguide surface. Thus, antigen-antibody interaction in surface V, where the receptor is linked through APTES moiety, would occur closer to the surface than that for surface VI, in which a larger size cross-linker molecule (amine-dextran) have been used, leading to the observed enhance response.

In the light of the results obtained, surface V was selected as the most appropriate one for further biosensor development.

3.2. Biosensor optimization

To enhance the performance of the biosensor, different parameters during APTES silanization and immobilization of hapten *4e* affecting the functionalization yield were optimized (supplementary material section 4).

The concentration of the hapten *4e* during the chip functionalization was also evaluated. An increase of the hapten *4e* concentration up to 250 µg/mL did not led to a better performance of the sensor. Therefore a 50 µg/mL was chosen as the optimum immobilization concentration. On the other hand, a pre-incubation time of *4e* with N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide sodium salt (sulfo-NHS) helped to activate the carboxylic group of the hapten prior to covalently attach to the amine silane, enhancing the functionalization performance.

The effect of the antibody concentration was evaluated with different antisera dilutions between 1:1000 and 1:5000 (v/v). The best results were obtained for a 1:2000 (v/v) serum dilution, being high enough to give a good signal-to-noise ratio and sensitivity (B/B_0 ratio). Higher dilutions present weaker response in terms of absolute signal (Δφ) and a lower dilution, although slightly enhance the signal, is exceedingly decreasing the immunoassay sensitivity (Fig. S1). Therefore 1:2000 (v/v) dilution was selected for further analysis.

Another parameter with a direct effect on the sensor response is the incubation time of the antibodies with the sample prior to the sensor injection. This step is important in order to allow an appropriate interaction of the antibodies with the Irgarol 1051 present in the sample before the contact with the hapten onto the surface. This parameter was evaluated over a

range of 1 to 10 minutes. The minimum time was chosen as the optimal, as longer times do not render in better sensitivities.

The chosen regeneration solution was NaOH because other solvents, i.e HCl, do not dissociate the antibody-antigen interaction or may destroy the bioreceptor activity (results not shown). Four different concentrations of NaOH with fixed flow (25 µL/min) and time (120 s) were tested for sensor regeneration. The efficiency of the regeneration step was evaluated as the recovery of the same absolute signal (Δφ) for antisera dilution 1:2000 (v/v) in consecutive measurements under the same conditions, both without Irgarol, and in the presence of a high concentration of Irgarol 1051.This indicates an effective removal of the antibody bound to the sensor with minimal surface damage. When regenerating with a low concentration of NaOH (25 mM) the absolute signal decreased a 55 ± 10 % in the absence of Irgarol 1051 just after 5 different measurement-regeneration cycles. The decrease in the absolute signal response in consecutive measurements was attributed to antibodies bound to the sensor surface not effectively removed. On the opposite site, when regenerating with a high concentration of NaOH (100 mM) the performance of the sensor improved, but the signal still decreased a 20 ± 3 % after 5 cycles in the absence of Irgarol 1051. This decrease was possibly due to damages on the surface biolayer. In the case of 50 mM NaOH the sensor retains 95 ± 6 % of the initial response after 10 cycles, indicating an efficient regeneration of the sensor surface (Fig. S2).

Some preliminary studies of the shelf life of the biosensor were performed. The same chip was evaluated using different sensor waveguides functionalized the first day and stored in air at room temperature. The same performance was obtained for the different waveguides sensors evaluated in consecutive days (see Fig. S3). Finally, the effect of the flow rate was analysed in the range of 15 to 25 µL/min. When the flow was 20 µL/min, the absolute signal with antisera dilution 1:2000 (v/v) and in the absence of Irgarol 1051 was 3.3 ± 0.2 rad. A faster flow, 25 µL/min, decreased the absolute signal to 10 ± 2 % and lower flows did not show better sensitivities. Therefore, the chosen flow rate was 20 µL/min. With the above mentioned the complete measurement-regeneration cycle takes around 20 minutes.

A summary of the different parameters affecting the immunoassay performance are shown in the supplementary material (Table S1).

3.3. Analytical performance of the BiMW immunosensor

Using the optimised conditions established before, the sensor response was evaluated for Irgarol 1051 standard solutions in the concentration range from 0 to 100 µg/L in buffer conditions. Figure 4 shows the calibration curve obtained for this range of concentrations. From here, we deduce that the half maximal inhibitory concentration (IC_{50}) and LOD were 99 ng/L and 3 ng/L, respectively. The DR was from 9 to 1190 ng/L. The sensor was stable up to 30 different measurements-regeneration cycles using the same sensor surface.

