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Sensitive analytical performance of folding based biosensor using methylene blue tagged aptamers

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Abstract:

This work demonstrates the development of a folding based electrochemical aptasensor using methylene blue (MB) tagged anti-Ochratoxin A (OTA) aptamers. Different aptamer coupling strategies were tested using Hexamethylenediamine, polyethylene glycol, simple adsorption and diazonium coupling mechanism. The best sensitivity was recorded by oxidation of amines using hexamethylenediamine (HDMA) on screen printed carbon electrode (SPCE). To achieve the direct detection of OTA, aptamer conjugated redox probe was used and detection was demonstrated based on the conformational changes in aptamer structure upon OTA sensing. Signaling in this class of sensors arises from changes in electron transfer efficiency upon target-induced changes in the conformation/flexibility of the aptamer probe. These changes can be

readily recorded electrochemically. The developed aptasensor is unique in its own mechanism as redox probe tagged aptamer coupling such as MB has never been tried to immobilize using long carbon chain spacers as, addition of spacers would provide more sensitive detection methods. A good dynamic range 0.01-5 ng/ml was obtained for OTA with Limit of detection (LOD) 0.01 ng/ml and Limit of quantification (LOQ) of 0.03 ng/ml respectively. The good reproducibility was recorded with RSD% of 3.75. The obtained straight line equation was y=0.4035x+0.90311, r=0.9976. We believe that the sensor design guidelines outlined here represents a general strategy for developing new folding-based electrochemical aptasensors. The developed aptasensor was extended to screen cocoa samples for OTA contamination. The cocoa samples were extracted and purified using molecular imprinted polymer (MIP) columns. The aptasensor displayed good recovery values in the range 84-85% thus, exhibited the effectiveness of proposed aptasensor for such complex matrices.

Key words: Aptamer, OTA, DPV, cocoa, redox probe, methylene blue

1. Introduction:

Electrochemical, aptamer-based (EAB) biosensors employing structure-switching aptamers represent a propitious stage for the rapid and sensitive quantification of target molecules [1]. To date, aptamer based biosensors have been reported against a wide range of targets including bacterial, proteins [2-5], ions, small molecules [6], and mammalian cells [7, 8]. This is achieved through the utilization of single-stranded DNA or RNA aptamers that have been selected for binding to a specific target [8]. The principal signaling mechanism in this class of sensors is a result of conformation and/or flexibility changes in the electrode bound aptamer [4, 9]. Typically, the aptamer is modified at the 5′-end for electrode attachment (e.g., thiolated) and at the distal, 3′-end, with a redox probe molecule such as methylene blue (MB) or ferrocene [10-

14]. Because the electron transfer efficiency is a function of the distance and collision frequency between the redox reporter and the electrode surface, changes in faradaic current upon target binding is readily measured electrochemically [15, 16]. Target detection is performed by measuring changes in voltammetric peak current, typically defined as percent signal change [17, 18]. Using this sensing strategy, EAB sensors have been reported to exhibit typical limits of detection (LOD) from micromolar down to picomolar levels when detecting, for example, proteins [19] or small molecules [11]. In addition, sensors are able to perform such detection in complex sample matrices, whole blood or physiological matrix [5, 20, 21].

Although such kind of electro-chemical aptasensors has been fabricated for selective and sensitive detection of many target molecules, they required a large scale conformational change of the aptamer to modulate the distance of redox tags from the electrode to change the signal [20]. Additionally, these aptasensors generally undergo the problem of substantial background current [22]. To circumvent these drawbacks, herein, we described a new simplified approach using spacer attached aptasensors. In this fabrication scheme, hetero bifunctional linear and long spacer arm of different molecular weight Poly ethylene glycol (PEG) and hexamethyldiamine (HMDA) was obtained on electrode surface via electrochemical oxidation of NH_2-PEG-COOH. The amino-aptamer was linked flexibly to terminal carboxylic group of PEG spacer forming a diblock macromolecule that is immobilized on screen printed carbon electrode (SPCE) surface. The diblock macromolecules were expected to form clusters of long chain spacer arms on the substrate surface, leaving long hole for the redox probe to reach the electrode surface. In the absence of target analyte, the aptamer remained unfold, thereby allowing the passage of redox probe to electrode surface [23]. Upon target analyte binding, the electron transfer is inhibited due to binding conformational changes of aptamer from random coils to G-quadruplex structure that

significantly covers the entrance of long tunnel, blocking redox probe to reach the electrode surface [24].

It is difficult to achieve ultrasensitive detection of small molecules by a basic aptasensor because of the low size of analytes. Thus, in order to carry out the sensitive detection, the exploration of novel methods is essential. Here, we employed our designed platform to develop a simple and ultrasensitive electrochemical aptasensor for the small size target analyte. Ochratoxin A (OTA) (408 MW), it contaminates a variety of food commodities [25]. Cocoa and various derivatives contamination (International Cocoa Organization (ICCO) with OTA has been revealed by numerous studies using conventional techniques [26-28]. OTA has several toxicological effects such as nephrotoxic, hepatotoxic, neurotoxic, teratogenic and immunotoxic, and it is believed to cause increased oxidative stress at cellular level [29, 30]. Recently, numerous aptamer based biosensors based on enzyme-labels, nano particles and label free detection methods have been exploited for OTA detection [31-34]. By employing this strategy, we demonstrated that our designed electrochemical aptasensor is simple, sensitive and stable. Compared to existing aptasensors based on conformational changes, the proposed strategy offers substantial advantages because the target induced conformational changes is used to cover the entrance of a long tunnel to detect the target analyte, eliminating the size factor of molecules.

In this work, we have demonstrated the OTA detection in cocoa samples. Cocoa beans after post-harvest treatments are mainly exported to Europe and North America to be turned into liquor, butter and cocoa powder [35]. Cocoa and various derivatives contamination (International Cocoa Organization [ICCO] with OTA has been revealed by numerous studies using conventional techniques. Hence, there is a need to screen cocoa beans for OTA quantitation before consumption. Cocoa is a complex matrix with tannin and a thick surface resulting in turbid

samples even after the extraction using suitable chemicals. Herein, we have used 1% sodium hydrogen carbonate ($NaHCO_3$) for extraction and Molecular imprinted polymer (MIP) columns were employed for sample clean-up. MIP columns are cost effective, chemically stable and compatible with all solvents [36]. The developed method combines the significant advantages of differential pulse voltammetry (DPV) with a rapid and real-time monitoring of OTA using aptamers. DPV offers high sensitivity, low limit of determination, easy operation, and the use of simple instrumentation [34, 37]. The exploration of MIP columns, SPEs and the optimization of appropriate aptamer concentrations will certainly make an impact on real tests.

2. Chemicals and materials

Hexamethylenediamine, N-Hydroxysuccinimide (NHS), N-(3-dimethylaminopropyle)-N'-ethyle-carbodiimide hydrochloride (EDC), were purchased from sigma (France). Ochratoxin A (from *A. ochraceous*) and Ochratoxin B (OTB) procured from Sigma was first dissolved in methanol and then diluted in binding buffer. The anti-OTA aptamer tagged with redox probe methylene blue was ordered from Microsynth, Switzerland. The aptamer sequences are shown below as 5' end is modified with $COOH^-$ and 3' was methylene blue tagged.

5'-GAT CGG GTC TGG GTG GCG TAA AGG GAG CAT CGG ACA-3' (COOH-OTA-MB).

Aptamer solutions were prepared in binding buffer (BB), pH7.4 containing 1mM $MgCl_2$, 140 mM NaCl, 2.7 mM KCl, 0.1mM Na_2HPO_4 and 1.8 mM KH_2PO_4 were used. NH_2-PEG-COOH (300) and PEG 24-Amine (MW 1146.25), 4-amino benzoic acid, sodium nitrite ($NaNO_2$), hydrochloric acid ethanolamine and bovine serum albumin were purchased from Sigma (France).

2.1 Apparatus

All electrochemical measurements were carried out using an AUTOLAB PGSTAT100 potentiostat/galvanostat equipped with a frequency response analyzer system (Eco Chimie, Netherlands) controlled by General purpose Electrochemical system (4.9) for voltammetry. SPCEs were fabricated using a DEK 248 screen-printing system [38]. The SPCE consists of conventional three electrode configuration with graphite as working (4 mm diameter disk) and counter (16 mm×1.5mm curved line) electrode, and Ag/AgCl (16mm×1.5mm straight line) as pseudo-reference electrode.

2.2 Electrochemical oxidation of amines on a SPCE surface

Electrochemical grafting of long spacer chains to SPCE was carried out in 0.05M of Tetrabutylammonium tetra-fluoroborate 99% ($NBu4BF_4$)-0.5M acetonitrile solution containing 6 mM hexamethyldiamine (HMDA), 6 mM NH_2-PEG-COOH (MW3400) or carboxy PEG amine (10000). Then the potential was cycled between 0.3 V and 1.7 V (vs. SPCE) at a scan rate of 0.5 V/s for the electrochemical oxidation of amino groups. After washing the electrode surface with water, the modified electrodes were used directly for aptamer immobilization or stored at room temperature without loss of any immobilization efficiency. After rinsing the electrode with water to remove the unbound aptamer, the anti-OTA-aptamer modified SPCEs can be used directly for aptasensing or stored dry at 4°C for several days without any decrease in the sensitivity. For the electrochemical experiments, a 100 mL of OTA dissolved in BB was dropped onto the surface of the sensor, followed by incubation for 45 min in different concentrations of OTA.

2.3 Covalent immobilization of MB tagged aptamer

The redox probe MB was tagged on 3'of the aptamer whereas 5' was modified with $COOH^-$. Prior to the immobilization, the terminal carboxylic group of aptamer at 5' was activated by 100

mM EDC and 25 mM NHS in 100 mM MES buffer for 60 min. After the activation process, 20 mL of activated aptamer solution (2µM) were incubated onto the SPCE surface for 45 min under water saturated atmosphere. After rinsing the electrode with water to remove the unbound aptamer, the anti-OTA-aptamer modified SPCEs can be used directly for aptasensing, or stored dry at 4°C for several days without any decrease in the sensitivity. For the electrochemical experiments, a 100 mL of OTA dissolved in BB was dropped onto the surface of the sensor, followed by incubation for 45 min with different concentrations of OTA.

2.4 Diazonium coupling and adsorption

In another approach, the clean SPCE surface modification was also performed in the diazotation mixture following the procedure described by Baranton and Belanger (2005) [39], using 1:1 equiv. of $NaNO_2$ and 4-aminobenzoic acid. In brief, 400 µL of 0.1M $NaNO_2$ solution (final concentration, 2mM) added to the electrolytic solution (20 mL) containing 2 mM 4-aminobenzoic acid and 0.5M HCl. The mixture was stirred and left to react for about 5 min at room temperature. The electrochemical reductive modification of SPCE with *in situ* generated 4-carboxyphenyl diazonium salt (4-CPDS) was carried out by linear sweep voltammetry from 0.6-0.8V. The terminal carboxylic groups on SPCE surface were activated by immersing the 4-CP/SPCE in a solution of 100 mM EDC and NHS in 100 mM MES buffer (pH 6.8) for 60 min. After washing the electrode surface with water, 30µL of anti OTA-aptamer tagged with MB solution was incubated on to the activated SPCE surface. After incubation, the electrode was rinsed with BB water to remove unbound aptamer, modified SPCE was incubated with 30µL of 1M ethanolamine solution for 45 min to deactivate the remaining succinimide group and block unreacted sites. It confirms that the aptamers were attached only to the modified 4-CPDS modified sites on the surface. After rinsing the electrodes, 30 µL 1% BSA solution was also

incubated for 60 min to completely block the unbound sites of the SPCE surface. The OTA-aptamer modified SPCEs can be used directly as aptasensor, or stored dry at 4°C for several days without any decrease in the sensitivity. The simple adsorption was also performed by putting the aptamer solution on to the SPCE for 30 mins. After the washing steps, the electrodes were directly used for biosensing experiments.

2.5 Cocoa sample preparation

The method used in OTA extraction from cocoa samples was as follows: A portion of 50 g of contaminated cocoa sample was weighed in a beaker and then OTA was extracted with 200 mL of a 1% aqueous solution of sodium hydrogen carbonate ($NaHCO_3$) rotating with magnetic stirring for 45 min. The extract was filtered and centrifuged at 5000 rpm for 10 min. An aliquot of 50 mL (filtered and centrifuged sample) was taken and passed through molecular imprinted polymer (MIP) column, at a flow rate of 1 drop/sec. The column was later washed with 20 mL of 50 mM PBS containing 0.1% tween 20. OTA was eluted with 2 mL of methanol and collected in a clean vial. The collected OTA was 12.5 ng/mL. The collected sample was further diluted in binding buffer for recovery studies.

2.6 DPV measurements

After immobilization of aptamers and incubation of OTA on SPCE surface, DVP measurements were carried out using BB solution under the following conditions; modulation time, 0.02 interval time 0.5s, initial potential 0.4V, end potential 0.15V, step potential 0.005 V, modulation amplitude 0.10005 V, stand by potential 0 V. Upon the oxidation of MB tagged on aptamers, the height of the resulting peak wave form was recorded and plotted against OTA concentration to construct a calibration curve. All the tests were performed in triplicate.

3. Results and Discussions

3.1 Design strategy of folding based aptasensor

Scheme 1 represents the outline of the strategy used in this work to fabricate the folding based electrochemical aptasensor. The aptamer immobilization via electrochemical oxidation of long chain HMDA or PEGs on the SPCE surface formed a cluster of long spacer arms on the transducer surface, leaving long holes for the redox probe to reach the electrode surface. Here in the designed strategy, aptamers were employed as gates of long tunnels. The aptamers were expected to keep their random coiled structure in the absence of target, thus leaving the gate opened for flow of electrons to the transducer surface. However, the addition of target analyte closed the gates of tunnels by transforming the conformation of the aptamer from random coil to G-quadruplex structure that closed the entrance of long tunnels, resulting in the blockage of the electron flow to the electrode surface. The increasing concentrations of the target analyte increased the formation of aptamer gate and subsequently decreased the number of open tunnel, resulting in decreased electrochemical signal. In our designed strategy, two factors; aptamer gate formation and exposure of negative charged bone, contributed to enhance the sensitivity of the system by decreasing the electrochemical signal upon target analyte recognition.

3.2 Electrochemical grafting of spacers to the SPCE surface

In order to optimize the concentration, experiments with 0.32 mM to 6 mM of HMDA and PEGs were performed and the resulting electrochemical oxidation peak current was measured. The oxidation peak current was observed with concentration of 6 mM for all the tested spacers as this concentration provides optimal electrochemical signal. Fig.1 shows consecutive cyclic voltammograms of 6 mM HDMA and two PEGs at the SPCE surface. The oxidation peaks at 0.9-1.2 V (vs. SPCE) is assigned to amine oxidation into its analogous cation radical that could

form a strong and chemically stable N-C covalent bond on the carbon electrode surface by one electron oxidation process.

3.3 Coupling of aptamers using different approaches

It was explained in section 2.1 about the strategy of aptamer immobilization by amine oxidation using three spacers, diazonium chemistry and simple adsorption technique. The MB tagged aptamer-OTA binding response was recorded using all the tested coupling strategy and it was revealed from the results that the maximum response was obtained using amine oxidation by HMDA. It provides more sensitive detection of OTA as compare to other tested approaches because of its long carbon chain length, which could be brought down close to the SPCE surface. Moreover, it provides very rapid detection as compare to diazonium approach. Thus resulting in rapid (two step) detection strategy. The response with simple adsorption was also significant but the problem associated is leaking of biomolecules and stability in results. Therefore, further experiments were conducted with HMDA spacer to obtain the high magnitude of detection for real sample cocoa.

3.4 Optimization of various parameters (incubation time, aptamer concentrations)

Immobilization of aptamer is very important and crucial factor for aptasensor development. Higher aptamer concentrations decrease the detection value and lower concentrations may reduce the signal [25]. Experiments with different concentrations of anti-OTA-aptamer tagged with MB were performed. As illustrated in the Fig. 2a, various redox probe labelled aptamer concentrations such as 0.15, 0.3, 0.5, 1, 2 and 3 µM were tested to immobilize on to the SPCE. The original current response was presented in the inset in Fig 2a. The best concentration was chosen to be 2 µM for folding based aptasensor development. In this study, the choice of aptamer concentration was more than our previous study. [25, 40] In this study, we deal with

redox probe MB tagged with aptamer, in turn responsible for conformational changes in aptamer structure upon target recognition. In order to enhance the sensitivity, optimization of incubation time was also investigated. The incubation time is another important parameter to affect the analytical performance of the proposed aptasensor. As shown in the Fig 2b, the experiments were carried out with varying incubation times from 15, 20, 30, 45 and 60 min. But the fact that, OTA-aptamer response reached to an equilibrium at 45 min. Thus, 45 min was chosen as the optimal incubation time.

3.5 DPV based detection of OTA using redox probe MB tagged aptamers

After the demonstration of immobilization strategy for redox probe labelled aptamers by various strategies, such as linking of aptamers on spacers HMDA and PEGs, diazonium coupling chemistry and adsorption, a label free DPV based electrochemical detection was carried out. Using the optimized parameters, a calibration curve was performed based on the change in the peak current in the range 0.01 to 5 ng/ml with 45 min of incubation. As shown in the Fig. 3, the increase in peak current was observed upon increasing OTA concentration. Owing to OTA and aptamer strong specific interaction, the quantity of OTA on the aptasensor surface generated substantial resistance to the electron transfer reaction of the redox probe. Hence, at a low OTA concentration, the electron transfer resistance was expected to be less relative to high OTA concentration. Therefore, a smaller difference in peak current was observed at low OTA concentration, and a larger difference in peak current was observed at high OTA concentration before and after OTA-aptamer complex formation. As demonstrated in the figure, there is a steady linear increase in the current difference with OTA concentration from 0.01-5 ng/ml. The original response of current changes upon potential applied was recoded and presented as Fig 4.

This behavior is in agreement with other reports (18, 20, 37, and 38). The linear regression equation was calculated to be y=0.4035x+0.90311, r=0.9976 with an estimated LOD of 0.01 ng/ml, calculated as the concentration of toxin corresponding to the 3 times "s/m" ratio, where "s" is the standard deviation of the blank DPV (three replicates) and "m" is the slope of the related calibration curve. The calculated RSD value is 3.75%, this value represents the mean RSD obtained from five different OTA concentrations, where five different modified SPCE are used as replicates for each concentration. The obtained values of r and RSD including the wide range of response demonstrate the good performance of this innovative approach. Further improvements are previewed so as to get better repeatability between the different electrodes. This aptasensor for OTA detection presents high analytical performance in terms of linear range, sensitivity (0.4035 ng/ml), and LOD (0.01 ng/ml). We reached a very low LOD without the need of neither amplification, nor MB intercalation, taking advantage of a novel folding based electrochemical platform. As result, our approach is simpler and cheaper, allowing us to achieve the wide range of response (0.01-5 ng/ml) and one of the best LOD reported so far for folding based aptasensors using MB tagged aptamers. These data demonstrates that rational engineering of the aptamer structure to create large conformation changes upon OTA binding leads to improved aptasensor performance.

3.6 Real sample analysis (recovery)

Recovery is the ratio of concentration of analyte quantified by a given method in a said sample to the actual concentration by the standard method. When this ratio is multiplied by 100, it is expressed as percentage recovery. The developed method was extended to analyze OTA in real samples; hence experiments were carried out with raw cocoa samples in order to validate the developed method. It is worthy to mention that in this work, a DPV based electrochemical

aptasensor was developed wherein; a redox probe, MB tagged aptamers were fixed on a surface using spacers. Such approach is applied for the first time to detect OTA in raw cocoa samples at the cutoff level of 2 µg/kg meets EU standard. This exercise also determines the matrix interferences during the analysis. Firstly, the raw cocoa samples were extracted using MIP columns and further applied for the recovery studies. It was noticed that cocoa samples were retained the turbidity which possibly deteriorates immobilized aptamer on the electrode surface, hence extraction was needed. Cocoa samples were spiked with three known concentrations of OTA (2, 5 and 10 µg/kg) after a short pretreatment of samples described in Section 2.5. The RSD was calculated to be 4.72% indicating the precision and reproducibility of the developed aptasensor. The accuracy (A.E. %) was obtained by comparing the spiked (2, 5 and 10 µg/kg) and the measured values (1.67, 4.25 and 8.6 µg/kg). The analytical results and the obtained recoveries were shown in Table 1. The spiked values were consistent with the obtained concentrations of OTA. The developed aptasensor could therefore be applied in a rapid and simple detection of OTA in real cocoa samples. The recovery was calculated on the basis of calibration curves performed in BB. The recovery results validated the suitability of the method to real samples. The precision was determined by RSD% for the triplicate measurements.

3.7 Specificity and analytical features of the sensor

Concerning the practical point of view, a biosensor should not only be sensitive but also specific. In order to evaluate the selectivity of the developed aptasensor, control experiments were performed using OTA and OTB. The spacer modified electrodes were incubated for 45 min with three different concentrations (1.25, 2.5, and 5 ng/ mL) of OTB as non-specific analyte and blank. As shown in Fig. S1, incubation with OTB did not produce significant changes of the ratio response as against the OTA. These results demonstrated that the developed aptsensor has

sufficient specificity to its target molecule. The reproducibility of the developed aptasensor was also investigated with inter-assay precision. The precision test was evaluated with the same OTA concentrations (ng/mL) using three aptasensor independently prepared in the same experimental conditions.

4. Conclusions

In order to function in real-world applications, a sensor should achieve acceptable sensitivity, specificity, and selectivity. