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Potentiometric chemical sensors for the detection of paralytic shellfish toxins

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Abstract

Potentiometric chemical sensors for the detection of paralytic shellfish toxins have been developed. Four toxins typically encountered in Portuguese waters, namely saxitoxin, decarbamoyl saxitoxin, gonyautoxin GTX5 and C1&C2, were selected for the study. A series of miniaturized sensors with solid inner contact and plasticized polyvinylchloride membranes containing ionophores, nine compositions in total, were prepared and their characteristics evaluated. Sensors displayed cross-sensitivity to four studied toxins, i.e. response to several toxins together with low selectivity. High selectivity towards paralytic shellfish toxins was observed in the presence of inorganic cations with selectivity coefficients ranging from 0.04 to 0.001 for Na^+ and K^+ and $3.6*10^{-4}$ to $3.4*10^{-5}$ for Ca^{2+}. Detection limits were in the range from 0.25 to 0.9 $\mu molL^{-1}$ for saxitoxin and decarbamoyl saxitoxin, and from 0.08 to 1.8 $\mu molL^{-1}$ for GTX5 and C1&C2, which allows toxin detection at the concentration levels corresponding to the legal limits. Characteristics of the developed sensors allow their use in the electronic tongue multisensor system for simultaneous quantification of paralytic shellfish toxins.

Graphical abstract

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Keywords

chemical sensors, paralytic shellfish toxins, potentiometry, ionophores

Introduction

Marine toxins are chemical compounds biosynthesized by a few phytoplankton species that cause negative impacts on marine organisms and, in severe cases, mortality of fish, birds and mammals. In addition, filter-feeding bivalves can accumulate toxins in their tissue and provoke human poisoning when consumed [1]. Proliferation of toxic phytoplankton leading to shellfish poisoning are called toxic algal blooms [2-3]. As occurrence of toxic algal blooms is unpredictable, routine monitoring of the presence of marine toxins in bivalves and toxic phytoplankton species in seawater near bivalve catching and production areas are necessary. To address this need, monitoring programs have been established in several coastal countries. EU monitoring programs currently include three groups of toxins, divided according to the symptoms in humans: diarrheic shellfish toxins (DSTs), paralytic shellfish toxins (PSTs) and amnesic shellfish toxins (ASTs), and also some other lipophilic toxins [4-5]. The occurrence of PSTs in Portuguese coastal waters is less frequent compared to the other types of toxins [6], however they are of particular concern due to the life-threatening neurological symptoms they can cause in humans. In severe cases respiratory paralysis and death may occur, with overall mortality estimated to be about 8.5 -9.5 % [7-8].

According to the EU legislation, the official reference method for the detection of PSTs is the Liquid Chromatography (LC) with Fluorimetric Detection (FLD) [9-10]. As LC-FLD is a laboratorial technique involving the use of expensive apparatus, which must be operated by highly skilled personnel, development of less costly and less complex assays and probes for marine toxins detection is of practical interest.

Several biosensors and immunoassays have been proposed for individual PSTs' detection, along with nerve cell and sodium channel based assays [11]. Antibody-based assays and biosensors can achieve very low limits of detection, but usually only for a small number of known PSTs, since the antibodies employed have a low cross-reactivity. Additionally, antibodies require an animal host for their production. Nerve cell and sodium channel based methods are of particular interest as they produce toxicity estimate of PSTs, which was found to be well correlated with animal tests. Furthermore, nerve cell and sodium-channel based methods have

higher selectivity than animals as the latter can respond to other contaminants besides PSTs. Direct measurements of toxicity instead of concentration is also more relevant in the monitoring as toxins are always present as a mixture of compounds with toxicity varying up to two orders of magnitude. However, both nerve cells and sodium channels involve laborious preparation procedures, have long response times and, most importantly, lack stability, which leads to low reliability and reproducibility of measurements [12-13].

Chemical sensors represent an interesting alternative to the methods described above, mainly due to their robustness and low cost. However, there are only few reports on the chemical sensors for PST detection. Detection of one of the PSTs, saxitoxin, using surface plasmon resonance sensor with calix[4]arene derivatives as a recognition element was reported in [14]. Aza and diaza crown ethers modified with anthracene and coumaril moieties were proposed as receptors for fluorescent detection of saxitoxin in a series of publications [15-19]. It is important to note that these works were mostly of exploratory nature and only one study [14] reports a calibration curve. Furthermore, development of the sensors for PSTs detections, including chemical sensors, biosensors, immunoassays, etc., targeted mainly only one toxin – saxitoxin, which is the most common PST worldwide. However, typical profile of PSTs detected in bivalves in Portuguese coast differ and comprises mainly decarbamoylated and N-sulfocarbamoylated toxins [6, 20-21].

The purpose of the present work was the development of potentiometric chemical sensors for PSTs typically present at Portuguese coast. No previous reports of potentiometric chemical sensors for PST detection nor chemical sensors for detection of PSTs other than saxitoxin were reported.

