Author's Accepted Manuscript

www.elsevier.com/locate/talanta

Sensor system based on flexible screen-printed electrodes for electrochemical detection of okadaic acid in seawater

B. Molinero-Abad, L. Perez, D. Izquierdo, I. Escudero, M.J. Arcos-Martinez

PII: S0039-9140(18)30981-0
DOI: https://doi.org/10.1016/j.talanta.2018.09.072
Reference: TAL19086

To appear in: *Talanta*

Received date: 16 May 2018
Revised date: 14 September 2018
Accepted date: 19 September 2018

Cite this article as: B. Molinero-Abad, L. Perez, D. Izquierdo, I. Escudero and M.J. Arcos-Martinez, Sensor system based on flexible screen-printed electrodes for electrochemical detection of okadaic acid in seawater, *Talanta,* https://doi.org/10.1016/j.talanta.2018.09.072

Sensor system based on flexible screen-printed electrodes for electrochemical detection of okadaic acid in seawater

B. Molinero-Abad [a], L. Perez[a,b], D. Izquierdo[a], I. Escudero[b] and M. J. Arcos-Martinez[a*]

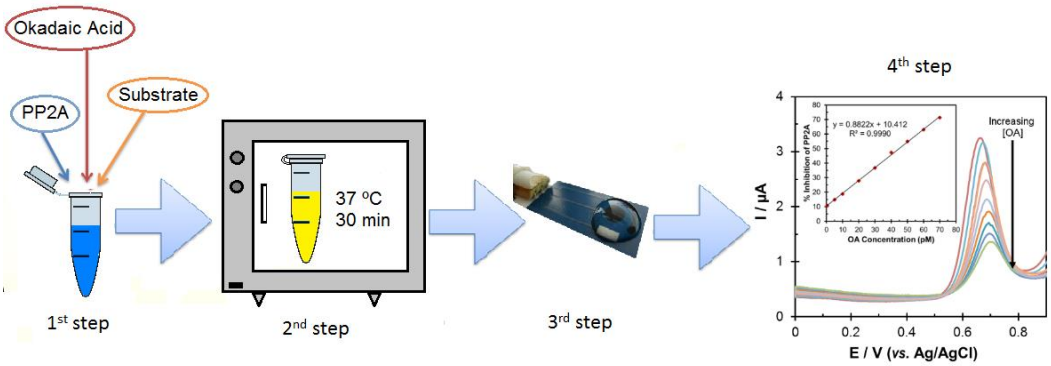
Departmento de Quimica[a] and Departmento de Biotecnologia[b], Facultad de Ciencias, Universidad de Burgos. Plaza Misael Bañuelos s/n, 09001 Burgos, Spain,

*jarcos@ubu.es

Abstract

The monitoring of marine dinophysistoxin okadaic acid in seawater can serve as an early alert system for preventing the potential negative effects this toxin can have on the food industry and human health in general. A disposable sensor system for electrochemical detection of this toxin has been developed using screen-printed electrodes. The method described is based on the inhibition of protein phosphatase type 2A by okadaic acid, evaluating the enzyme activity. p-Nitrophenyl phosphate, 4-methylumberilphenyl phosphate and phenyl phosphate have been tested as substrates achieving good limits of detection of $2.7 \cdot 10^{-12}$ M of okadaic acid. The proposed method has been successfully applied to okadaic acid determination in seawater samples. A study of divalent cations present in seawater that can interfere with the measurement has been carried out. The electrode systems were printed on both rigid and textile backing materials to observe the influence of those materials on the final performance of the sensor.

Graphical abstract



Keywords

Okadaic Acid, Screen Printed Electrodes, Textile Sensors, Seawater, PP2A

1. Introduction

Okadaic acid (OA) is a marine lipophilic toxin produced by toxicogenic dinoflagellates, mainly belonging to the genera Dinophysis and Prorocentrum [1], which may accumulate in the digestive glands of shellfish when they feed on these kinds of microalgae [2, 3]. Although OA and its dinophysistoxin derivatives produce no harmful effect in shellfish, it is the major diarrhetic shellfish poisoning (DSP) toxin in humans, causing gastrointestinal troubles such as diarrhea, nausea, vomiting and abdominal pain. The toxicity mechanism of OA and of all DSP toxins lies in blocking the active sites of the enzymes and therefore the consequent inhibition of serine/threonine protein phosphatases type1 (PP1) and type 2A (PP2A), resulting in an over-phosphorylation of proteins in cells [4-6]. Human intoxication by DSP can have a disastrous effect on the shellfish industry resulting in huge financial losses [7]. Early detection of these toxins in shellfish tissue, or even directly in the seawater they inhabit prior to collection, could be key to minimizing the risk of collecting infected shellfish. In this regard, the European Commission Regulation EU No 786/2013 establishes a maximum permitted concentration of 160 µg of OA equivalents per kg of live bivalve molluscs for human consumption [8].

Several methods for OA detection have been reported in an effort to protect people from the potential health risks of eating contaminated shellfish. One of the most commonly used methods for years has been mouse bioassay (MBA) [9, 10]. However, due to the ethical concerns surrounding animal testing and the poor specificity of this method, new methods have been developed in recent years.

Immunological assays such as the enzyme-linked immunosorbent assay (ELISA) have been used due to their high sensitivity and stability, since they rely on the structure of the toxin for detecting the interaction between antibodies and toxins [11]. Immunosensors follow the main principle as that of ELISA methods, linking antibodies to the electrode surface and obtaining the OA signal through electrochemical detection [12-15]. Eissa and co-workers developed an immunosensor based on a screen-printed electrode modified with graphene on which OA antibodies were covalently immobilized, providing a limit of detection (LOD) of 19 ng L^{-1} [16]. Dominguez developed another immunosensor with an LOD of 150 ng L^{-1} under flow conditions [17].

