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Saxitoxin aptasensor based on attenuated internal reflection ellipsometry for seafood

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ABSTRACT

In this study, we proposed label-free saxitoxin (STX) sensor using STX specific aptamer in combination with spectroscopic ellipsometry (SE) and attenuated internal reflection (AIR) spectroscopic ellipsometry method which is operated under surface plasmon resonance (SPR) conditions. Besides the other surface plasmon resonance-based applications, AIR-SE applications have unique advantages in terms of sensitivity and it was used herein for real-time detection of STX in real samples. Another method, SE, was also used and compared with AIR-SE. Analytical performances were satisfactory with low detection limits and a wide detection range. Limit of detection was 0.01 ng/mL for AIR-SE and 0.11 ng/mL for SE. Both proposed sensors were operable in 0.01 nM–1000 nM STX range. These methods were also used for the accurate, selective, and sensitive detection of STX from fish and shrimp samples.

1. Introduction

Paralytic shellfish poisoning (PSP) toxins are neurotoxins synthesized by microscopic dinoflagellates (Campbell et al., 2011b). Organisms that consume dinoflagellates, such as molluscan shellfish may accumulate the PSP toxins and potentially transfer them through the trophic chain (Deeds et al., 2008). These toxins are not harmful directly for the shellfish; however, it may be fatal for other organisms through toxin contaminated shellfish consumption. PSP toxins are stable, and cannot be thermally denaturized by cooking (Etheridge, 2010). After the consumption, the toxin bind with a high affinity to muscle and nerve cells, blocking the influx of sodium and preventing the generation and propagation of action potentials (Cestèle and Catterall, 2000). Symptoms appeared after intake of saxitoxin (STX), one of the PSP toxins, are numbness of lips and tongue, following by the numbness of fingers and the control loss in the arms and legs. Breathing difficulty after being paralyzed muscles of the chest and abdomen results in respiratory distress, and finally to death. Lethal levels of less than 1 mg of STX per kg of shellfish have been reported (van den Top et al., 2011). For that reason, STX contamination in seafood is frequently monitored by regulatory authorities. The permitted PSP residual limit for the shellfish meat is 800 μg PSP toxin/kg meat, in the EU, which is deemed as appropriate for eliminating acute poisoning (Campàs et al., 2007).

The reference method for PSP toxin analysis is the mouse bioassay or the chromatographic analysis (Cao et al., 2018). However, ethical issues relating to mouse bioassay as well as time-consuming, labor-intensive, and sophisticated method requirement characteristics of chromatographic assays are directing the search for alternative methods for STX analysis (Campbell et al., 2011b; Lawrence et al., 2005; Turner et al., 2011).

In the last three decades, various techniques, such as cell-based bioassays (Okumura et al., 2005; Wang et al., 2015a), receptor binding assays (RBA) (Doucette et al., 1997; Van Dolah et al., 2012), fluorometric assays (Louzao et al., 2003), and enzyme-linked immunosorbent assay (ELISA) (Chu and Fan, 1985; Garet et al., 2010) have been reported by researchers. Cell-based bioassays are good candidates for STX detection since cell growth and viability are inhibited by STX contaminated samples. In a study, where mammalian neuronal networks have been utilized to screen the toxin activity of STX using an electrode array, 9.3 pg/mL detection limit (LOD), and a detection range of 0.003–0.3 ng/mL STX were reported (Kulagina et al., 2006). Wang et al. developed a cardiomyocyte cell-based electrochemical sensor with 0.087 ng/mL LOD and with two linear response range at 0.03–300 ng/mL and ~0.3–30 μg/mL STX (Wang et al., 2015b). The same research group has also reported another cardiomyocyte cell-based STX sensor with 0.35 ng/mL LOD value (0.3–300 ng/mL detection range),

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where the potentiometric approach was being used (Wang et al., 2015a). A neuroblastoma cell-based electrochemical STX sensor was successfully used for the STX detection with 0.03 ng/mL LOD and a detection range of 0.03 ng/mL - 30 μg/mL (Zou et al., 2015). Although the analytical performances of cell bioassays are satisfactory, they still suffer from some problems including the influenced cellular functions by constituents of the extracellular medium, and the necessity to reduce the ionic load in the natural samples to the desired levels (Banerjee et al., 2013).

