



Immunoassay and amperometric biosensor approaches for the detection of deltamethrin in seawater

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Received: 27 April 2018 / Accepted: 19 June 2018
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Abstract

The study of an enzyme-linked immunosorbent assay (ELISA) and an amperometric biosensor for the detection of the pyrethroid deltamethrin in seawater is reported. The preparation of specific polyclonal antibodies is addressed using two immunizing haptens based on deltamethrin and cypermethrin compounds, with a spacer arm placed at the cyano residue in the pyrethroid structure. Different conjugates based on bovine serum albumin and aminodextran are prepared depending on the lipophilic profile of the competitor haptens studied. A reproducible and sensitive indirect competitive ELISA is developed, reaching a limit of detection of $1.2 \pm 0.04 \mu\text{g L}^{-1}$ and an IC_{50} value of $21.4 \pm 0.3 \mu\text{g L}^{-1}$ (both $n = 3$). For validation of the assays described, artificial seawater samples fortified with deltamethrin are analyzed. For the ELISA assay, these accuracy studies reported a slope of 0.904. An amperometric immunosensor is developed using the same immunoreagents and achieving a comparable detectability in terms of LOD of $4.7 \mu\text{g L}^{-1}$, measuring seawater without any pretreatment. These results suggest that both techniques can be used as rapid and simple analytical methods for deltamethrin quantification in seawater samples, which are great candidates for initial environmental screening programs.

Keywords Immunoassay · Amperometric biosensor · Seawater · Deltamethrin

Introduction

Nowadays, α -cyano pyrethroids are being extensively used as insecticides in domestic, agriculture, horticulture, public health, and veterinary applications, mainly due to its low mammalian

toxicity and its activity against a broad range of pests [1–3]. Cyphenothrin, λ -cyhalothrin, cypermethrin, and deltamethrin (see Fig. 1) among other synthetic pyrethroids have reported improved physicochemical properties and biological actions compared to their natural analogues. Among them, deltamethrin have been presented as one of the more lipophilic and stable insecticide-permethrins, and in consequence, its residues have been detected in agricultural products [4], food [5, 6], and surface waters [7, 8]. This pyrethroid has been classified as highly toxic to aquatic life [9], and although α -cyano pyrethroids are thought to be safe for human health, some negative related effects have been reported, encouraging the need of creation of control platforms. During the last decades, a wide range of global and regional environmental monitoring programs have been developed [10, 11], aiming for early warning systems that can provide extreme sensitivity and selectivity information about contaminants. Together with the REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) law (EC 1907/2006) and the Drinking Water Directive (DVD, the Council Directive 98/83/EC), new initiatives for the regulation of the pollutant's presence in aquatic environments have been developing during the last years.

Philipp Fruhmann and Ana Sanchis contributed equally to this work.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00216-018-1209-1>) contains supplementary material, which is available to authorized users.

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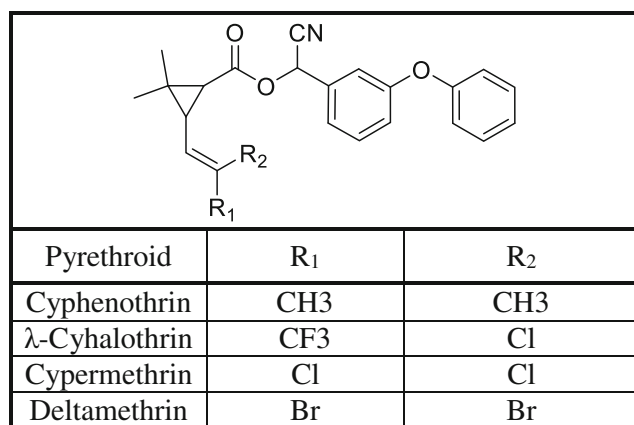


Fig. 1 Chemical structures of some of the most used synthetic pyrethroids

Current analytical methods for deltamethrin detection are usually based on chromatographic techniques usually coupled to universal detectors such as UV or mass spectrometry [12–21], achieving good levels of sensitivity and accuracy but at the expense of being time-consuming, expensive, not suitable for the analysis of large numbers of samples, and incapable of being easily field-deployable.