Chromatographic techniques have also been widely used in the detection of DSP toxins, often coupled with other techniques to improve detection. Such techniques include liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) [18], ion spray mass spectrometry [19], dual solid phase extraction coupled with liquid chromatography-tandem mass spectrometry (SPE-LC-MS/MS) for the simultaneous determination of multi-class cyanobacterial and algal toxins in water [20], high performance liquid chromatography (HPLC) [21], and liquid chromatography-linked protein phosphatase bioassay [22].

Serine/threonine protein phosphatase inhibition has commonly been detected using colorimetric and fluorometric techniques by monitoring changes of absorbance or fluorescence between the substrate and the product of the enzymatic reaction [23-25]. The most common protein phosphatase used are PP1 and PP2A, due to the high inhibition capability of OA on these enzymes.

The use of electrochemical techniques with screen-printed electrodes presents a promising alternative to other costly techniques, offering many advantages such as low costs, inherent miniaturization and portability, simplifying the measurement process. In fact, electrochemical biosensors have also been used to monitor protein phosphatase inhibitions [26-30].

In this study, an electrochemical sensor system was developed based on the detection of the product of the PP2A enzymatic reaction in the presence of OA with different substrates: p-nitrophenyl phosphate (p-NPP), 4-methylumbelliferyl phosphate (4-MUP) and phenyl phosphate (PP). The proposed method is a very simple and sensitive procedure, detecting a concentration of $2.7 \cdot 10^{-12}$ M (2.17 ng L^{-1}). To the best of our knowledge, this value is less than the lowest values obtained by other electrochemical methods mentioned in the bibliography, even those that use immunosensors (Table 1). Only some chromatographic techniques coupled with other techniques such as LC-HRMS reported by Bosch-Orea [18] are able to provide a better limit of detection (0.3 ng L^{-1}). However, they require tedious sample treatments and expensive equipment, and have only been used to detect OA in bivalve samples but not in seawater, which is a considerably more difficult matrix to directly analyze using chromatographic techniques.

2. Experimental

2.1 Reagents and materials

All chemicals used in this study were of analytical-reagent grade or above and were used as received without further purification. High-quality water (Milli Q A10 system, Millipore, Bedford, USA) was employed for preparing all solutions.

OA in ethanol solution was purchased from Cayman (Michigan, USA), sodium chloride was provided by Panreac (Barcelona, Spain), and p-nitrophenol (p-NP), 4-methylumbelliferone (4-MU), p-NPP, 4-MUP, phenol and PP were all obtained from Sigma-Aldrich (St. Louis, MO, USA).

Lyophilized Protein Phosphatase A from human blood cells obtained from Zeulab (Zaragoza, Spain) was rehydrated according to the procedure describe by Smienk [31], by adding 2 mL phosphate buffer solution and mixing gently at 60 rpm for 60 minutes at room temperature on a New Brunswick Scientific orbital shaker (Endfield, CT, USA).

Metal salts solutions for the synthetic sea water preparation and interferences study were prepared with $MgCl_2$, $CaCl_2$ and $Cd(NO_3)_2$ provided by Panreac (Barcelona, Spain), and $Zn(NO_3)_2$, $CuSO_4$, $Pb(NO_3)_2$ and $HgSO_4$ purchased from Merck (Darmstadt, Germany). KCl was provided by Scharlau (Barcelona, Spain),

$NaHCO_3$ was obtained from Fluka (Steinheim, Germany), and KBr and $SrCl_2$ were purchased from Merck (Darmstadt, Germany).

Synthetic seawater was prepared according to the procedure that can be found in [32], by dissolving 23.926 g of NaCl, 4.008 g of Na_2SO_4, 0.026 g of H_3BO_3, 10.831 g of $MgCl_2 \cdot 6H_20$, 1.518 g of $CaCl_2 \cdot 2H_20$, 0.677 g of KCl, 0.196 g of $NaHCO_3$, 0.098 g of KBr and 0.140 g L^{-1} of $SrCl_2$ in 1 L of high-quality water.

Seawater samples were collected from mussel farms located in the Pontevedra coast, Spain. They were stored at -20°C in high-density polyethylene bottles until analysis.

C10903P14 (carbon/graphite ink) and D2071120D1 (dielectric ink) were purchased from Gwent Electronic Materials (Torfaen, U.K). Electrodag 6037 SS (Ag/AgCl ink) and Electrodag 418 (Ag ink) were supplied by Acheson Colloiden (Scheemda, Netherlands).

2.2. Screen-printed carbon electrode (SPCE) fabrication

Home made SPEs, used in this work, consist of a carbon working electrode (area, 12.56 mm^2), a carbon counter electrode and a Ag/AgCl reference as a pseudoreference electrode. Ag ink was used in conductive paths of SPEs. These electrodes were produced on a DEK 248 screen-printer (DEK, Weymouth, UK) by sequential deposition of different commercial inks according to the procedure described in a previous work [33]. The electrodes were printed using three different backing materials (BM): a 500 µm thickness polyester film (PMX981) provided by HiFi Industrial film (Dardilly, France), a Gore-Tex® Pro-Shell Nylon Liner and a 2-Layer Gore-Tex® Paclite® Shell Ripstop, both purchased from Rockywoods Fabrics, LLC (Lovelan, USA). These will be referred to hereinafter as backing material 1 (BM1), backing material 2 (BM2) and backing material 3 (BM3), respectively.

2.3. Instrumentation and measurements

Electrochemical measurements were performed with a PalmSens® portable electrochemical potentiostat (PalmSens® Instruments BV, Houten, The Netherlands) controlled by PS Trace software to collect the data register. All measurements were carried out at room temperature using a screen printed Ag/AgCl pseudoreference electrode.