On the STX detection at the point of analysis, immunoassays come to the forefront. For instance, electrochemical sensors are a good candidate for this purpose. In an early report, an amperometric immunosensor design has performed a good limit of detection (LOD, 15 ng/mL) and stability (more than 120 days) (Carter et al., 1993). Recently, a potentiometric STX sensor incorporated with a lipid membrane, and graphene have been reported, where the LOD of the assay was 0.3 ng/mL STX for shellfish samples (Bratakou et al., 2017). Furthermore, an inhibition immunoassay platform SPR based sensor with 1 ng/mL detection limit has also been reported (Campbell et al., 2007; Fonfria et al., 2007). Photoinduced electron transfer fluorescence chemosensors with several μM STX detection limits have been reported for STX detection (Gawley et al., 2007; Kele et al., 2002). Despite the successful analytical performance of the immunoassays mentioned here, the same successful analytical result was unfortunately not achieved in real applications. In a comparative study to evaluate four commercial immunological test kits on the PSP toxin detection in Tasmanian shellfish, poor analytical results were reported in terms of detection limits, and high false negative and positive results (Dorantes-Aranda et al., 2017).

In this work, an antiSTX aptamer that has been reported before (Handy et al., 2013) was used in combination with an ellipsometric method. Aptamers are nucleic acids selected in vitro from a random oligonucleotide library via a protocol described elsewhere (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Aptamers show good affinity to their targets and have been applied for the detection of various targets such as organic dyes, metals, drugs, carbohydrates, amino acids, nucleotides, peptides, enzymes, and antibodies (Cho et al., 2009). They also have several advantages such as stability, low cost, and ease of production (Song et al., 2012). There are various studies where STX specific aptamers have been employed, and there are various antiSTX aptamer selection studies (Handy et al., 2013; Hu et al., 2013). A comprehensive review of aptamer-based biosensors for aqua-toxins can also be found in the literature (Cunha et al., 2018). On the other hand, there is a limited number of studies on aptasensors for STX detection, and only one study was mentioned in that review (Alfaro et al., 2015). In that study, a fluorometric approach has been implemented to achieve 7.5 ng/mL LOD and 15 ng/mL to 3 μg/mL linear range of detection. A label-free and real-time optical biolayer interferometric biosensor for the detection of saxitoxin using aptamer has been reported which exhibited a broad detection range from 10 ng/mL to 2 μg/mL of saxitoxin with a relatively low LOD value of 0.5 ng/mL (Gao et al., 2017). In a recent study, highly sensitive surface-enhanced Raman scattering (SERS) based aptasensor has also been reported, with 11.7 nM LOD and 10–200 nM linear range, using Au nanoparticles as SERS tags (Cheng et al., 2019).

Ellipsometry is an optical method, which is based on the determination of changes in the polarization state of reflected light from thin films (Poksinski and Arwin, 2004). Its sensitivity highly relies on the configuration of the ellipsometer in use. For instance, highly accurate goniometer (e.g. as low as 0.01°/movement) either in combination with a spectrophotometric light source (e.g. 200 nm–1200 nm light source with a suitable monochromator) results in extremely high sensitivity and accuracy, in terms of dielectric functions of the surface under inspection. This, in turn, gives good sensitivity in (bio)sensor applications. When SE is used, ellipsometric functions (i.e. the delta (Δ) and the psi (Ψ)) of an over-layer on the substrate where molecular interaction occurs, change intensely. The former one, the Δ, is more sensitive to dielectric function change on the substrate and shifts to lower degrees when molecular deposition occurs due to molecular recognition of the analyte via recognition element (Fig. 1). As known, the surface plasmon resonance (SPR) is a powerful and label-free technique that is used for real-time monitoring of biomolecular interactions. In SPR, monochromatic and polarized light (which is also used in the ellipsometry) coupled with plasmons on the ultrathin metal surface is highly sensitive to the changes in dielectric functions of the sensor surface. SPR based biosensor has also been used for the development of a rapid assay for PSP toxin (Haughey et al., 2011). However, since the molecular mass of common PSP toxins is low, and detection via SPR is difficult, all SPR optical biosensor method for PSP toxin determination has been designed as an inhibition assay (Campbell et al., 2011a). In this study, the analytical performance of the attenuated total internal reflection-spectroscopic ellipsometry (AIR-SE) method under SPR conditions was evaluated to search for a better SPR based STX assay which may overcome low molecular weight target recognition problems via an optical-based sensor. To achieve this, a flow-cell experimental setup was assembled to run AIR-SE under SPR conditions using AIR geometry and optical coupler which results in a dramatic change in ellipsometric function, especially in Δ (Fig. 1).