Immunoassays have become great candidates to complement chromatographic techniques in environmental studies because of its attractive features, including rapid detection, low cost, the possibility of analysis of complex matrices without extensive pretreatment, variety of platforms, and high-throughput capabilities. Detection of pyrethroids by immunochemical procedures has been widely explored [22]. Antibodies addressing specific detection of deltamethrin or cypermethrin [3, 23–25] or the pyrethroid family [7, 26] have been produced and used to develop a broad variety of immunochemical techniques [27, 28]. However, few examples of immunosensors for the detection of deltamethrin have been reported. A surface plasmon resonance (SPR) based on antibody-antigen recognition with enhancement with nanoparticles has been described for the detection of deltamethrin, achieving a LOD of $0.01 \mu\text{g L}^{-1}$ [29]; however, the need of operation in flow conditions and using magnetic nanoparticles to enhance the final signal can be a limitation in terms of cost and usability in field assays. On the other hand, an electrochemical impedance spectroscopy (EIS) assay has also been described for the detection of deltamethrin, and although it showed a great LOD of $0.303 \mu\text{g L}^{-1}$, the authors stated that their technique exhibited large dispersion in most of the calibration points and real samples, decreasing the accuracy and precision of the insecticide measuring [30].

Considering all mentioned points, we therefore set out to develop an immunosensor with electrochemical readout for the detection of deltamethrin in different water environments. Electrochemical biosensors are based on antigen-antibody assays, which eventually produce a change in an electrical

parameter that can be measured by the transducer. They are considered attractive options due to its advantages in rapidness, reliability, low costs, and efficiency for monitoring (organic) contaminants in aquatic environments [26, 31]. In comparison to optical sensors, which an example has been described for deltamethrin detection, the electrochemical variants usually are considered to be cheaper alternatives in addition to being more suitable for “on site” measures [22, 31]. In addition to this and to the best of our knowledge, there is no immunosensor with *amperometric* readout described for deltamethrin in the literature, which makes such a sensor highly desirable.

Materials and methods

Chemicals and biochemicals

The chemical reagents used were obtained from Aldrich Chemical Co. (Milwaukee, WI, USA) and from Sigma Chemical Co. (St. Louis, MO, USA). The preparation of the described bioconjugates and antibodies has been performed with the support of the U2 of the ICTS “NANBIOSIS,” more specifically by the Custom Antibody Service (CAbS, CIBER-BBN, IQAC-CSIC). The synthesis of the immunizing haptens D133 and C134 and competitors used in this paper are described in the Electronic Supplementary Material (ESM). The immunogens and bioconjugates prepared are also described in the same section. The antisera (As) were obtained by immunizing female white New Zealand rabbits with C134-HCH (cypermethrin immunogen; As358, As359, and As360) and with D133-HCH (deltamethrin immunogen; As361, As362, and As363) following the protocol usually used in our group [32]. Deltamethrin standard was purchased from Sigma Chemical Co. (St. Louis, MO, USA). For the matrix studies, artificial seawater (aSW) was purchased from Sigma Chemical Co. (St. Louis, MO, USA), prepared at 40 mg mL^{-1} in Milli-Q water; its pH and conductivity were monitored being 8.14 ± 0.27 and 49.69 ± 1.51 , respectively ($n = 20$).

Instruments and general methods

The pH and the conductivity of all buffers and solutions were measured with a pH meter pH 540 GLP and a conductimeter LF 340, respectively (WTW, Weilheim, Germany). Polystyrene microtiter plates were purchased from Nunc (Maxisorp, Roskilde, Denmark). Dilution plates were purchased from Nirco (Barberà del Vallés, Spain). Washing steps were performed on a Biotek ELx465 (Biotek Inc.). Absorbances were read on a SpectramaxPlus (Molecular Devices, Sunnyvale, CA, USA). The competitive curves were analyzed with a four-parameter logistic equation using the software SoftmaxPro v4.7 (Molecular Devices) and

GraphPad Prism 5.03 (GraphPad Software Inc., San Diego, CA, USA).