Materials and methods

Reagents

Sodium hydrogen phosphate and dihydrogen phosphate, aniline, tris(hydroxymethyl) aminomethane (BioPerformance Certified) were from Sigma Aldrich, ethanol, sodium hydroxide, hydrochloric acid, sulfuric acid, sodium nitrate, potassium nitrate and calcium nitrate were from Panreac, tetrahydrofuran (Chromasolv) was from Fisher. All reagents were p.a. (for analysis) unless stated otherwise. High molecular weight polyvinyl chloride (PVC), dibutyl phthalate (DBP), potassium tetrakis(4-chlorophenyl)borate (KTPB), tridodecylmethylammonium chloride

(TDMACl) and ionophores were from Fluka. Screen-printed electrodes (SPE) with gold working and auxiliary electrodes and silver reference electrode were from DropSens (Spain). Ultrapure water produced by Merck Millipore Water System (18 $M\Omega cm^{-1}$) was used for solution preparation and sensor washing.

Solutions of PSTs, namely saxitoxin (STX), decarbamoyl saxitoxin (dcSTX) and N-sulfocarbamoyl toxins gonyautoxin GTX5 and C1&C2, were certified reference material from the Institute for Marine Biosciences, National Research Council, Halifax, Canada. When working with PSTs, long sleeved lab coat and non-permeable nitrile or latex gloves should be used. Toxin containing waste should be decontaminated using a 10% solution of sodium hypochlorite during 30 minutes and disposed of down the drain with plenty of water.

Sensor fabrication and potentiometric measurements

Potentiometric sensors with solid inner contact were fabricated using SPE. Firstly, surface of SPE working electrode was rinsed with ethanol and water and cleaned by cycling potential for 5 cycles between -0.2 and +1.2 V at 50mV/s in 50 $mmolL^{-1}$ sulfuric acid. Solid contact was prepared by electropolymerization of aniline in deaerated aqueous solution of 50 mmol L^{-1} aniline in 1 mol L^{-1} hydrochloric acid by cycling potential for 100 cycles between -0.23 and +0.85 V at 50 mV/s. Sensors were washed with deionized water, conditioned for 2 h in 1 mmol L^{-1} hydrochloric acid and dried. All controlled-potential experiments were performed with an EZstat-Pro EIS (NuVant Systems Inc., Indiana, USA). Platinum wire served as the counter electrode and Ag/AgCl (KCl 3 $molL^{-1}$) served as a reference electrode.

Membrane mixtures were prepared by dissolving PVC (33 %w/w), dibutyl phthalate (66 %w/w), ionofore (1 %w/w) and lipophilic salt (0.5 %w/w) in tetrahydrofuran. Membrane compositions are listed in the Table 1. Membrane mixture was drop casted on the solid contact of the SPE and left to dry at room temperature. Prior to use, the sensors were conditioned in water for 2 h.

Calibration measurements were carried out in the solutions of STX, dcSTX, sodium, potassium and calcium nitrates on the background of 0.25 mmol L^{-1} Tris-HCl buffer and in the solutions of GTX5, C1&C2 and sodium chloride on the background of 1 mmol L^{-1} phosphate buffer. Both buffers had pH 7. Calibration measurements in sodium, potassium and calcium nitrate solutions were made in the concentration range from 1 $\mu molL^{-1}$ to 1 $mmolL^{-1}$.

Calibration solutions of PSTs were prepared by diluting toxin standards in buffer to the final concentrations from 0.1 to 6.8 μmol L^{-1}. Sensor response to pH was studied for pH range from 3 to 9. Measurements were started in the 1 mmol L^{-1} solutions of HCl with pH 3, to which 0.1 mmolL^{-1} Tris-base solution was gradually added. Selectivity was evaluated using two solution method as described in [22] considering STX as a primary ion. Concentrations of STX and dcSTX were 2 μmol L^{-1}, of sodium and potassium were 1mmol L^{-1} and calcium - 10 mmol L^{-1}.

Potentiometric measurements were carried out using custom-made high input impedance digital voltmeter (Sensor Systems LLC., St. Petersburg, Russia) connected to a PC for the data acquisition. Sensor potential was measured vs. SPE own pseudo-reference electrode. pH of the solutions was measured using combination pH glass electrode (Metrohm, Switzerland). Sensor potentials were recorded after stable readings were reached, typically after 5 minutes. At least three replicated calibration measurements were made. Between measurements, sensors were washed with deionized water until stable potential readings were reached. Typically about 30 minutes were necessary for potential recovery. When not in use, sensors were kept dry at room temperature and prior to measurements were soaked during 1 h in buffer solution.

Table 1. Compositions of the sensing membranes. DBP - dibutyl phthalate, KTPB - potassium tetrakis(4-chlorophenyl)borate; TDMACl - tridodecylmethylammonium chloride.

Sensor	Ionophore	Lipophilic salt	Plasticizer
1	Calix[6]arene	KTPB	DBP
2	Calix[4]arene-25,26,27,28-tetrol	KTPB	DBP
3	1,4,7,10,13-pentaoxa-16-azacyclooctadecane	KTPB	DBP
4	1,4,10,13-tetraoxa-7,16-diazacyclooctadecane	KTPB	DBP
5	Calix[6]arene-hexaacetic acid hexaethylester	KTPB	DBP
6	Octadecyl 4-formylbenzoate	KTPB	DBP
7	4,6,11,12-tetrahydro-3-methyl-1-phenyl-1H-pyrazolo[3′,4′:4,5]pyrimido[1,2-b]quinazolin-5-ium tetrafluoroborate	KTPB	DBP
8	Octadecyl 4-formylbenzoate	TDMACl	DBP
9	4,6,11,12-tetrahydro-3-methyl-1-phenyl-1H-	TDMACl	DBP