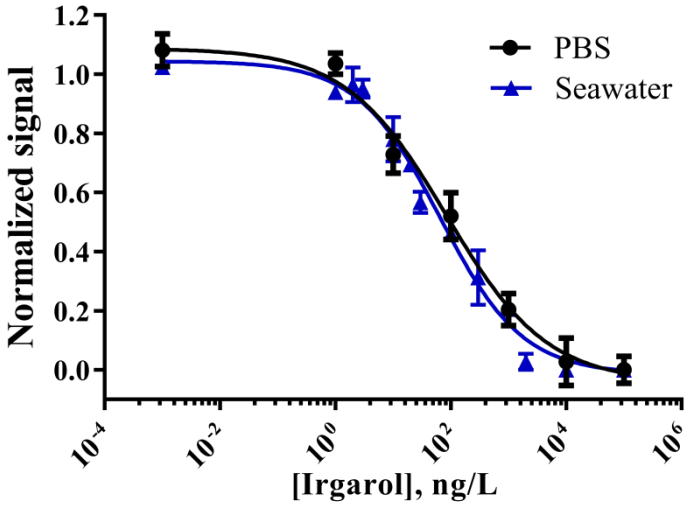

Figure 43. *Competitive calibration curve obtained in Irgarol 1051 standard solutions in PBS pH 7.4 and in spiked seawater.*

For the analysis of Irgarol 1051 in seawater, we first evaluated the sensor response to the flow of seawater alone in order to check the presence of a background signal due to any matrix effect (Fig. 5). The seawater sample was previously checked that it does not contain detectable levels of Irgarol 1051. When flowing the seawater sample onto the sensor surface, the sensor response shows the same phase change at the entrance and at the exit, a stable line during the flow of seawater alone and the recovery of the base line. That indicates that the surface biofunctionalization is resistant to non-specific adsorptions due to the sea matrix.

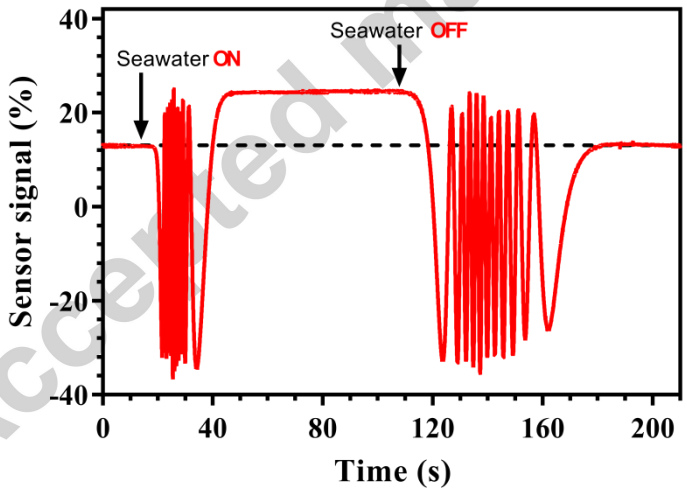

Figure 5. *Sensor signal response to the flow of seawater.*

Seawater samples were then spiked with increasing concentrations of Irgarol 1051 in the range from 0 to 100 µg/L and incubated with a constant concentration of antibody. The good performance of the antibody As87 was reported in a previous immunoassay where it was concluded that salinity did not significantly affect their performance (Sanvicens et al., 2012) and with an amperometric biosensor monitoring in seawater (Salvador and Marco, 2016). The obtained responses are represented in Figure 4, showing the calibration inhibition curve. Measured under the optimized conditions, the IC_{50} and LOD were 66 and 3 ng/L, respectively. The LOD is even as good as some of the reported chromatographic methods (Biselli et al.,

2000; Gimeno et al., 2001; Readman et al., 1993). The DR was from 9 to 478 ng/L. Although, the DR is reduced with seawater due to some matrix effect, the absolute signal and sensitivity remains stable, therefore the BiMW immunosensor allows the direct detection of Irgarol 1051 in seawater. The pH of seawater is usually in the range of 7.5 to 8.4 (Chester and Jickells, 2012). As previously reported using ELISA assays, the immunocomplex is stable for a wide pH range. Both, the reported ELISA assays (Ballesteros et al., 1998) and current results, corroborate the inherent stability of our nanoimmunosensor in the seawater range of pH 7.5 to 8.4. In light of the aforesaid, there is no need for sample pre-treatment and the BiMW sensor is stable up to 30 different measurements-regeneration cycles with seawater samples (Fig. S4).

3.4. Analyses of spiked seawater samples

In order to test the robustness and trueness of the biosensor results, several blind spiked seawater samples were analysed both, with the BiMW biosensor and by a confirmatory method, based on chromatography and mass spectrometry.

For the preparation and fortification of the samples, pristine seawater was taken in the north of the Catalan litoral using Pyrex borosilicate glass bottles and filtered with nylon filters (0.45 µm mesh size, Whatman). Aliquots were spiked with certain concentrations of Irgarol 1051 in the facilities of the Institute IDAEA-CSIC (Barcelona, Spain). The concentrations of Irgarol 1051 in the blind samples were unknown during the analysis with the BiMW biosensor.