All amperometric measurements were performed with an Autolab PGSTAT302N equipped with an ECD module (Utrecht, NL) and recorded using Nova software. Screen-printed electrodes (SPE, AT220) consisting of a 4-mm smooth Au working electrode, Au counterelectrode, and Ag pseudo-reference electrode were bought from a commercial source (Dropsens, Spain) and functionalized prior to their use. For the flow cell setup, the corresponding flow-cell (DRP-FLWCL, DropSens, Spain) was used. Initial cleaning of the electrodes was performed via a UV/Ozone Procleaner™ unit (BioForce Nanoscience, Ames, IA, USA) and further functionalization was done using different functionalized PEG chains from Polypure (Oslo, Norway). Within the setup, a peristaltic pump from Ismatec (ISM597D) (Wertheim, Germany) and Tygon tubes (inner diameter = 0.75 mm) were used. The connection between the peristaltic pump and the flow cell was made with PTFE tubes with an inner diameter of 0.5 mm. The calibration curves were fitted to a four-parameter logistic equation using OriginPro software (Northampton, MA, USA).

Buffers

Unless otherwise indicated, phosphate buffer saline (PBS) is 0.01 M phosphate buffer in a 0.8% saline solution, pH 7.5. Coating buffer is a 0.05 M carbonate-bicarbonate buffer, pH 9.6. PBST consists of PBS with 0.05% Tween 20 with a pH of 7.5. PBST optimized was PBS with 0.005% Tween 20, 1.6% saline solution with a pH of 7.5. PBT optimized was PB (no saline solution) with 0.01% Tween 20 with a pH of 7.5. Citrate buffer was 0.04 M sodium citrate, pH 5.5. The substrate solution contains 0.01% 3,3',5,5'-tetramethylbenzidine (TMB) and 0.004% H₂O₂ in citrate buffer. Borate buffer is 0.2 M boric acid/sodium borate, pH 8.7.

ELISA C134-AD/As360 protocol

Microtiter plates were coated with the antigen C134-AD (0.45 µg mL⁻¹ in coating buffer, 100 µL well⁻¹), overnight at 4 °C, and covered with adhesive plate sealers. The next day, the plates were washed four times with PBST (300 µL well⁻¹), and the deltamethrin standards (from 10,000 to 0 µM in DMSO, and diluted 200 times in PBST or aSW just prior the assay) or the samples were added (50 µL well⁻¹), followed by the solution of antisera As360 (1/8000 both in PBST, and aSW, 50 µL well⁻¹). After 30 min at RT, the plates were washed as mentioned before, and a solution of anti-IgG-HRP (1/6000 in PBST) was added to the wells (100 µL well⁻¹) and incubated for 30 min at RT. The plates were washed again, and the substrate solution was added (100 µL well⁻¹). Color development was stopped after

30 min at RT with 4 N H₂SO₄ (50 µL well⁻¹), and the absorbance was read at 450 nm. The standard curves were fitted to a four-parameter equation according to the following formula: $y = (A - B/[1 - (x/C)^D]) + B$, where A is the maximal absorbance, B is the minimum absorbance, C is the concentration producing 50% of the maximal absorbance, and D is the slope at the inflection point of the sigmoid curve. Unless otherwise indicated, data presented correspond to the average of at least two well replicates.

Specificity studies

Solutions of different structurally related compounds such as cypermethrin, cypermethrin fractions (F1 and F2), λ-cyhalothrin, esfenvalerate, permethrin, and 3-phenoxybenzaldehyde (see ESM Fig. S4). The standard curves were performed following the protocol described before. The cross-reactivity (CR) values were calculated according to the equation: $IC_{50} \text{ [nM]} (\text{Deltamethrin}) / IC_{50} \text{ [nM]} (\text{cross-reactant}) \times 100$.