The goal of this study is to determine the STX detection ability of an antiSTX aptamer using ellipsometric techniques that rely on the measurement of the polarization state change of a polarized light reflecting from the sensor surface. Moreover, the possible detection limit improvement is also being considered by implementing surface plasmon resonance conditions. A novel technique for the diagnostic assay of a marine toxin is also reported.

2. Materials and methods

2.1. Instrumentation and reagents

Optosense, OPTS9000 model spectroscopic ellipsometer (Optosense, Turkey) was used for SE measurements, while a total internal reflection assembly (i.e. simple Kretschmann geometry, BK7 prism and immersion oil with flow cell, n = 1.58 at 25 °C) was used for AIR-SE measurements. Two different immobilization techniques and sensor chip substrate were used for the ellipsometric measurements. Silicon wafer was the substrate for the SE technique, while gold-coated glass slide was used for AIR-SE measurements. Glass slide surface was coated with 3 nm Cr and 50 nm Au film by high vacuum physical vapor deposition system (Nanovak, Turkey) to achieve SPR conditions. Blocking agent (6-Mercapto-1-hexanol, MCH), immobilization solution (KH2PO4 1 M, pH 3.8) and hybridization buffer (NaCl 150 mM, Na2HPO4 20 mM, EDTA 0.1 mM, Tween 20 0.005%, pH 7.4) and cleaning purpose solvents (ethanol, isopropanol, etc.) were purchased from local representatives of Sigma-Aldrich or Merck. Coating materials Cr (as an adhesive layer) and Au (plasmon layer) was coating grade, having at least 99.95% purity. Si wafer was <100>, N-type, single side polished, and 0.7 mm thick (Sigma-Aldrich). All substrates were cleaned before any modification and treatment via an oxygen plasma system (RF plasma, Diener, Clairton, USA) for 30 min. All cleaning steps were finalized by drying/purging under the nitrogen atmosphere (99% purity). For all aqueous solutions and washing stages, MilliQwater (18 MΩ) was used. Optical thicknesses of over-layers formed on substrates were measured using an ellipsometer.

An antiSTX aptamer has been reported previously (Handy et al., 2013) having the following sequence were used: 3′-GGT ATT GAG GGT CGC ATC CCG TGG AAA CAT GTT CAT TGG GCG CAC TCC GCT TTC TGT AGA TGG CTC TAA CTC TCC TCT-5′ (antiSTX, see also Table 1). Functionalized antiSTX aptamers and control aptamers (CTRL, non-specific probe, 3′- GGT ATT GAG GGT CGC ATC TAG TAG AAA AGT GCT GAG TAG TTT TAC CTG GTA GAT ATG CGA TGG CTC TAA CTC TCC TCT-5′) were purchased from Molbiol (Germany).

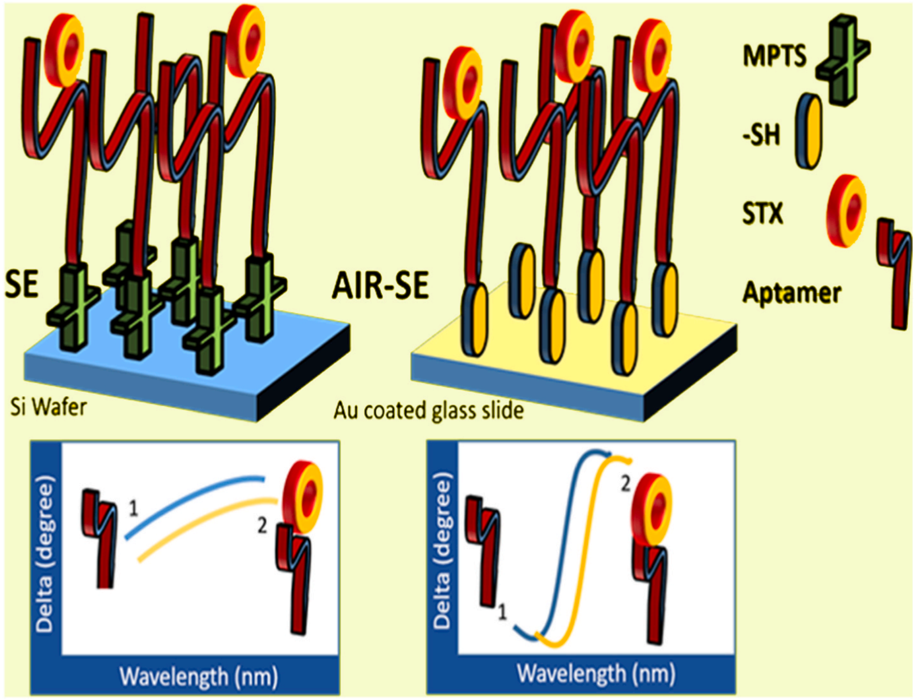

Fig. 1. Schematic representation of aptasensor structure for SE and AIR-SE-SPR method.