The electrochemical measurements were performed by differential pulse voltammetry (DPV) from 0.00 V to +1.20 V at 0.030 $V \cdot s^{-1}$, applying a potential pulse of 0.028 V with a time pulse of 0.010 s in a 100 µL drop cast onto the electrodic screen-printed system. Blanks of 0.6 M NaCl were carried out between measures to ensure that no substance is adsorbed on the electrode surface.

Table 1 - Summary of analytical methods employed for OA detection in real samples

Method	Detection Technique	Linear range	LOD	Applicability	Reference
LC	HRMS	0.5 ─ 50 ng L^{-1}	0.3 ng L^{-1}	Filtered seawater	[18]
SPE-LC	MS/MS	5 ─ 250 µg L^{-1}	10 ng L^{-1}	Lake water	[20]
Electrochemical biosensor	Amperometry	30 ─ 250 ng L^{-1}	─	Buffer solution	[27]
Electrochemical immunosensor	SWV	≤5 µg L^{-1}	19 ng L^{-1}	Mussels	[16]
Electrochemical immunosensor	Amperometry	0.19 ─ 25 µg L^{-1}	150 ng L^{-1}	Mussels	[17]
Electrochemical biosensor	Amperometry	0.1.─ 4.0 µg L^{-1}	100 ng L^{-1}	Buffer solution	[34]
Electrochemical immunosensor	DPV	─	380 ng L^{-1}	Mussels	[35]
Electrochemical immunosensor	DPV	0.78 ─ 500 µg L^{-1}	500 ng L^{-1}	Mussels	[36]
Electrochemical biosensor	DPV	0.41 ─ 56.3 ng L^{-1}	2.17 ng L^{-1}	Seawater	This work

LC liquid chromatography, HRMS high resolution mass spectrometry, SPCE-LC solid extraction phase coupled with liquid chromatography, MS/MS tandem mass spectrometry, SWV square wave voltammetry, DPV differential pulse voltammetry.

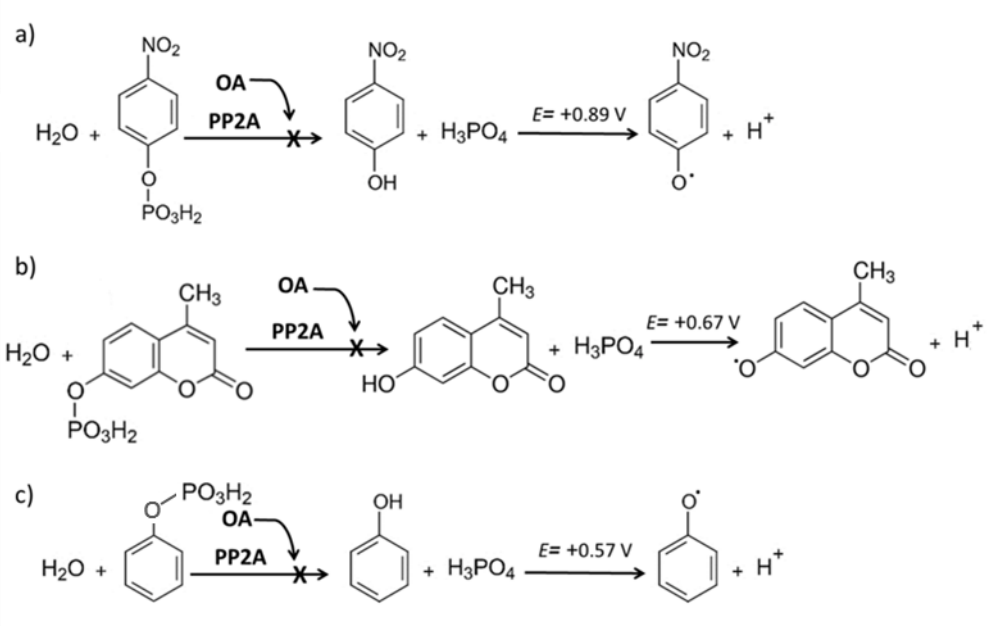
2.4. Enzyme incubation

Aliquots of 750 µL were prepared containing either $1 \cdot 10^{-2}$ M, $1 \cdot 10^{-3}$ M or $1.5 \cdot 10^{-2}$ M of p-NPP, 4-MUP and PP substrate respectively, 50 µL of enzyme solution (1.73 mU), and OA in a range from $5 \cdot 10^{-13}$ M to $7 \cdot 10^{-1}$ M for spiked measurements. Lastly, the final volume was made up with 0.6 M NaCl solution or synthetic seawater. In the case of the real seawater samples, 750 µL aliquots contained the same amount of substrate and enzyme solution as described above, and 300 µL of the seawater sample. The final volume was also made up with 0.6M NaCl. The aliquots were incubated for 30 minutes at 37°C in an oven and then cooled down to 0°C to stop the enzymatic reaction until the electrochemical analysis.

3. Results and discussion

3.1. Optimization of the experimental parameters

The determination of OA is based on the electrochemical detection of products of enzymatic dephosphorylation (p-NP, 4-MU and phenol), since the presence of OA implies a decrease in the concentration of these products due to the enzymatic inhibition that it produces (Scheme 1).



Scheme 1. Enzymatic reaction of the substrates a) p-NPP, b) 4-MUP and c) PP with PP2A enzyme followed by the corresponding electrochemical oxidation of the products

The inhibition by OA was calculated as shown in Eq 1 below.

$$\% \text{ Inhibition} = \left(\frac{I_0 - I_i}{I_0}\right) \times 100 \qquad (1)$$

Where I_0 represents the oxidation peak current of the control sample, that is, the product of the enzymatic reaction without the addition of OA, I_i means the oxidation peak current of the product of the enzymatic reaction in the presence of the selected concentration of OA.

The precision of the measurements was calculated in terms of reproducibility considering the relative standard deviation (RSD) of the slopes of three calibration curves. From each calibration curve, LOD has been calculated according to $3\sigma/m$ (where σ is the standard deviation of the blank, and m represents the slope

of the calibration curve) [37, 38]. In this case, σ is the estimated standard deviation of the % inhibition caused by the lowest concentration that was detected.

The enzymatic products p-NP, 4-MU and phenol were firstly studied using cyclic voltammetry between -1.50 V and +1.50 V. The 4-MU product exhibits an irreversible oxidation peak at around +0.67 V (vs screen-printed pseudoreference Ag/AgCl electrode) due to the oxidation of its hydroxyl group. However, p-NP and phenol show both oxidation and reduction peaks. The oxidation peaks of p-NP and phenol appear at around +0.89 V and +0.57 V respectively, corresponding to the oxidation of the hydroxyl group of each molecule. The reduction peak of p-NPP at around –0.89 V is related to the reduction of the nitro group. In the case of p-NP, although it exhibits two electrochemical peaks, the reduction peak is observed both in the substrate and in the product. Therefore, the oxidation peak has been selected for the DPV study since it is only present in the enzymatic product, with the aim of reducing the possible interferences that the unreacted substrate may cause.

Incubation time and incubation temperature were optimized to obtain the optimum electrochemical signal. An incubation temperature range from +25°C to +90°C was checked; obtaining the most suitable operating temperature so that the enzyme transforms the substrate to product at +37°C. The electrochemical signal increases from +25°C to +37°C, where a maximum signal intensity is achieved, with enzyme activity then starting to decrease, indicating that the optimal temperature is +37°C.

The enzyme/substrate ratio was also examined and the amount of PP2A and the concentration of substrate for p-NPP, 4-MUP and PP were optimized. Different concentrations of substrate from $1 \cdot 10^{-4}$ M to $5 \cdot 10^{-2}$ M and different amounts of buffered enzyme solution from 25 µL to 200 µL in a total volume of 750 µL were studied. The optimized values were 50 µL of enzyme PP2A and a substrate concentration of $1 \cdot 10^{-2}$ M, $1 \cdot 10^{-3}$ M and $1.5 \cdot 10^{-2}$ M for p-NPP, 4-MUP and PP, respectively.

3.2 Proof of concept

Under the optimized parameters, a first electrochemical study was carried out in a 0.6 M NaCl solution. This electrolyte solution has been selected as proof of concept to carry out the electrochemical measurements, due to the high concentration of NaCl in seawater. With this aim, calibration curves of 0.6M NaCl samples spiked with OA were performed in a linear range from $5 \cdot 10^{-13}$ M to $7 \cdot 10^{-11}$ M of OA for p-NPP and 4- MUP,

and from $5 \cdot 10^{-12}$ M to $7 \cdot 10^{-11}$ M of OA for PP, as it cannot detect $5 \cdot 10^{-13}$ M of OA. The analyses were performed in the three electrodic systems with different backing materials. Figure 1 shows DPV curves after the incubation process with different concentrations of OA, where current peaks appear centered at +0.89 V (Figure 1.a), at around +0.67 V (Figure 1.b) and at around +0.57 V (Figure 1.c), which correspond to the oxidation of p-NP, 4-MU and phenol respectively. As can be observed, the height of the oxidation peak decreases as OA concentration increases for each product, confirming the inhibition suffered by PP2A in the presence of OA. As can be seen in Table 2, the developed sensor system is able to detect an OA concentration in the picomolar range using any of the studied substrates, displaying good reproducibility with RSD values of less than 10 %. The use of one support material in place of another does not provide great differences in terms of LOD. However, it was observed that BM1 provides a lower RSD value, which could be explained by the better reproducibility of the electrode surfaces printed on rigid material compared to the flexible fabric material BM2 and BM3.

Table 2 – Parameters obtained from calibration curves for the OA inhibition of PP2A enzyme versus OA concentration in 0.6 M NaCl solution.

Substrate	**Backing material**	**IC_{50} (pM)**	**Calibration Range (pM)**	**Sensibility (nM/nA) (n=3)**	**RSD (%) (n=3)**	**LOD (pM)**	**S_{LOD} (pM)**
p-NPP	BM1	86.1	3.7 – 70.0	555.6	2.6	3.7	0.1
p-NPP	BM2	69.4	6.9 – 70.0	614.8	9.4	6.9	0.7
p-NPP	BM3	60.6	3.3 – 70.0	676.2	8.1	3.3	0.3
4-MUP	BM1	40.7	5.2 – 70.0	976.8	6.9	5.2	0.4
4-MUP	BM2	45.8	3.3 – 70.0	1013.0	9.1	3.3	0.3
4-MUP	BM3	44.9	2.7 – 70.0	882.2	8.2	2.7	0.2
PP	BM1	39.2	7.6 – 70.0	795.9	4.0	7.6	0.3
PP	BM2	40.8	8.8 – 70.0	800.1	4.7	8.8	0.4
PP	BM3	49.4	6.3 – 70.0	926.5	5.1	6.3	0.3

3.3 OA detection in synthetic seawater

After observing the results obtained in the 0.6 M NaCl solution, a closer approach was taken in synthetic seawater to emulate real-life conditions and check the possible matrix effect that can take place in a more complex system. As in the previous case, several samples with synthetic seawater, PP2A and p-NNP, 4-MUP or PP containing spiked OA in a range from $5 \cdot 10^{-13}$ M to $7 \cdot 10^{-11}$ M were incubated and used to perform the calibration curves with the three different BMs. The results obtained show that the synthetic seawater does not produce a significant matrix effect, providing similar RSD and LOD values, as shown in Table 3.