pyrazolo[3′,4′:4,5]pyrimido[1,2-b]quinazolin-5-ium tetrafluoroborate

Results and discussion

Selection of the ionophores for the sensors for the detection of PSTs was carried out taking into account literature data and properties of toxins. Paralytic shellfish toxins have two guanidinium moieties in their structure as shown in the Fig. 1 [8]. These guanidinium groups are basic and their pKa have been determined experimentally for STX and dcSTX: pKa of the group at C2 was found to be 8.22 and 8.10, respectively, and at C8 - 11.28 and 10.84, respectively [23]. Both of these toxins exist as doubly charged cations at pH below 7, uncharged species at pH above 13, and as mixture of forms in the pH range between 7 and 13, with predominance of single charged cation at pH between 9 and 10.5. Thus, at neutral pH both these toxins exist as doubly charged cations. N-sulfocarbamoyl toxins, GTX5 and C1&C2, due to the presence of acidic groups, sulfamate group at C13 and, in the case of C1&C2 sulfate group at C11, can exist as positively charged, negatively charged or zwitterionic species depending on pH of the aqueous solutions [24]. pKa values of 1,2,3 guanidinium group of GTX5 was estimated to be 8.5 while pKa of sulfamate group to be about 2 [24]. That means that GTX5 is principally positively charged at a pH below 4 and negatively charged at pHs above 6. No experimental data on pKa values of toxins C1 and C2 are available. pKa values predicted using ALOGPS 2.1 software [25] were close to the ones reported for GTX5. Thus, C1&C2 would be expected to exist as doubly charged anions at neutral pH above 4.7, as positively charged ion at pH below 3.5. Toxins C1 and C2 are stereoisomers with respect to the sulfate group at C11. As they are usually present as a mixture in bivalves and in the commercially available standards (77% of C1 and 23% of C2), they were considered as one compound in this work.

Fluorimetric and SPR sensors for STX detection have been developed using ligands, which are known to bind to guanidinium cation, such as crown ethers and calixarenes [14-19]. Accordingly, crown ethers and calixarenes were used as ionophores in membranes of potentiometric sensors in this work (sensors 1 – 5, Table 1). It was expected that these sensors would respond primarily to the cationic STX and dcSTX. Dibutyl phthalate was used as a solvent and tetraphenylborate salt as an anionic lipophilic salt in the membrane cocktails as they ensured the Nernstian response to guanidinium and the highest selectivity according to [26]. Sensors

based on anion-exchangers with expected sensitivity to GTX5 and C1&C2 have been tested as well (sensors 6-9, Table 1).

Taking into account complex speciation chemistry of PSTs, sensitivity of the sensors to pH was assessed with the aim to optimize measurement conditions. At the optimum pH toxins should be ionized and preferably exist as only one charged specie while sensor sensitivity to pH should be minimal. Such conditions could be important in practice as small changes of sample pH would not disturb toxin speciation and, consequently, sensor response. All studied sensors responded to pH at least at some pHs (Table 2 and Fig. 1S). Except sensor 6 that responded to pH in all studied pH range, other sensors were sensitive only at acidic pH, between 3 and 5. Sensors based on azo crown ethers, i.e. 3 and 4, also responded to more alkaline pH, between 7 and 9. Slopes were well below Nernstian for all sensors, except for the sensor 7 that displayed response closer to the theoretical, of 46 mV/pH, in the pH range between 3 and 6. Range of pH, in which most of the sensors did not respond to pH or had very low sensitivity was between 6 and 7. In this pH range dominant forms of toxins are double charged cations for STX and dcSTX, single charged anion for GTX5 and double charged anion for C1&C2. Therefore, pH 7 was selected for all further experiments.

Table 2. Sensitivity of the sensors to pH (average slopes and standard deviations).

Sensor	pH interval	Slope, mV/pH
1	3 a 4	-16 ± 2
	4 a 9	5 ± 1
2	3 a 4	-19 ± 2
	4 a 9	7 ± 1
3	3 a 4	-28 ± 4
	4 a 8	1.0 ± 0.6
	8 a 9	-17 ± 3
4	3 a 7	-7 ± 1
	7 a 9	-14 ± 1
5	3 a 4	-19 ± 3
	4 a 9	-8 ± 1

6	3 a 9	-20 ± 3
7	3 a 6	-46 ± 3
	6 a 9	-10 ± 1

Slopes of the electrode functions of all sensors and responses in the solutions of PSTs are shown in the Fig. 2 and 2S, respectively. All studied sensors responded to saxitoxin and decarbamoyl saxitoxin. Only sensors containing anion-exchangers (6 and 7) displayed sensitivity to gonyautoxin 5. Sensors containing calix[6]arenes (1 and 5), diaza-crown ether (4) and one of the anion exchangers (6) also displayed sensitivity to the toxins C1&C2. Responses mostly diverged from the theoretical values with exception of responses of the sensor based on aza-crown ether (3) to saxitoxin and decarbamoyl saxitoxin and sensors based on calix[6]arene (1) and diaza-crown ether (4) to C1&C2 that were close to the theoretical value of -29 mV/pX for doubly charged anions. Responses of the other sensors to saxitoxin and decarbamoyl saxitoxin were superNersntian and close to the theoretical response to the single charged ion. Responses to the anionic toxins were subNernstian. These deviations from the theoretical values can be related to various factors. One of them is toxin lipophilicity as higher responses were observed to the more lipophilic toxins i.e. saxitoxin and decarbamoyl saxitoxin (according to the values predicted by the ALOGPS 2.1 software) [27]. Other factors may be association in the membrane phase, i.e. formation of single charge saxitoxin cation in the membrane phase instead of double charged in the aqueous phase [28] or formation of the complexes between ionophore and secondary ions present in solutions [29]. Sensors containing anion-exchangers and cationic lipophilic salt (8 and 9) displayed super Nernstian responses about 180 mV/pX to saxitoxin and decarbamoyl saxitoxin and no sensitivity to GTX5 and C1&C2 (data not shown). Responses were poorly reproducible so these sensor compositions were excluded from the further studies.

Stability of the developed sensors was evaluated in the dcSTX solutions. As an example of typical sensor behavior, potential of the sensor 6 in 2 μmol L^{-1} solution of dcSTX in Tris-HCl buffer and slope of the response of the same sensor in dcSTX solutions measured during 3 weeks are shown in the Fig. 1S a and b, respectively. Drastic changes of both potential and slope were observed on the first day, which is typical for sensors with PVC plasticized membranes, afterwards potential and slope stabilized. After 6 weeks of use, slope of the sensors started to decrease and they were discarded.

Selectivity of the sensors towards saxitoxin in the presence of alkali and alkali-earth cations and decarbamoyl saxitoxin was evaluated. Saxitoxin was selected as a primary ion as all sensors responded to it. Selectivity study was limited to the ions, to which sensors displayed cationic response. Thus, toxins GTX5 and C1&C2, to which sensor responses were anionic were excluded. Sodium, potassium and calcium were selected as interfering ions as they are likely to be present in bivalve extracts, samples for which the sensors are being developed. Selectivity coefficients, together with standard deviations are shown in the Fig. 3. Most of the sensors were not selective either to saxitoxin or to decarbamoyl saxitoxin with selectivity coefficients being close to 0. Sensors 5 and 7 displayed higher selectivity towards decarbamoyl saxitoxin, being capable of detecting it in the presence of STX in 25 and 4 times excess , respectively. All sensors were selective to saxitoxin in the presence of 100 to 1000 excess of sodium and potassium and ca. 10000 excess of calcium.

All of the developed sensors displayed sensitivity to several of the studied PSTs, with the sensor 6 responding to all four toxins, together with low selectivity to these compounds or in other words displayed cross-sensitivity towards PSTs. Therefore, these sensors are not suitable for selective detection of the PSTs, except in the cases when one of the toxins is dominant. However, due to their cross-sensitivity and different sensitivity and selectivity patterns, these sensors represent attractive candidates to be used in an electronic tongue multisensor system, which should allow quantification of all four toxins. Electronic tongue multisensory systems have been proposed to account for insufficient selectivity that many chemical sensors display in multicomponent media. This approach consists in using arrays of low selective or cross-sensitive sensors with varying sensitivity and selectivity patterns together with chemometric data processing for quantitative analysis and classification of multicomponent samples [30-31].

Detection limits to the PSTs below legal limits is an important requirement for the sensors, in order to be applicable to toxin determination. Due to the high toxicity of the PSTs, legal limits for them are very low, 800µg of STX equivalents per kg of bivalve meat. As detection of PSTs by the reference method (LC-FLD) is done in bivalve extracts, values in mg per kg of meat are calculated from concentration detected in extract taking into account weight of meat and volumes of solutions used for extraction and purification. STX equivalents are calculated using toxicity equivalence factors for each toxin. In bivalve extracts prepared according to the official method, concentrations corresponding to the legal limit are 0.27 µM for

saxitoxin and decarbamoyl saxitoxin and 2.7 µM for GTX5 and C1&C2 [32]. Detection limits to GTX5 and C12&C2 of all studied sensors sensitive to these toxins were below legal limit (Fig. 4). Detection limits of the sensors 1 and 4 to decarbamoyl saxitoxin and sensor 4 to saxitoxin were also below legal limit, while detection limits of the others were slightly higher. According to the literature using sensors as an array together with data processing by the chemometric techniques allows to extract information from the non-linear part of the sensor response curve, which effectively decreases detection limit [33]. Thus, multisensor system based on the developed sensors should be applicable to the detection of PSTs at the concentration levels corresponding to the legal limits for bivalves.

Conclusions

A series of nine potentiometric chemical sensors with plasticized PVC membranes for the detection of PSTs were developed. The main focus was on the detection of toxins typically found in the Portuguese waters such as GTX5, C1&C2 and dcSTX. STX was included in the study because it is one of the most common PST worldwide. Optimal pH for detection of toxins was found to be between 6 and 7 as in this pH range sensors displayed the lowest response to pH, while all four studied toxins are present in ionized form. Characteristics of seven sensor compositions, i.e. cross-sensitivity and low selectivity to PSTs, high selectivity to potential interferents (sodium, potassium and calcium) and low detection limits, make them promising to be used in the electronic tongue multisensor system for simultaneous quantification of PSTs.

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References

1. A. Gersen, I.E. Pol-Hofstad, M. Poelman, P.P.J. Mulder, H.J. van der Top, J. Boer, Marine toxins: chemistry, toxicity, occurrence and detection, with special reference to the Dutch situation, Toxins 2 (2010) 878-904.
2. A. Zingone, H.O. Enevoldsen, The diversity of harmful algal blooms: a challenge for science and management, Ocean Cost. Manag. 43 (2000) 725-748.
3. D.M. Anderson, Approaches to monitoring, control and management of harmful algal blooms (HABs), Ocean Coast. Manag. 52 (2009) 342-347.
4. Anon., 1998. Decreto- Lei nº 293/98; http://www.esac.pt/noronha/legislalimentar/DL%20293_98.pdf
5. Anon., 2004. Regulation (EC) no. 854/2004 of the European Parliament and of the Council of 29 April 2004. Official Journal of European Communities L139, 88–102.
6. P. Vale, M.J. Botelho, S.M. Rodrigues, S. Sousa Gomes, M.A. M. Sampayo, Two decades of marine biotoxin monitoring in bivalves from Portugal (1986–2006): A review of exposure assessment, Harmful Algae 7 (2008) 11-25.
7. M.N. Mons, H.P. van Egmond, G.J.A. Speijers, Paralytic shellfish poisoning: a review. Report nr. 388802005, National Institute of Public Health and the Environment, Bilthoven, The Netherlands, 1998, 1-47.
8. L.E. Llewellyn, Saxitoxin, a toxic marine natural product that targets a multitude of receptors, Nat. Prod. Rep. 23 (2006) 200-222.
9. J.F. Lawrence, B. Niedzwiadek, C. Menard, Quantitative determination of paralytic shellfish poisoning toxins in shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection: collaborative study, J. AOAC Int. 88 (2005) 1714-1732.
10. Anon. AOAC Official Method 2005.06. Quantitative determination of paralytic shellfish poisoning toxins in shellfish using prechromatographic oxidation and liquid chromatography with fluorescence detection. AOAC International, 2005, Gaithersburg, MD, USA.
11. A.R. Humpage, V.F. Magalhaes, S.M. Froscio, Comparison of analytical tools and biological assays for detection of paralytic shellfish poisoning toxins, Anal. Bioanal. Chem. 397 (2010) 1655-1671.
12. L.E. Llewellyn, J. Doyle, J. Jellett, R. Barrett, C. Alison, C. Bentz, M. Quilliam, Measurement of paralytic shellfish toxins in molluscan extracts: comparison of the microtiter

plate saxiphilin and sodium channel radioreceptor assays with mouse bioassay, HPLC analysis and a commercially available cell culture assay, Food Addit. Contam. 18 (2001) 970-980.

13. K. Campbell, L.D. Stewart, J.G. Doucette, T.L. Fodey, S.A. Haughey, N. Vilarino, K. Kawatsu, C.T. Elliott, Assessment of specific binding proteins suitable for the detection of paralytic shellfish poisons using optical biosensor technology, Anal. Chem. 79 (2007) 5906-5914.
14. H. Chen, Y. Sook Kim, S.-R. Keum, S.-H. Kim, H.-J. Choi, J. Lee, W.G. An, K. Koh, Surface plasmon spectroscopic detection of saxitoxin, Sensors 7 (2007) 1216-1223.
15. R.E. Gawley, Q. Zhang, P.I. Higgs, S. Wang, R.M. Leblanc, Anthracylmethyl crown ethers as fluorescence sensors of saxitoxin, Tetrahedron Lett. 40 (1999) 5461-5465.
16. R.E. Gawley, S. Pinet, C.M. Cardona, P.K. Datta, T. Ren, W.C. Guida, J. Nydick, R.M. Leblanc, Chemosensors for the marine toxin saxitoxin, J. Am. Chem. Soc. 124 (2002) 13448-13453.
17. P. Kele, J. Orbulescu, T.L. Calhoun, R.E. Gawley, R.M. Leblanc, Coumaryl crown ether based chemosensors: selective detection of saxitoxin in the presence of sodium and potassium ions, Tetrahedron Lett. 43 (2002) 4413-4416.
18. R.E. Gawley, M. Shanmugasundaram, J.B. Thorne, R.M. Tarkk, Selective detection of saxitoxin over tetrodotoxin using acridinylmethyl crown ether chemosensor, Toxicon 45 (2005) 783-787.
19. R.E. Gawley, H. Mao, M.M. Haque, J.B. Thorne, J.S. Pharr, Visible fluorescence chemosensor for saxitoxin, J. Org. Chem. 72 (2007) 2187-2191.
20. M.A. Artigas, P.J.V. Vale, S.S. Gomes, M.J. Botelho, S.M. Rodrigues, A. Amorim, Profiles of paralytic shellfish poisoning toxins in shellfish from Portugal explained by carbamoylase activity, J. Chromatogr. A, 1160 (2007) 99-105.
21. M.J. Botelho, C. Vale, J.G. Ferreira, Profiles of paralytic shellfish toxins in bivalves of low and elevated toxicities following exposure to *Gymnodinium catenatum* blooms in Portuguese estuarine and coastal waters, Chemosphere 138 (2015) 1028-1036.
22. Y. Umezawa, P. Bühlmann, K. Umezawa, K. Tohda, S. Amemiya, Potentiometric selectivity coefficients of ion-selective electrodes Part I. Inorganic cations (Technical report), Pure Appl. Chem. 72 (2000) 1851-1856.

23. R.S. Rogers, H. Rapoport, The pKa's of saxitoxin, J. Am. Chem. Soc. 102 (1980) 7335-7339.
24. P. Eangoor, A. Indapurkar, J.S. Knaack, Development of a solid phase extraction method to extract gonyautoxins from urine, Ely J. Pharm. Res. 1 (2015) 101.
25. I.V. Tetko, J. Gasteiger, R. Todeschini, A. Mauri, D. Livingstone, P. Ertl, V.A. Palyulin, E.V. Radchenko, N.S. Zefirov, A.S. Makarenko, V.Y. Tanchuk, V.V. Prokopenko, Virtual computational chemistry laboratory - design and description, J. Comput. Aid. Mol. Des. 19 (2005) 453-63.
26. F.N. Assubaie, G.J. Moody, J.D.R. Thomas, Comparative study of polyether-type neutral carriers for the potentiometric sensing of guanidinium ions, Analyst 114 (1989) 1545-1550.
27. B. Fu, E. Bakker, V.C. Yang, M.E. Meyerhof, Extraction thermodynamics of polyanions into plasticized polymer membranes doped with lipophilic ion exchangers: a potentiometric study. Macromolecules 28 (1995) 5834-5840.
28. T. Ito, H. Radecka, K. Tohda, K. Odashima, Y. Umezawa, On the mechanism of unexpected potentiometric response to neutral phenols by liquid membranes based on quaternary ammonium salts - systematic experimental and theoretical approaches. J. Am. Chem. Soc. 120 (1998) 3049-3059.
29. P. Buhlmann, Y. Umezawa, Apparently “non-Nernstian” equilibrium responses based on complexation between the primary ion and a secondary ion in the liquid ISE membrane. Electroanal. 11 (1999) 687-693.
30. Yu. Vlasov, A. Legin, A. Rudnitskaya, C. Di Natale, A. D’Amico, Nonspecific sensor arrays (“electronic tongue”) for chemical analysis of liquids (IUPAC Technical Report), Pure Appl. Chem. 77 (2005) 1965–1983.
31. A. Legin, A. Rudnitskaya, Yu. Vlasov Electronic tongues: new analytical perspective for chemical sensors. In S. Alegret Ed. Integrated Analytical Systems, Comprehensive Analytical Chemistry XXXIX, Elsevier, 2003, pp.437-486.
32. Anon., Committee on toxicity of chemicals in food, consumer products and the environment. Statement on risk assessment and monitoring of paralytic shellfish poisoning (PSP) toxins in support of human health. COT Statement 2006/08, July 2006.
33. A.V. Legin, A.M. Rudnitskaya, Yu.G. Vlasov, C. Di Natale, A. D'Amico, The features of the electronic tongue in comparison with the characteristics of the discrete ion-selective sensors, Sens. Actuat. B-Chem. 58 (1999) 464-468.

Figure legends.

Fig. 1. Structure of saxitoxin and its analogues studied in this work:

	R1	R2	R3
STX	$OCONH_2$	H	H
dcSTX	OH	H	H
GTX5	$OCONHSO_3$	H	H
C1	OCONHSO3	OSO_3	H
C2	$OCONHSO_3$	H	OSO_3

Fig. 2. Slopes of the electrode functions of the sensors in the solutions of PSTs. Measurements were carried out in buffer solutions with pH 7. Mean values of three measurements with standard deviations are shown.

Fig. 3. Logarithms of selectivity coefficients (log $K_{A/B}$) of the sensors to saxitoxin (STX) in the presence of dcSTX, Na^+, K^+ Ca^{2+}. Measurements were carried out in buffer solutions with pH 7. Mean values of three measurements with standard deviations are shown.

Fig. 4. Limits of detection of the sensors in the solutions of PSTs. Measurements were carried out in buffer solutions with pH 7. Mean values of three measurements with standard deviations are shown.

Highlights

- Potentiometric electronic tongue for detection of paralytic shellfish toxins
- Joint Y-PLS effective for calibration transfer from buffers to bivalve extract
- Electronic tongue quantifies three toxins in clean acidic mussel extracts
- Individual sensor quantifies dcSTX toxin in acidic mussel extracts
- Four paralytic shellfish toxins most abundant at Portuguese coast selected

Fig. 1

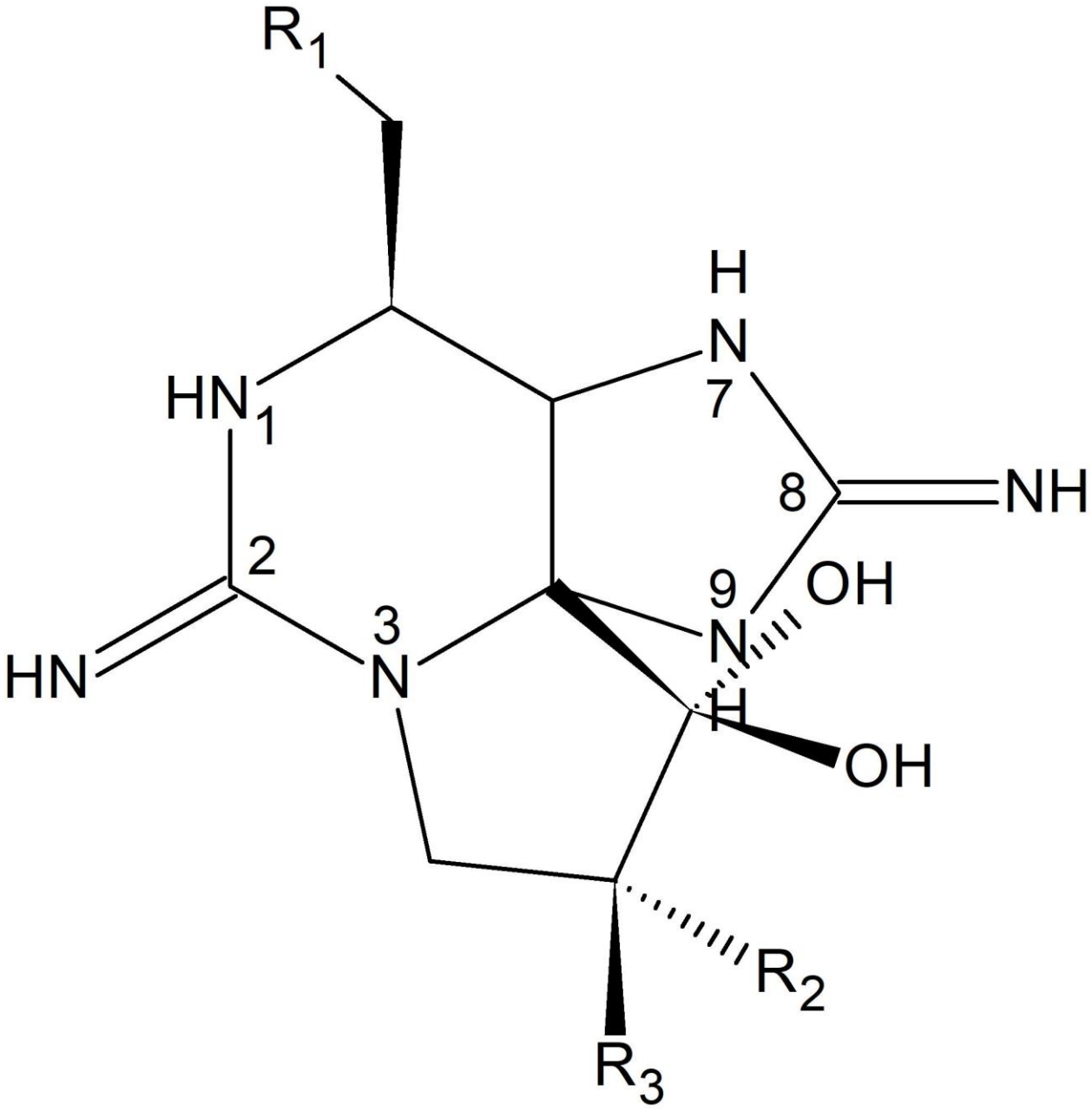


Fig 2

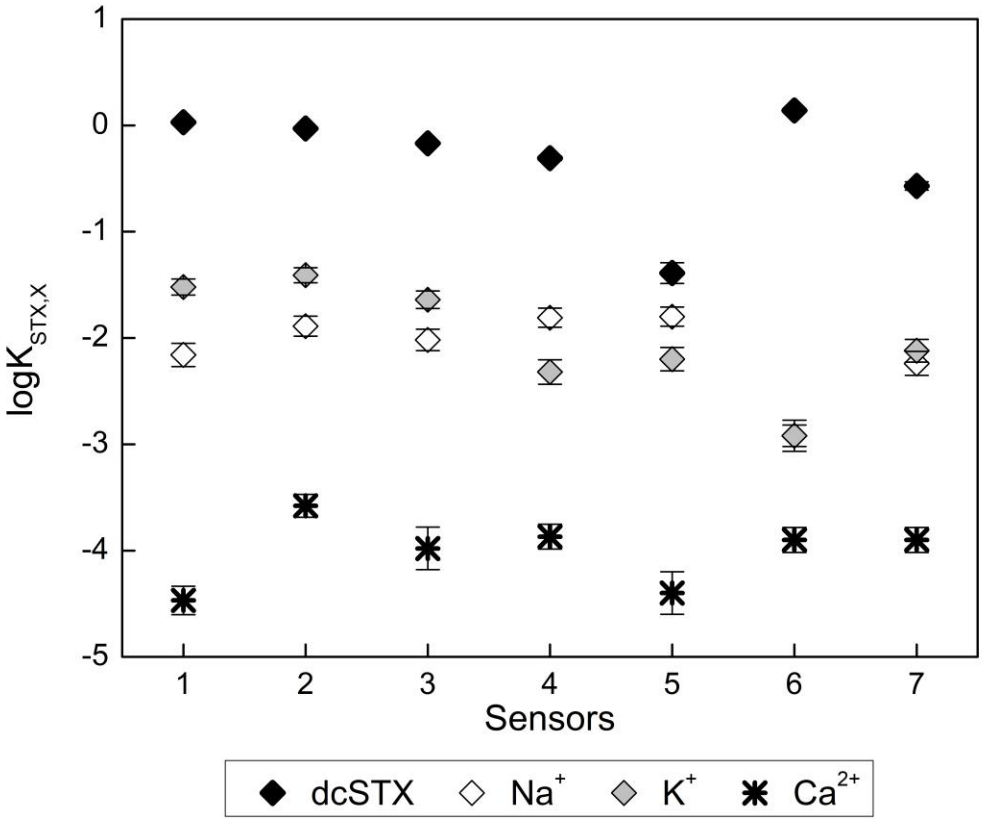


Fig 3

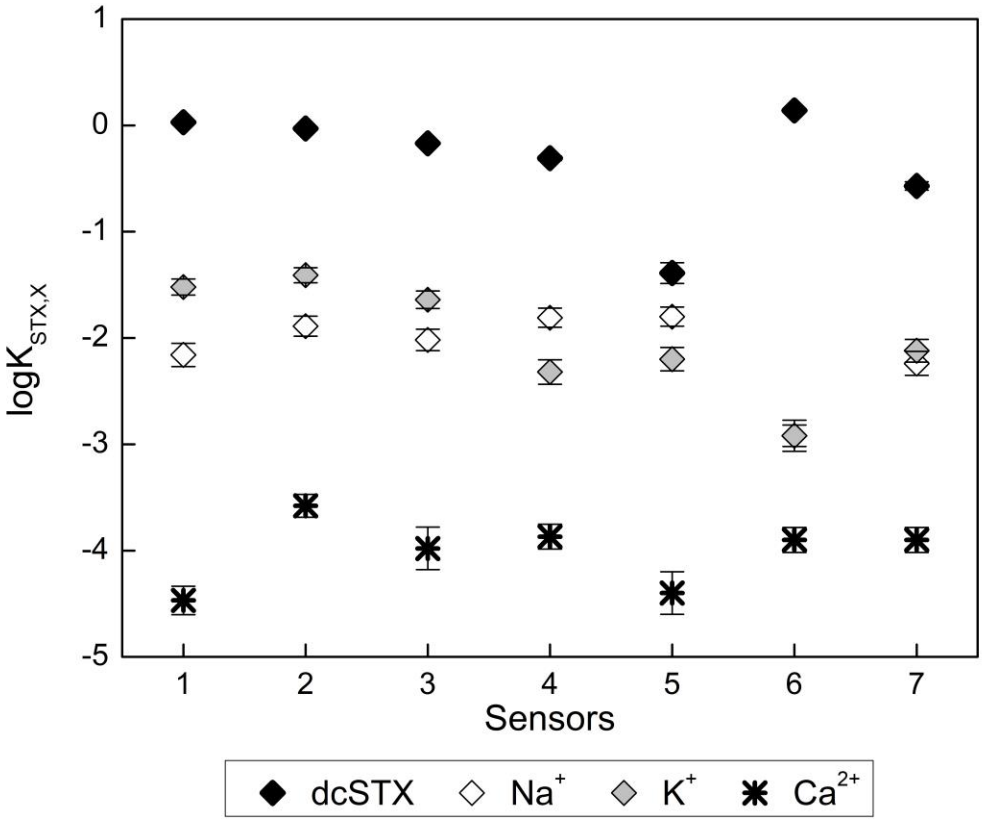
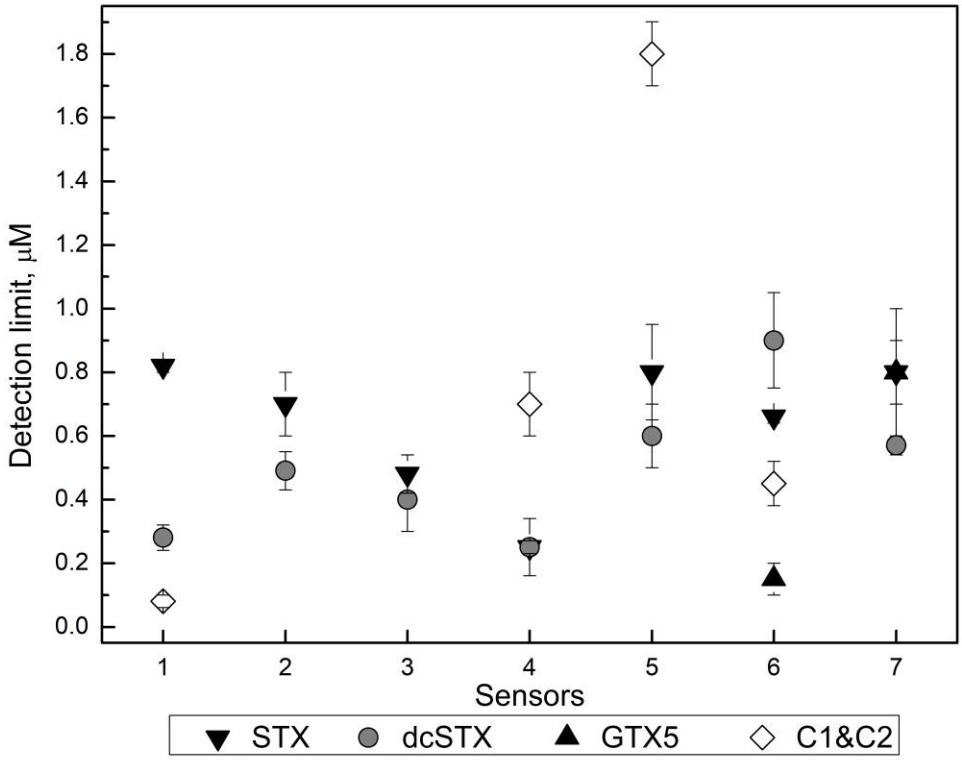


Fig 4



Graphical abstract