Biofunctionalized SPE

The gold SPE were rinsed with water and ethanol, and cleaned in a UV/Ozone chamber for 15 min prior to their functionalization. A mixed self-assembling monolayer (m-SAM) was prepared by immersing the electrodes on an ethanolic solution consisting on a mixture of 2.5 mM O-(2-carboxyethyl)-O'-(2-mercaptoethyl)-heptaethylene glycol (acid-PEG-thiol) and 7.5 mM O-(methyl)-O'-(2-mercaptoethyl)-hexaethylene glycol (m-PEG-thiol) for 2 h at RT. Afterwards, the electrodes were washed with ethanol, dried, and biofunctionalized placing on the surface of the electrode a solution of 25 µL of the competitor C134-AD (200 µg mL⁻¹) and 25 µL of a mixture of EDC/NHS (200 mM) in PBS. After 3 h at RT, the electrodes were rinsed with PBS and the remaining reactive carboxylic acids were capped by immersing the electrodes in a 1 M ethanolamine solution in PBS for 1 h at RT. After this treatment, the functionalized electrodes were washed with water, dried, and stored in a desiccator until use.

Immunosensor protocol

The used immunosensor protocol was similar to one recently developed [33] using a flow rate of 100 µL min⁻¹ during the whole procedure. The electrodes were placed in a flow cell and flushed for 10 min with PBST buffer. Deltamethrin standards (prepared in aSW or PBS) or samples (600 µL) were mixed with a solution of As360 (1/500 in PBST, 600 µL) and the mixture (1 mL) flushed over the sensor and subsequently washed with PBST buffer (5 min). Afterwards, a solution of antiIgG-HRP (1/1000 in PBST, 1 mL) was flushed through the cell, followed by another washing step with PBST (5 min).

After this time, a solution of citrate buffer was passed (5 min) and the amperogram (C) was recorded (60 s) followed by the substrate solution ten times diluted in citrated buffer (0.001% TMB and 0.0004% H_2O_2 in citrate buffer, 3 min) and another amperogram was recorded (S). The amperograms were acquired applying a potential of -0.10 vs. the Ag pseudo reference electrode and recording the signal for a period of 60 s. The chip was regenerated by passing a solution of citrate buffer (5 min), 0.3 M NaOH solution (10 min), and PBST (5 min) buffer, and used for the next measurement.

Results and discussion

Immunoreagent preparation and characterization

The immunizing haptens D133 (for deltamethrin) and C134 (for cypermethrin) were selected and synthesized according to Skeritt et al. [34] with slight modifications (see ESM Fig. S1). HCH conjugates were prepared for raising antibodies As358–360 (C134) and As361–363 (D133) while BSA and AD bioconjugates were prepared for their use as competitors. The highly hydrophilic polysaccharide AD was proposed instead of BSA in some cases, to minimize the potential risk of hapten folding. Deltamethrin and cypermethrin are highly lipophilic (see ESM Table S2) and have a tendency to be buried into the hydrophobic core of proteins; therefore, the hydrophilic character of AD could prevent the hapten folding, being an alternative option to the ones based on the use of short or rigid spacer arms [3, 35, 36]. Furthermore, the amino groups allow covalent coupling to the carboxylic groups on the haptens. Confirming our hypothesis, the antibodies evaluated during the first titer screening did not show any signal when exposed to C134 and D133 coupled to BSA, but higher responses at higher antiserum dilutions were obtained when the non-competitive assays were performed against aminodextran conjugates (data not showed).

The avidity of antisera raised towards homologous and heterologous bioconjugate competitors was tested aiming to obtain the best analytical performance of the competitive immunochemical assay, since it has been reported that the nature of the coating antigen can modulate such parameters of an assay [36]. Conjugates based on cypermethrin and deltamethrin moieties (F1 and F2 haptens) (for exploring the importance of the halogen atoms on the pyrethroid structure over the antibody recognition profile, and F3 and 3-phenoxybenzaldehyde (3-PBA) for the double-ring structure with the phenoxy benzene fragment) were proposed as heterologous competitors. Moreover, bioconjugates with different hapten densities were also prepared (see ESM Table S1), because lower densities have been often related to better sensitivities [37]. The appropriate dilutions of the antisera (As358–363) and the coated antigens (C134-AD, D133-AD, F1-BSA, F2-BSA, F3-BSA,

and 3-PBA-BSA) were established after two-dimensional checkerboard titration assays performed as described in [38]. It was found some good combinations that allowed the detection of deltamethrin (see ESM Table S3); however, despite the precedents, the best As/bioconjugate combination consisted on As360 (C134-HCH) with the homologous competitor C134-AD. The ELISA developed using these immunoreagents detected deltamethrin directly in seawater with an IC_{50} value of $21.4 \pm 0.3 \mu\text{g L}^{-1}$ and a limit of detection of $1.21 \pm 0.04 \mu\text{g L}^{-1}$. Under these conditions, cypermethrin was detected in this assay with an IC_{50} of $78.6 \pm 0.7 \mu\text{g L}^{-1}$ and a LOD of $4.56 \pm 0.05 \mu\text{g L}^{-1}$. The fact that the antiserum raised against a chlorinated compound (cypermethrin, $X=\text{Cl}$, see ESM Fig. S1) recognizes better the brominated derivative (deltamethrin, $X=\text{Br}$) has been previously reported [37, 39]. Between the hypotheses to explain this fact, there is the size of the bromine atoms, bigger than the chlorine atoms, and therefore, fitting better into the antibody binding cavities, favoring the antibody recognition of the deltamethrin. Another explanation relies in the differences of the electronic distribution between chlorinated and brominated analogues, which could create differences in dipole-dipole interactions in the antigen-antibody complex.

The effects of different physicochemical parameters related to the final matrix and the buffer selected for the development of the assays were tested introducing variations in the initial conditions of the assay (see ESM Fig. S3). According to this optimization, a calibration curve of the deltamethrin assay after optimization is shown in Fig. 2 and the final features of the assay are summarized in Table 1. The reproducibility of the assay has been demonstrated by evaluating the intraday and interday variability at the IC_{50} value and was found below 5% in all the cases.

The specificity of the immunoreagents was assessed by testing structurally related compounds such as cypermethrin, cypermethrin fractions (F1 and F2), λ -cyhalothrin, esfenvalerate, permethrin, and 3-phenoxybenzaldehyde (see

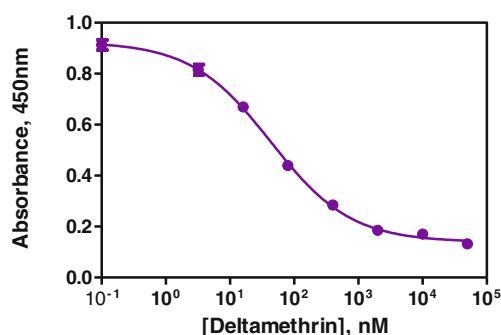


Fig. 2 Calibration curve of the As360/C134-AD ELISA. The data shown correspond to the average of three assays performed on three different days. Each assay was built using three well replicates. LOD corresponds to limit of detection, calculated as the concentration given at 90% of the maximum signal. The information of the different parameters of the assay is summarized in Table 1

Table 1 ELISA As360/C134-AD

$$[\text{As}] = 1/8000, [\text{competitor}] = 0.45 \mu\text{g mL}^{-1}$$

Absorbance _{min}	0.162 ± 0.024	Tween 20	0.005%
Absorbance _{max}	0.944 ± 0.020	pH	7.5
Slope	− 0.714 ± 0.022	Ionic strength	28.6 mS cm ^{−1}
IC ₅₀ , nM	42.4 ± 0.59 (21.4 ± 0.30)	Competition time	30'
(μg L ^{−1})		Preincubation time	No
LOD, nM	2.39 ± 0.85 (1.21 ± 0.04)		
(μg L ^{−1})			
R ²	0.998 ± 0.001		

ESM Fig. S4). Table 2 summarizes the IC₅₀ and cross-reactivity results for each compound, showing that the antibody raised and selected for this study was specific for deltamethrin, although a slight recognition (CR < 25%) of pyrethroids sharing the 2,2-dimethyl-cyclopropane group or terminal halogen atoms was also observed. Additionally, other contaminants usually found in environmental samples such as antibiotics (sulfapyridine, chloramphenicol), pesticides (Irgarol 1051®), polybrominated flame retardants (BDE-47), hormones (17β-estradiol), or toxins (domoic acid) did not interfere with the assay (data not shown).