Table 3 – Parameters obtained from calibration curves for the OA inhibition of PP2A enzyme versus OA concentration in synthetic seawater solution.

Substrate	**Backing material**	**IC_{50} (pM)**	**Calibration Range (pM)**	**Sensibility (nM/nA) (n=3)**	**RSD (%)**	**LOD (pM) (n=3)**	**S_{LOD} (pM)**
p-NPP	BM1	60.8	6.3 – 70.0	688.8	8.5	6.3	0.5
p-NPP	BM2	44.8	3.2 – 70.0	923.4	1.4	3.2	0.1
p-NPP	BM3	49.7	8.3 – 70.0	954.7	4.2	8.3	0.3
4-MUP	BM1	53.1	6.1 – 70.0	781.6	8.7	6.1	0.5
4-MUP	BM2	47.1	6.5 – 70.0	742.4	6.9	6.5	0.4
4-MUP	BM3	45.8	7.7 – 70.0	918.5	6.8	7.7	0.5
PP	BM1	83.9	7.0 – 70.0	593.8	6.3	7.0	0.4
PP	BM2	60.8	6.3 – 70.0	809.3	4.0	6.3	0.3
PP	BM3	55.0	8.0 – 70.0	905.8	8.7	8.0	0.7

3.4. Study of the inhibition type

The inhibitory effect of OA on different enzyme-substrate complexes was examined through the kinetics parameter of the Lineweaver-Burk plots, Figure S1. For that, three regression curves were performed for each substrate; one without OA, one with $5 \cdot 10^{-11}$ M OA, and the last one in the presence of $7 \cdot 10^{-11}$ M OA. Lineweaver-Burk plots showed that OA produces a mixed inhibition, indicating that these substrates bind at a different active site than OA, which may result in their inhibition through changes in the three-dimensional shape of the enzyme, decreasing its activity. This is in keeping with other studies referenced in the bibliography that also support the claim that OA produces a mixed inhibition on the PP2A enzyme [4, 6, 39]. K_M values were calculated, resulting in the following order: $K_{M\ p\text{-}NPP} > K_{M\ PP} > K_{M\ 4\text{-}MUP}$. These results suggest that 4-MUP is the substrate with the most affinity for PP2A, while p-NPP has the least affinity for it among the three substrates studied.

3.5. Interference study

Seawater is a complex matrix where metal ions of oligo-elements or heavy metal contaminants may be present, and they may also induce the inhibition of PP2A. Figure 2 compares the inhibition- of different divalent cations with that caused by OA. The results indicate that a concentration of $1 \cdot 10^{-6}$ M of mercury or copper ions produce a strong inhibition of the enzymatic dephosphorylation of PP2A, similar to the inhibition produced by $7 \cdot 10^{-11}$ M of OA. However, the inhibition is not observable at lower concentrations of $1 \cdot 10^{-8}$ M of these cations. On the other hand, Mg^{2+}, Ca^{2+}, $Pb^{2+,}$ Cd^{2+} and Zn^{2+} exhibit either only a small sign of inhibition or none at all. In fact, some of the cations exhibit a slight increment of the enzymatic products, which may be due to the catalytical and central role that metal ions play in the activation of a water molecule for the dephosphorylation reaction [40]. Furthermore, the purification and long-term storage of PP2A may render the enzyme metal dependent to maintain the activity [41], broadening its substrate specificity [42]. It should also be noted that when p-NPP is employed as the substrate, the inhibition by metal ions is generally higher than when 4-MUP is used. This may be explained by the specificity between enzyme and substrate, showing that PP2A has a higher affinity for 4-MUP than p-NPP.

3.6. OA detection in real seawater samples

Two seawater samples collected on the same day from the Atlantic Ocean off the Galician coast during red tide were analysed, in order to determine the OA present in this water. The collected samples were incubated with the enzyme and the substrate, and measured in triplicate using DPV for each of the three substrates. Given that the tested BMs do not offer any significant differences, the samples were measured using only BM1 as backing material of the electrodic system. The intensity of the current (peak height) allowed for the determination of OA in the problem samples using the previous calibration curves performed, obtaining the results shown in Table 4. The samples were also analysed using the ELISA [31] method, providing a concentration of OA ≤ 50 pM. This demonstrates that all three substrates exhibit good capability in the determination of OA.

Table 4 – OA concentration measured in seawater samples, with the different substrates.

Substrate	**Seawater Sample 1**	**Seawater Sample 2**
p-NPP	49± 4 pM	48 ± 10 pM
4-MUP	48 ± 5 pM	47 ± 5 pM
PP	48 ± 7 pM	50 ± 5 pM

Conclusions

In this work, an electrochemical method using disposable electrodes was developed based on the inhibition of the PP2A enzyme by okadaic acid (OA), and applied to the detection of this marine diarrheic shellfish poisoning toxin in seawater. p-nitrophenyl phosphate, phenyl phosphate and 4-methylumbelliferyl phosphate demonstrated to be appropriate substrates to monitoring the inhibition by measuring the intensity decrease of the oxidation peak of their enzymatic reaction products. Okadaic acid was seen to behave as a mixed inhibitor in the presence of the three substrates.

The method uses only a small amount of enzyme and is highly sensitive, simple and rapid. It has proven to be capable of detecting low OA concentrations (LOD = $2.7 \cdot 10^{-12}$ M) in seawater samples that have not received any treatment. An interference study was also performed, showing that metals ions present in seawater do not interfere with the determination of OA.

The influence of the backing material of the electrodic system was also studied, observing that flexible textile supports offer results comparable to those obtained with rigid supports.

The method allows the determination of OA at concentration levels of 10^{-11}M in seawater, the aim of which is to provide an early warning method to prevent the contamination of mussels with OA.