Table 1
The antiSTX aptamer and non-specific probe used in this study.

Name	Sequence	Description
antiSTX-SE	3′- NH_2- A_{10}- antiSTX- 5′	SE probe that was immobilized on Si wafer
antiSTX-AIR	3′- SH- $(CH_2)_6$- A_{10}- antiSTX- 5′	AIR-SE probe that was immobilized on gold coated glass slide
CTRL-SE	3′- NH_2- A_{10}- CTRL -5′	Non-specific SE probe that was immobilized on Si wafer
CTRL-AIR	3′- SH- $(CH_2)_6$- A_{10}- CTRL-5'-	Non-specific AIR-SE probe that was immobilized on gold coated glass slide

2.2. Preparation of sensor chips

The antiSTX-AIR and CTRL-AIR probe were immobilized on the BK7 substrates coated with Au by –SH binding route. For this purpose, substrates were cleaned by ethanol-isopropanol rinsing and by plasma cleaning to remove residues from the surface. Cleaned substrates were then immersed into antiSTX-AIR or CTRL-AIR probe solution in immobilization buffer (1 M KH2PO4, pH 3.8) with various concentrations between 0.05 μM and 2.0 μM. Incubation was carried on 4 h under a dark environment at room temperature. The optical thickness of the over-layer on the substrate was measured by ellipsometry using BK7/Cr/Au/Organic (n = 1.48) layer assumption. The antiSTX-SE and CTRL-SE probe were immobilized on the Si wafer surface via aminopropyl triethoxysilane – EDC route which has been described elsewhere (Caglayan and Üstündağ, 2020a, b). Before the immobilization step, Si-wafers and Au coated sensor surface was cleaned via water rinse – drying - plasma cleaning – nitrogen purging steps.

2.3. Evaluation of sensor performance

STX solution (0.01–600 ng/mL) in the buffer (NaCl 150 mM, Na2HPO4 20 mM, EDTA 0.1 mM, Tween-20 0.005%, pH 7.4) were interacted with the probe in an ellipsometer having AIR setup, and real-time binding was monitored to evaluate sensor performance of AIR-SE system. Then, sensor response against target concentrations (i.e. calibration curve) was calculated using 60 min data from the real-time data. The SE sensor performance was evaluated also using STX-aptamer interaction with STX solution in phosphate buffer (0.1–1000 ng/mL). After interacting anti-STX immobilized Si-wafer pieces with STX solutions, cleaning was performed using buffer solution. Ellipsometric parameters of the dried wafer were then measured using ellipsometry without using the AIR assembly. The most vulnerable parameter to the molecular recognition, Δ (delta) was used to construct the calibration curve.

In and after all immobilization and cleaning steps, layer thickness over both the Au-coated slides and the Si-wafers were determined by ellipsometric measurements. Layer thicknesses as “optical thickness of layers” were measured using SE with an angle of incidence of 60°. It is well known that ellipsometry is an indirect technique that is used for measuring two ellipsometric parameters (i.e. ψ and Δ) which cannot be converted directly into the optical parameters of the sample investigated except for isotropic, homogenous and extremely thin films. Therefore, building an optical model that describes the sample structure should be considered. With this aim, a four-phase model consisting of a glass slide (BK7)/Au film/overlayer (as organic material)/air for AIR-SE chips, and Si/SiO_2/overlayer (as organic material)/air were applied.

2.4. Real sample measurements, selectivity, and accuracy

STX containing samples were prepared according to the procedure described elsewhere (Pinto et al., 2019). A portion of 5 g from each fish or shrimp samples purchased from the local market was extracted with 5 mL of 1.0% acetic acid by vortex for 120 s and heating for 10 min in a water bath (at 85 °C). Then, digested real samples were cooled down to room temperature while vortexed for 60 s at every 5 min intervals. Homogenized samples were then centrifuged (Hermle Z36HK, Germany) at 7000 rpm for 10 min. This supernatant was evaporated then transferred to the buffer solution and used as-is for all measurements. Spiked samples were prepared as described above to achieve 10, 100, and 1000 ng/mL final STX concentration in real samples.