The confirmatory method was as follows: samples were extracted using a method based on that published by Gros et al. (Gros et al., 2006), with some modifications. Briefly, 500 mL of seawater were acidified with formic acid to pH 2.0 and extracted by solid phase extraction (SPE) using Oasis® HLB cartridges (Waters). After the loading, the cartridges were washed with 5.0 ml ultrapure water, previously adjusted at pH=2 with formic acid, and eluted with 5.0 mL of methanol. The extracts were evaporated to 100 µL, under a gentle flow of nitrogen, and 900 µL of methanol were added to each vial. The extracts were analysed by liquid chromatography coupled to high resolution mass spectrometry with electrospray ionisation (HPLC-ESI-HRMS). The chromatographic separation was achieved by reverse-phase chromatography with a C18 column. 20 µL of extract were injected each run. The electrospray ionisation source worked in positive mode, with the source temperature set at 300^{o} C and a spray voltage of 2.500 KV. Acquisition was carried out in full scan mode with a resolution of 70.000 (full width at half maximum, measured at m/z=200).

In order to prevent cross contamination and the presence of interferences, all the bottles and the glass material employed in their analysis was previously rinsed with ultrapure water and methanol and heated at 400^{o} C overnight. Samples were stored at -20^{o} C until their analysis.

Five seawater samples were fortified at concentrations that ranged from 25 ng/L to 100 µg/L. The nominal concentrations of the blind samples were 100 µg/L (far above the sensor limit of linearity), 25 ng/L, 1.0 µg/L, 200 ng/L and 500 ng/L. After being spiked, the seawater samples were labelled as blind samples and analysed by the BiMW immunosensor in duplicate. In parallel, the chromatographic method confirmed, within an error margin of <10 %, the trueness and stability of the spiked concentrations. This discarded potential losses of Irgarol 1051 due to degradation, sorption or any other miscellaneous factor.

Overall, a good agreement was obtained between the BiMW immunosensor and the confirmatory method (see Table 1) The most contaminated sample (#1, $c_{nominal}$=100 µg/L) offered an instrumental response far above the limit of linearity, as expected. All the other samples could be correctly quantified by the biosensor, offering results close to the spiked concentrations.

Table 1. *Analyses of spiked seawater samples.*

Sample	$C_{nominal}$ (ng/L)	C_{sensor} (ng/L)	error (%)
#1	10^5	>LoL	N.A.
#2	30	25	16
#3	1000	1177	-18
#4	200	131	34
#5	500	505	-1

LoL: limit of linearity; N.A.: Not Applicable

These results correlated linearly with the concentration of LC-MS analyses, with a Pearson correlation coefficient of r=0.996 and a p=0.004, respectively. The slope of the regression line was close to the unit (slope= 1.219±0.081), suggesting a good correspondence between both techniques.

4. Conclusions

A bimodal waveguide interferometer (BiMW) immunosensor for real-time and label-free detection of Irgarol 1051 directly in seawater has been successfully developed. Special attention has been placed to the surface functionalization by evaluating different silanization protocols and activation chemistries. Another crucial step for the development of the biosensor was the optimization of the immunoassay conditions. The working principle is based on a competitive inhibition format assay with the covalent attachment of an Irgarol 1051 derivative to the sensor surface via an aminosilane. Results show a LOD and IC_{50} of 3 ng/L and 66 ng/L in seawater, respectively, clearly lower than the 16 ng/L set by the EU as the maximum allowable concentration. The sensor surface is stable up to 30 measure-regeneration cycles of seawater samples and each measure-regeneration cycle takes only 20 minutes. Finally, the immunosensor performance has been evaluated with blind samples without any pre-treatment and by a confirmatory method based on chromatographic tools. The immunosensor was confirmed to exhibit a satisfactory performance and showed to be a sensitive and straight-forward methodology in comparison with the LC-MS method, which was more time-consuming, less cost-effective and involved the use and waste of significant volumes of environmentally unfriendly organic solvent. To our knowledge this is the most sensitive biosensor for the detection of Irgarol 1051 directly in seawater. The low LOD is as good as some of the reported chromatographic techniques but without the need of clean-up and pre-concentration steps. This study was part of the European project BRAAVOO (*Biosensors, Reporters and Algal Autonomous Vessels for Ocean Operation*) for the development of biosensors for real time monitoring of biohazard and man-made chemical contaminants in the marine environment. As part of the project, the developed immunosensor can be further miniaturized and integrated in unmanned marine vessels or buoys for the autonomous monitoring of Irgarol 1051 in seawater. Other future improvements will include a multiplexed functionalization of each of the waveguide sensors within the same chip for the multiple evaluation of different chemical pollutants present in the same sample of seawater.

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Highlights

- Implementation of an effective nanobiosensor for the label-free and real-time monitoring of Irgarol 1051 directly in seawater.
- The biosensor is able to directly detect Irgarol 1051 in undiluted seawater with no sample pre-treatment.
- The limit of detection in real sweater samples is 3 ng/L, well below the 16 ng/L set by the EU as the maximum allowable concentration in seawater.
- The biosensor is able to carry out up to 30 measurement-regeneration cycles in 20 min each.