Potential non-specific interferences caused by the sample matrix were studied by preparing a standard curve directly in seawater; however, as can be seen in Fig. S5 (see ESM), the assay performed very well in seawater, observing only a mild signal loss with respect to the assay carried out in PBST, while the detectability was in the same order.

Accuracy studies performed using blind spiked samples of artificial seawater demonstrated that the results obtained match the spiked values (see Fig. 3). The sample concentrations were calculated interpolating the results to deltamethrin standard curves prepared in aSW, showing in the linear regression analysis a very good correlation value ($R^2 = 0.995$) with a slope of 0.904, close to the perfect correlation.

Table 2 Cross-reactivity of related compounds in the As360/C134-AD^a

	IC ₅₀ (nM)	% CR
Deltamethrin	42	100
Permethrin	307.1	13
Cypermethrin	189.1	22
Esfenvalerate	218.1	19
λ-Cyhalothrin	185.8	23
3-Phenoxybenzoic acid	> 10,000	< 0.05
F1	> 10,000	< 0.05
F2	> 10,000	< 0.05

^a Cross-reactivity is expressed as a percentage of the relation between the IC₅₀ (nM) of deltamethrin and the IC₅₀ (nM) of the other compounds tested. Data obtained using ELISA

Amperometric flow detection

With the immunoreagents completely characterized, we proceeded to their implementation on an amperometric sensor platform. Amperometric immunosensors consisted in the acquisition of a current when a potential is applied. The acquisition of the current is made using gold screen-printed electrodes which can allow the immobilization of biomolecules by thiol chemistry. Specifically, those gold SPE can be used several times after a step of regeneration, being cost per analysis lower than disposable SPE. Moreover, the fact that it can be reusable makes it possible to develop a remote device that can work automatically. The biofunctionalization of the electrodes was accomplished through the formation of a stable mixed-self-assembled monolayer with a ratio of 1:3 between acid-PEG-thiol/m-PEG-thiol as described in [40] and based on previous studies [33, 41]. The biofunctionalization of the SPE was monitored by measuring CV. The experiments were carried out in order to determine the effect on the rate of the electron transfer in the surface of the coated SPE. The CVs obtained for the SPE in different

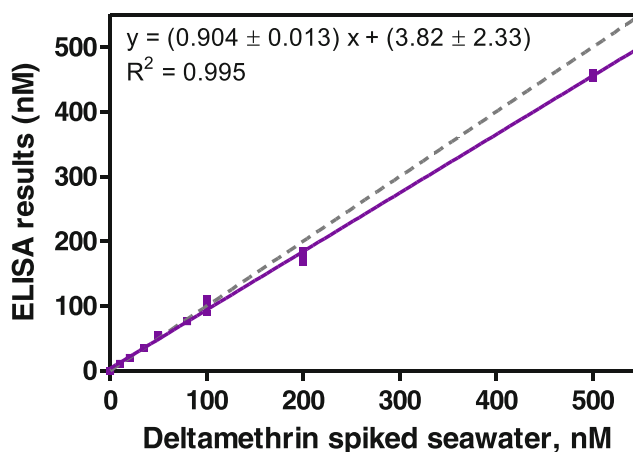


Fig. 3 Correlation studies performed to assess accuracy of the ELISA in artificial seawater. The graph shows the linear regression analysis. The dotted line corresponds to a perfect correlation ($m = 1$). The data correspond to the average of at least three well replicates from three different days

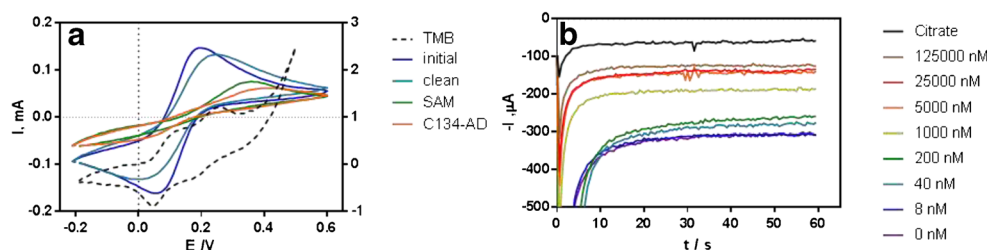


Fig. 4 (A) CV for initial, clean, formation of SAM and immobilization of C134-AD stages in 10 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ in PBS (scan rate $100 \text{ mV} \cdot \text{s}^{-1}$ from -0.2 to 0.6 V); CV for TMB 0.001% and H_2O_2 0.0004% in citrate

buffer (scan rate $50 \text{ mV} \cdot \text{s}^{-1}$ from -0.2 to 0.6 V). (B) Amperometric response of antibody binding As360 at -0.1 V at different concentrations of deltamethrin

stages in $\text{Fe}(\text{CN})_6^{3-/4-}$ solution are shown in Fig. 4A. As it can be seen, a substantial effect on the electron transfer was observed after the formation of the pegylated SAM, according to the full passivation of the surface. The immobilization of the coating antigen C134-AD on the functionalized surface has also affected the electron transfer; however, the highest impact was observed in the SAM formation. According to these results, the biofunctionalization took place successfully and the electrodes were ready to test the immunochemical assay.

Initial experiments were addressed to assess if the biofunctionalized electrode was able to detect deltamethrin. The binding of the antibody As360 with the immobilized coating antigen C134-AD was monitored at -0.1 V as described by Esteban-Fernandez et al. [42], using TMB as a mediator for HRP. The redox profile of TMB/ H_2O_2 was measured by CV showing the characteristic peaks (see Fig. 4A). The signal was acquired following the procedure in the experimental section and the different amperograms were acquired for each concentration of deltamethrin (see Fig. 4B). As can be observed, the steady-state of the signal was reached after 30 s; however, the final signal was obtained after 60 s. Thus, a single concentration of the analyte was passed through the flow cell to assess inhibition

of the binding of the antibodies to the surface of the electrode. In the absence of the analyte, an average absolute maximum signal of $256.0 \pm 46.4 \text{ nA}$ ($N=3$) was obtained, while in the presence of deltamethrin (125,000 nM), the signal was reduced to $94.7 \pm 23.1 \text{ nA}$ ($N=3$). The reproducibility of the electrodes was deeply studied by the analysis of two different SPE coated with the C134-AD, being an average absolute maximum signal of $285.0 \pm 33.5 \text{ nA}$ ($N=4$) and $245.5 \pm 62.33 \text{ nA}$ ($N=6$). According to these results, it can be stated that the regeneration of the surface can take place using the same SPE, and it can be reproduced in different SPE. When the measurements were performed using seawater, the maximum signal was $201.3 \pm 60.9 \text{ nA}$ ($N=7$), slightly lower than in buffer, and in the presence of deltamethrin, the amperometric signal was $103.7 \pm 19.9 \text{ nA}$ ($N=7$), which is in the same range as the results of our PBST measurements. With these precedents, we proceeded to build standard curves and measure them with the biofunctionalized electrodes. The results obtained are shown in Fig. 5 and the parameters of the assay in Table 3. The data shown corresponds to the measuring of the standard curve for at least three consecutive days. The amperometric signal within the recorded cycles showed to be very similar, and the standard deviation for the different normalized measurement points was also satisfying with 3 to 10% for buffer and 5 to 15% for artificial seawater. The IC_{50} obtained resulted in 272 and 230 nM (116 and $137 \mu\text{g L}^{-1}$) and a LOD of 14.9 and 9.3 nM (7.5 and $4.7 \mu\text{g L}^{-1}$) in buffer and in seawater, respectively.

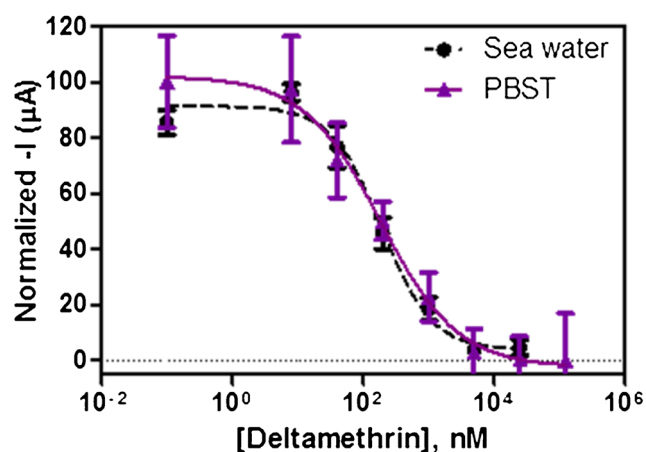


Fig. 5 Calibration curve obtained in the immunosensor for deltamethrin detection in PBST and in artificial seawater. The data correspond to the average of at least three different days. The information of the different parameters of the assay is summarized in Table 3

Table 3 Immunosensor As360/C134-AD

	PBST	Seawater
Norm- $I_{\min}$, μA	4.39 ± 5.62	2.68 ± 4.15
Norm- $I_{\max}$, μA	99.20 ± 0.45	93.29 ± 6.40
Slope	-0.76 ± 0.15	-1.40 ± 0.64
IC_{50} , nM ($\mu\text{g L}^{-1}$)	272.5 ± 206.4 (137.6 ± 104.2)	230.8 ± 162.3 (116.5 ± 81.9)
LOD, nM ($\mu\text{g L}^{-1}$)	14.9 ± 16.1 (7.5 ± 8.13)	230.8 ± 162.3 (4.69 ± 3.31)
R^2	0.97 ± 0.03	0.96 ± 0.02

Conclusions

An ELISA platform for the direct determination and quantification of a potential pollutant of the ocean, deltamethrin, has been developed and its suitability to analyze seawater samples has been evaluated, allowing a quick and cheap first step in the field of pyrethroids' monitoring. The antiserum 360 raised against C134 demonstrated a great specificity for deltamethrin detection, with mild cross-reactivity to other members of the α -cyano pyrethroids, and the assay developed did not show any matrix-related effect when analyzing seawater-spiked samples directly. Good recoveries from seawater-spiked samples were obtained, demonstrating its suitability to analyze environmental samples. Finally, a proof-of-concept using an amperometric sensor was demonstrated, detecting deltamethrin with good detectabilities compared with those of the ELISA. Although the accuracy and the limit of detection of our device is lower than within chromatographic measurements, the developed sensor is cheaper and could be used for autonomous measurements within a prolonged time period. In addition to this, the simplicity of the device itself and the possibility for remote control is an advantage, since chromatographic methods also require skilled personnel. For this reason, the electrode could be used as an alarm system for water quality in harbors, fish farms, and coastal areas.

Funding information This work has been funded by SEA-on-a-CHIP project (FP7-OCEAN-2013, no 614168). The Nb4D group (formerly Applied Molecular Receptors group, AMRg) is a consolidated research group (Grup de Recerca) of the Generalitat de Catalunya and has support from the Departament d'Universitats, Recerca i Societat de la Informació de la Generalitat de Catalunya (expedient: 2014 SGR 1484). CIBER-BBN is an initiative funded by the Spanish National Plan for Scientific and Technical Research and Innovation 2013–2016; Iniciativa Ingenio 2010, Consolider Program, and CIBER Actions are financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund. The ICTS “NANOBIOSIS,” and particularly the Custom Antibody Service (CAbs, IQAC-CSIC, CIBER-BBN), is acknowledged for the assistance and support related to the immunoreagents used in this work.

Compliance with ethical standards

Rabbit immunizations included in this study were carried out in the animal facility of the Research and Development Center (CID) from the Spanish Research Council (CSIC) -Registration Number B9900083. All efforts were made to minimize suffering of the animals. The protocol used for the production of antibodies was conducted in accordance with the institutional guidelines under a license from the local government (DAAM 7463) and was approved by the Institutional Animal Care and Use Committee at the CID-CSIC.

Conflict of interest The authors declare that they have no conflict of interest.

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