To confirm anti-STX aptamer selectivity neo-STX (NEO), Gonyautoxin-1&4 (GTX 1&4), and okadaic acid (OA) were used as non-specific targets. Selectivity measurements were performed using STX and non-specific target solution at 1/10 final ratio (i.e. 100 ng/mL STX and 1000 ng/mL NEO, GTX1&4 or OA).

Otherwise stated, all experiments in this study were performed using five replicates in series. Results were reported as arithmetical mean of the measurements with a standard deviation of these data (1σ).

3. Results and discussion

3.1. Optimum immobilization conditions

First, the immobilization time was changed between 10 and 360 min. Here, the Au coated surfaces were treated with 1 μM antiSTX solutions at 25 °C, and then 1 mM MCH was introduced to these surfaces for 60 min. The ellipsometric thickness of the over-layer formed after 120 min was about 3.76 ± 0.47 nm. This thickness increased only up to about 4.12 ± 0.51 nm even for 360 min. It seems that in our case, the optimum density of self-assembled aptamer probes was achieved after 120 min which was chosen as an optimal time for immobilization of the probes on the Au surface. Similarly, the optimal time for antiSTX aptamer immobilization was also found as 120 min on Si-wafer. Then, we changed the aptamer concentration in the incubation medium (in the range of 0.05–2.0 μM), but kept the incubation time for 120 min to detect optimum probe concentration. The thickness of the aptamer over-layers formed on the Au-surface first increased significantly and reached almost a plateau in a concentration of 1 μM. Then, 1 μM was deemed as optimal (i.e. effective but not full-coverage) for 120 min immobilization of antiSTX or CTRL aptamers on both Au and Si surfaces.

3.2. Analytical performance of the aptasensor

We first investigated the interaction between anti-STX and STX in buffer solution. Aqueous solutions of the STX prepared in the buffer (for AIR-SE system 0.01–600 ng/mL and SE system 0.1–1000 ng/mL) were used for this purpose. AIR-SE real-time sensor response (as Δ change, degree) is shown in Fig. 2.

The interaction was monitored for about 120 min. As seen here, the relative phase shift (Δ) follows interaction kinetics between STX in solution and anti-STX immobilized on the surface. This interaction becomes more favorable at higher STX concentrations while it is less favorable at low STX concentrations (e.g. 5 ng/mL). Kinetics were drastically become favorable at 300 and 600 ng/mL STX.

The calibration curve for AIR-SE was constructed using 60 min data from the real-time monitoring curve, discussed above. After reaching a plateau-like steady rate, it is assumed that available sites on the anti-STX aptamers have been filled by STX in buffer solution. Then, these responses at 60 min were selected to obtain a calibration curve for AIR-SE aptasensor (Fig. 3). The calibration curve carries a semi-logarithmic behavior intrinsically, due to single or multiple site interactions between the immobilized antiSTX aptamer and STX molecules in solution.

The semi-log calibration curve gave a linear response with a regression coefficient of 0.985 (see also Table 2). At higher concentrations the curve has tended to deviate from linearity, however, linearity was quite well and the fitted curve has represented all data in the graph.

The calibration curve for SE based aptasensor platform was also constructed. To do this, first, ellipsometric measurements were done directly on the dried sample surface for wavelengths between 300 and 1200 nm. The linear region of wavelength vs. Δ curve (approximately 600 nm region) was taken as a sensor response of SE aptasensor. Then, the calibration curve was constructed using these responses (relative change of Δ, degree). In Fig. 4, the SE calibration curve is given. As expected, the semi-log behavior was also observed here, due to the nature of the interaction. While aptamers have identical sequences and length, sensor responses were quite different for AIR-SE and SE type aptasensor. As shown in Fig. 4, when it's compared to the previous calibration curve, sensor response was at least three times lower than the AIR-SE response.

Furthermore, the linearity of the semi-log calibration curve was somewhat low with a regression coefficient of 0.966 while it has tended to deviate from linearity at higher STX concentrations. This observation is in concordance with the standard-deviation increase at single points of the curve (which is also in the margins of the points but is higher than the AIR-SE data), which may have resulted from non-uniformity of the surface and/or the sensitivity of the method.

Analytical performances of both sensors are summarized in Table 2. As shown in the Table, sensor sensitivity as the slope of the calibration curve is quite high (1.33 vs. 0.26) in the case of AIR-SE sensor methodology. This fivefold sensitivity increase is expected since SPR phenomena increase the equipment sensitivity under the same conditions. Because, Δ parameter of the ellipsometry is highly sensitive to the changes in dielectric parameters of the investigated surface, under SPR conditions. Limit of detection (LOD) of both techniques was also compared in Table 2. LOD of these methods was calculated from the sensor response equation by using 3σ of the sensor response (i.e. S/N ratio of 3). As expected, the LOD of AIR-SE (0.01 ng/mL) was higher than the SE (0.11 ng/mL) sensor. Besides, the linear range of the sensor was similar in both sensors, while the lower-margin of the linear range of

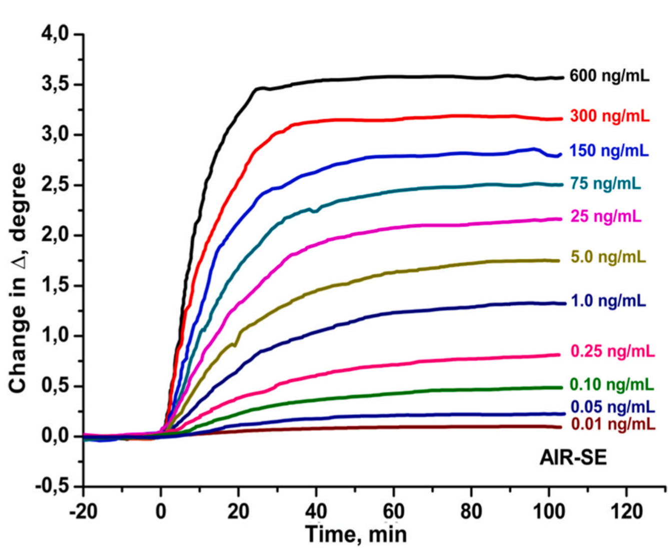

Fig. 2. Real-time sensor response (i.e. phase shift- Δ) for AIR-SE setup at 530 nm wavelength and 65° angle of incidence (for SPR conditions).

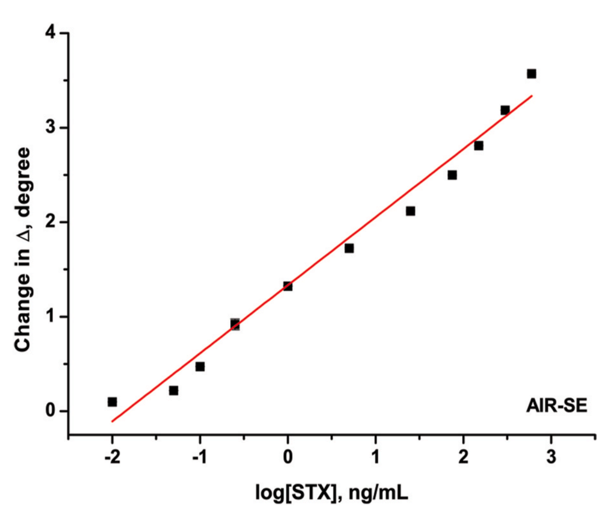

Fig. 3. The calibration curve of anti-STX aptamer at AIR-SE sensor (error bars are within the margins of the data points).

Table 2
Summary of analytical performance of the proposed method ($N = 5$).

Analytical characteristic	Values	
	AIR-SE	SE
Linearity range	0.01–600 ng/mL	0.1–1000 ng/mL
Regression equation, Δ (degree) and [STX] (ng/mL)	Δ = 1.33278 + 0.72031 [STX]	Δ = 0.26034 + 0.25675 [STX]
Standard error of the slope, ±	0.02781	0.01699
Standard error of the intercept, ±	0.04922	0.01537
R^2	0.98529	0.96603
LOD, ng/mL (S/N = 3)	0.01	0.11
LOQ, ng/mL	0.03	0.33

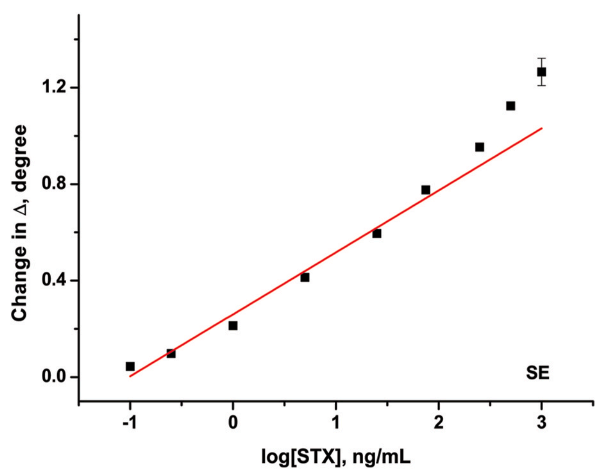

Fig. 4. The calibration curve of anti-STX aptamer at SE sensor (error bars are within the margins of the data points).

AIR-SE is 0.01 ng/mL, which is tenfold lower than the SE sensor.

3.3. Real sample measurements, selectivity, and accuracy

The specificity of the aptasensors was also investigated using a non-specific control aptamers which carries 24 randomly (but centered according to both ends of the aptamer sequence) distributed mutations. This CTRL aptamer immobilized sensor surface was also used for STX detection in buffer solution using the methods described above. Using both CTRL aptamers (i.e. CTRL-SE and CTRL-AIR) we observed a sensor response lower than 10% of the response of specific aptamer at each calibration point. These results are in conformation with the reported aptamer selectivity by several researchers (Zheng et al., 2015).

The selectivity of STX sensors was also crosschecked by some possible interferents including OA, NEO, and GTX1&4. For this purpose, analyte and possible interferents were simultaneously fed to the sensor surface. The influence of some selected interferents on the sensor response for both methods is given in Table 3. The observed signal from a tenfold higher concentration of possible interferents than the STX concentration gave a sensor response different than the STX only sensor signal. This difference was lower than +4.3%4 and + 3.7% for AIR-SE and SE, respectively.

Table 3
The influences of some analogous interferents on the Δ% change of the signal acquired from 100 ng/mL STX.

Interferents added*	Concentration (ng/mL)	Δ signal change upon interferent addition (%) – AIR-SE	Δ signal change upon interferent addition (%) - SE
OA	1000	−3.7	+1.2
NEO	1000	+4.3	+1.9
GTX1&4	1000	+2.6	+3.7

The precision and the accuracy of the AIR-SE method was evaluated using 5 ng/mL and 50 ng/mL of STX solution, while the SE method was evaluated using 50 ng/mL and 100 ng/mL of STX solution. For intra-day measurements, five independent series of sensor chips were used. For inter-day measurements, five independent series of chips were tested at five consecutive days. The precision and accuracy of both methods are given in Table 4. The accuracy of methods for intra-day measurements was in between −3.67% and +4.54%. Inter-day accuracies were in between −3.20% and +5.68%. Furthermore, intra-day precision of methods was in between 1.56% and 3.92%, while inter-day precisions were in between 2.27% and 4.75%. Both inter-day and intra-day accuracy of the AIR-SE method was better than the SE method and lower than 3.2%. Besides, the precision of the AIR-SE was higher than SE, which is in between 1.56% and 3.82%. For both methods, precisions and accuracies were quite satisfactory and even better for the AIR-SE method.

Analytical performances of the AIR-SE and SE aptasensors were evaluated using real-samples (Table 5). STX spiked seafood samples were tested using the procedures given above. Fish (trout, *Salmo trutta*) and shrimp (*Pandalus borealis*) samples were purchased from the local market. Then, for the AIR-SE method, STX was spiked into the real samples to get the final extracted amount will be 1.00, 10.0, and 100.0 ng/mL STX in buffer solution. Recoveries for the fish sample were between 96% and 105%. For shrimp samples, recoveries were between 98% and 104.1%. These results are very satisfactory in terms of real sample applicability. For the SE method, STX that was spiked to the real samples was a bit higher than the AIR-SE method, due to the lower sensitivity of the method. Recoveries obtained from the SE method were in between 94.8% and 105.4%, which is close to the AIR-SE recoveries. According to the FAO limits mentioned in the "Marine Biotoxins," document published by FAO (FAO, Food and Agriculture Organization of the United Nations, 2004) the lowest permissible level of STX equivalent toxin is 30 ng/mg tissue which is quite above the validated detectable concentration reported herein (i.e. 1 ng/mL). Both methods can be used for real sample (seafood) analysis reliably, using the advantages of both aptamer selectivity and the ellipsometry sensitivity.

4. Conclusions

We proposed an assay platform that would detect STX in a label-free manner from seafood samples. For this purpose, an aptamer in which selectivity is well known was used in combination with ellipsometric methods. Furthermore, we used total internal reflection ellipsometry in combination with the surface plasmon phenomena to improve the analytical performance of the proposed sensor. In a well-decorated manner, Au-coated glass slides for AIR-SE assay which were tuned for SPR phenomena, gave the best selectivity, sensitivity, accuracy, and precision, in this study.

Some of the STX detection methods reported in the literature are given in Table 6. The analytical performance of these methods together with our proposed method is applicable to detect minimum permissible limit for STX (or STX equivalent PSP toxin) in the fishery products, which is 30 ng/mg tissue. Among the other STX detection methods which have been reported in the literature, this method gave satisfactory and comparable results. Especially the AIR-SE aptasensor method could be an alternative for STX detection with the detection limit (0.01 ng/mL tissue extract) and a linear range of 0.01–600 ng/mL.

Ethical statement

We hereby declare that in this study, no animal/human subjects were used, and we followed the ethical standards in this study.

Table 4
Precision and accuracy results of the developed method for STX (N = 5).

Method	Added STX, ng/mL	Intra-day			Inter day[a]		
		Found Value, ng/mL	Precision (RSD, %)	Accuracy (ε, %)	Found Value, ng/mL	Precision (RSD, %)	Accuracy (ε, %)
AIR-SE	5	5.13 ± 0.08	1.56	+2.60	4.84 ± 0.11	2.27	−3.20
	50	48.51 ± 1.33	2.74	−2.98	51.36 ± 1.96	3.82	−2.72
SE	50	52.27 ± 1.82	3.48	+4.54	52.84 ± 2.08	3.94	+5.68
	100	96.33 ± 3.78	3.92	−3.67	104.36 ± 4.96	4.75	+4.36

[a] Five consecutive days.

Table 5
Analytical recovery of the proposed sensor in seafood samples (N = 5).

Samples	Spiked amount (ng/mL)	Detected by AIR-SE (ng/mL)	Recovery (%)	Spiked amount (ng/mL)	Detected by SE (ng/mL)	Recovery (%)
Fish	1.00	1.05	105.0	5.00	4.81	96.2
	10.0	9.6	96.0	50.0	47.4	94.8
	100.0	99.0	99.0	200.0	209.4	104.7
Shrimp	1.00	0.98	98.0	5.00	5.27	105.4
	10.0	9.8	98.0	50.0	52.4	104.8
	100.0	104.1	104.1	200.0	195.1	97.6

Table 6
Comparison of different methods for the detection of STX.

Method	Approach	Range (ng/mL)	LOD (ng/mL)	Selectivity against to STX	References
Fluorescence	Based on the pharmacological activity of toxins – Cell culture	0.08–8000	0.1	NO	(Louzao et al., 2003b)
Fluorescence	Based on the ability of PSP toxins to block veratridine-induced depolarization of synaptoneurosomes - mouse synaptoneurosome	0–10000	0.5	NO	(Nicholson et al., 2002)
Colorimetry	Based on vulnerability of NG108-15 cells to specified toxins	0.91–58.41 nM	0.14	NO	(Cañete and Diogène, 2008)
Electrophysiology	Based on monitoring the cardiomyocyte growth and beating status	−0.6 to 1.3 log STX (nM)	0.087	NO	Wang et al. (2015b)
Immunosensor	ELISA	0.020–0.80	0.019	NO	(Wharton et al., 2017)
Interferometry	Optical biolayer interferometry	100–800	0.5	YES	Gao et al. (2017)
Fluorescence	Evagreen fluorescence, double stranded DNA amplification	15–3000	7.5	YES	(Alfaro et al., 2015a)
Fluorescence	Circular dichroism spectra, fluorophore and quencher labeled aptamer	0–24	1.8	YES	(Cheng et al., 2019)
Electrochemical	Aptamer, carbon nanotube decorated electrode	0.27–9	0.11	YES	(Hou et al., 2016)
SE	Aptamer and ellipsometry	0.1–1000	0.11	YES	This study
AIR-SE	Aptamer and surface plasmon resonance enhanced internal reflection ellipsometry	0.01–600	0.01	YES	This study

CRediT authorship contribution statement

Mustafa Oguzhan Caglayan: Writing - original draft, Methodology, Data curation, Conceptualization, Investigation, Software, Visualization, Supervision, Writing - review & editing. **Zafer Üstündağ:** Methodology, Data curation, Investigation, Validation, Formal analysis, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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