Acknowledgements

Authors gratefully acknowledge financial support from Ministerio de Ciencia e Innovación (MICINN) (TEC2013-405661-P) Ministerio de Economía y Competitividad (MINECO) and European Regional Development Fund (FEDER) (MUSSEL RTC-2015-4077-2).

We would like to thank ANFACO for the shipment of seawater samples with OA, as well as the analysis of them using OKATEST.

Conflicts of interest

There are no conflicts to declare.

References

1. J.F. Dawson, C.F.B. Holmes, Molecular mechanisms underlying inhibition of protein phosphatases by marine toxins, Front. Biosci. 4 (1999) 646–658.
2. T. Yasumoto, M. Murata, Y. Oshima, M. Sano, G.K. Matsumoto, J. Clardy, Diarrhetic shellfish toxins, Tetrahedron 41 (1985) 1019–1025.
3. K. Tachibana, P.J. Scheuer, Y. Tsukitani, H. Kikuchi, D. Van Engen, J. Clardy, Y. Gopichand, F.J. Schmitz, Okadaic acid, a cytotoxic polyether from two marine sponges of the genus *Halichondria*,. J. Am. Chem. Soc, 103 (1981) 2469–2471.
4. C. Bialojan, A. Takai, Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. Specificity and kinetics, Biochem. J. 256 (1988) 283–290.
5. T.A.J. Haystead, A.T.R. Sim, D. Carling, R.C. Honnor, Y. Tsukitani, P. Cohen, D.G. Hardie, Effects of the tumour promoter okadaic acid on intracellular protein phosphorylation and metabolism, Nature 337 (1989) 78–81.
6. A. Takai, G. Mieskes, Inhibitory effect of okadaic acid on the p-nitrophenyl phosphate phosphatase activity of protein phosphatases, Biochem. J. 275 (1991) 233–239.
7. T.M. Hu, J. Doyle, D. Jackson, J. Marr, E. Nixon, S. Pleasance, M.A. Quilliam, J.A. Walter, J.L.C. Wright, Isolation of a new diarrheic shellfish toxin from Irish mussels, J. Chem. Soc. Chem. Commun. 1 (1992) 39.

8. The European Parliament and the Council of the European Union; Commission Regulation (EU) No 786/2013 of 16 August 2013 amending Annex III to Regulation (EC) No 853/2004 of the European Parliament and of the Council as regards the permitted limits of yessotoxins in live bivalve molluscs Text with EEA relevance. Off. J. Eur. 220 (2013) 14.
9. T. Yasumoto, Y. Oshima, M. Yamaguchi, Occurrence of a new type of shellfish poisoning in the Tohoku District, Bull. Japanese Soc. Sci. Fish. 44 (1978) 1249–1255.
10. E. Arteche, G. Strippoli, G. Loirand, P. Pacaud, L. Candenas, L. Souto, J. Fernandez, M. Norte, J.D. Marti, J. Savineau, B. Ii, G.L. France, An Analysis of the mechanisms involved in the okadaic acid induced contraction of the estrogen-primed rat uterus, J. Pharmacol. Exp. Ther. 282 (1997) 201–207.
11. A. Tubaro, C. Florio, E. Luxich, S. Sosa, R. Della Loggia, T. Yasumoto, A protein phosphatase 2A inhibition assay for a fast and sensitive assessment of okadaic acid contamination in mussels, Toxicon 34 (1996) 743–752.
12. M. Le Berre, M. Kilcoyne, M. Kane, Generation of a panel of high affinity antibodies and development of a biosensor-based immunoassay for the detection of okadaic acid in shellfish, Toxicon 103 (2015) 169–175.
13. L.D. Stewart, C.T. Elliott, A.D. Walker, R.M. Curran, L. Connolly, Development of a monoclonal antibody binding okadaic acid and dinophysistoxins-1, -2 in proportion to their toxicity equivalence factors, Toxicon 54 (2009) 491–498.
14. A. Tang, M. Kreuzer, M. Lehane, M. Pravda, G.G. Guilbault, Immunosensor for the determination of Okadaic acid based on screen-printed electrode, Int. J. Environ. Anal. Chem. 83 (2003) 663–670.
15. M.P. Kreuzer, M. Pravda, C.K. O'Sullivan, G.G. Guibault, Novel electrochemical immunosensors for seafood toxin analysis, Toxicon 40 (2002) 1267–1274.
16. S. Eissa, M. Zourob, A graphene-based electrochemical competitive immunosensor for the sensitive detection of okadaic acid in shellfish, Nanoscale 4 (2012) 7593–7599.
17. R.B. Dominguez, A. Hayat, A. Sassolas, G.A. Alonso, R. Munoz, J.-J.L. Marty, Automated flow-through amperometric immunosensor for highly sensitive and on-line detection of okadaic acid in mussel sample, Talanta 99 (2012) 232–237.
18. C. Bosch-Orea, J. Sanchís, M. Farré, D. Barceló, Analysis of lipophilic marine biotoxins by liquid chromatography coupled with high-resolution mass spectrometry in seawater from the Catalan Coast, Anal. Bioanal. Chem. 409 (2017) 5451–5462.
19. A.V. González, M.L. Rodríguez-Velasco, B. Ben-Gigirey, L.M. Botana, First evidence of spirolides in Spanish shellfish, Toxicon 48 (2006) 1068–1074.
20. S.K. Zervou, C. Christophoridis, T. Kaloudis, T.M. Triantis, A. Hiskia, New SPE-LC-MS/MS method for simultaneous determination of multi-class cyanobacterial and algal toxins, J. Hazard. Mater. 323 (2017) 56–66.
21. D.O. Mountfort, T. Suzuki, P. Truman, Protein phosphatase inhibition assay adapted for determination of total DSP in contaminated mussels, Toxicon 39 (2000) 383–390.
22. C.F.B. Holmes, Liquid chromatography-linked protein phosphatase bioassay; A highly sensitive marine bioscreen for okadaic acid and related diarrhetic shellfish toxins, Toxicon 29 (1991) 469–477.
23. N. Bouaïcha, I. Maatouk, G. Vincent, Y. Levi, A colorimetric and fluorometric microplate assay for the detection of microcystin-LR in drinking water without preconcentration, Food Chem. Toxicol. 40 (2002) 1677–1683.
24. D.O Mountfort, G. Kennedy, I. Garthwaite, M. Quilliam P. Truman, D.J. Hannah, Evaluation of the fluorometric protein phosphatase inhibition assay in the determination of okadaic acid in the mussels, Toxicon 37 (1999) 909–922.
25. K. Su, Y. Pan, Z. Wan, L. Zhong, J. Fang, Q. Zou, H. Li, P. Wang, Smartphone-based portable biosensing system using cell viability biosensor for okadaic acid detection, Sensors Actuators, B Chem. 251 (2017) 134–143.
26. M. Campàs, D. Szydłowska, M. Trojanowicz, J.L. Marty, Enzyme inhibition-based biosensor for the electrochemical detection of microcystins in natural blooms of cyanobacteria, Talanta 72 (2007) 179–186.
27. G. Volpe, E. Cotroneo, D. Moscone, L. Croci, L. Cozzi, G. Ciccaglioni, G. Palleschi, A bienzyme electrochemical probe for flow injection analysis of okadaic acid based on protein phosphatase-2A inhibition: an optimization study, Anal. Biochem. 385 (2009) 50–56.

28. M. Campàs, P. de la Iglesia, G. Giménez, M. Fernández-Tejedor, J.-L. Marty, J. Diogène, Marine biotoxins in the Catalan littoral: could biosensors be integrated into monitoring programmes? Contrib. to Sci. 4 (2008) 43–53.
29. M. Campàs, J.-L.L. Marty, Enzyme sensor for the electrochemical detection of the marine toxin okadaic acid, Anal. Chim. Acta. 605 (2007) 87–93.
30. J. Zhou, X. Qiu, K. Su, G. Xu, P. Wang, Disposable poly (o-aminophenol)-carbon nanotubes modified screen print electrode-based enzyme sensor for electrochemical detection of marine toxin okadaic acid, Sensors & Actuators b 235 (2016) 170-178.
31. H. Smienk, E. Domínguez, M.L. Rodríguez-Velasco, D. Clarke, K. Kapp, P. Katikou, A.G. Cabado, A. Otero, J.M. Vieites, P. Razquin, L. Mata, Quantitative determination of the okadaic acid toxins group by a colorimetric phosphatase inhibition essay: interlaboratory study, J. AOAC Int. 96 (2013) 77–85.
32. D.R. Kester, I.W. Duedall, D.N. Connors, R.M. Pytkowicz, Preparation of artificial seawater 1, Limnol. Oceanogr. 12 (1967) 176–179.
33. B. Molinero-Abad, D. Izquierdo, L. Pérez, I. Escudero, M. Arcos-Martínez, Comparison of backing materials of screen printed electrochemical sensors for direct determination of the sub-nanomolar concentration of lead in seawater, Talanta 182 (2018) 549-557.
34. N. Hamada-Sato, N. Minamitani, Y. Inaba, Y. Nagashima, T. Kobayashi, C. Imada, E.M. Watanabe, Development of amperometric sensor system for measurement of diarrheic shellfish poisoning (DSP) toxin, okadaic acid (OA), Sensors Mater. 16 (2004) 99–107.
35. A. Hayat, L. Barthelmebs, J.L. Marty, Enzyme-linked immunosensor based on super paramagnetic nanobeads for easy and rapid detection of okadaic acid, Anal. Chim. Acta, 690 (2011) 248–252.
36. A. Hayat, L. Barthelmebs, A. Sassolas, J.L. Marty, Development of a novel label-free amperometric immunosensor for the detection of okadaic acid, Anal. Chim. Acta. 724 (2012) 92–97.
37. J. Mocak, M. Bond, S. Mitchell, G. Scollary, Statistics and Chemometrics for Analytical Chemistry, Pure Appl. Chem. 69 (1997) 297–328.
38. E.R. Ziegel, Statistics and Chemometrics for Analytical Chemistry, Technometrics, 46 (2004) 498-499.
39. M. Rivas, C. García, J.L. Liberona, N. Lagos, Biochemical characterization and inhibitory effects of dinophysistoxin-1, okadaic acid and microcystine 1-r on protein phosphatase 2a purified from the mussel *Mytilus chilensis*, Biol. Res. 33 (2000) 197–206.
40. Y.G. Shi, Serine/threonine phosphatases: mechanism through structure, Cell 139 (2009) 468–484.
41. L.W. Cai, Y.F. Chu, S.E. Wilson, K.K. Schlender, A metal-dependent form of protein phosphatase 2A, Biochem. Biophys. Res. Commun. 208 (1995) 274–279.
42. T. Fellner, D.H. Lackner, H. Hombauer, P. Piribauer, I. Mudrak, K. Zaragoza, C. Juno, E. Ogris, A novel and essential mechanism determining specificity and activity of protein phosphatase 2A (PP2A) in vivo, Genes Dev. 17 (2003) 2138–2150.

Figure Captions

Fig. 1 Differential pulse voltammograms of PP2A enzymatic reaction products a) p-NP, b) 4-MU and c) Phenol after incubation in presence different concentration of OA. Insets show the corresponding calibration curves of PP2A inhibition rates versus OA concentration (0.5 – 70.0 pM in a) and b) and 5.0 – 70.0 pM in c)) in 0.6 M NaCl.

Fig. 2 Results of the interference study of the inhibition of PP2A activity by different divalent metal ions added in a concentration of $1 \cdot 10^{-6}$ M

Figures

Fig. 1

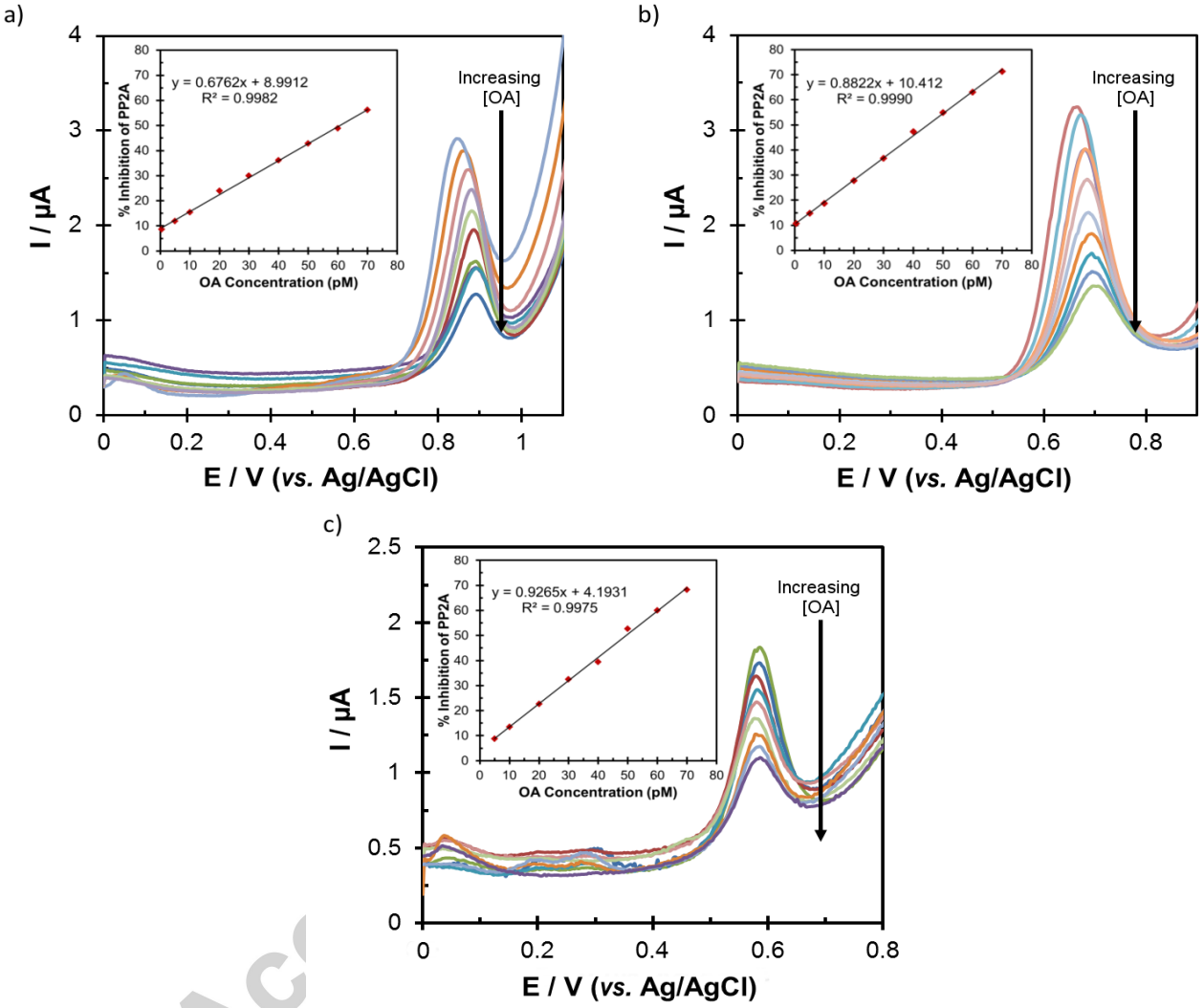
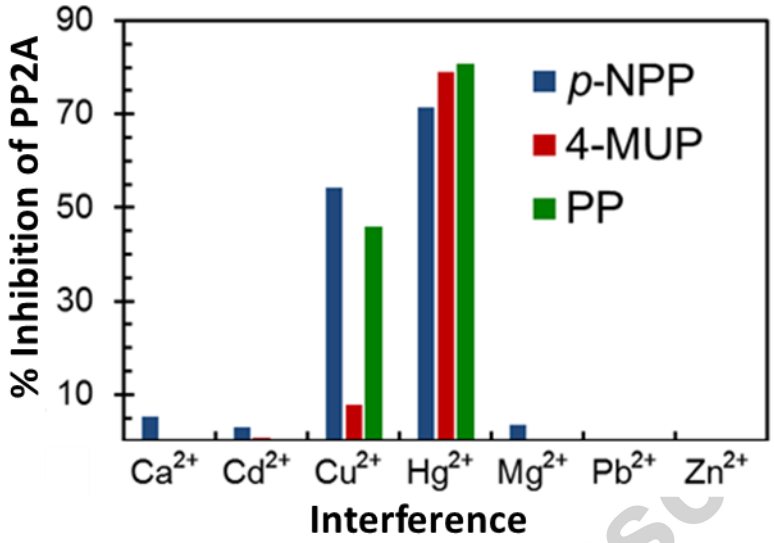


Fig. 2



HIGHLIGTHS

- Development of a simple, sensitive and inexpensive method for the voltammetric determination of OA using screen-printed carbon electrodes (SPCEs).
- Good analytical performance was obtained in the determination of OA.
- Possibility of determining OA in seawater without any treatment at very low concentration.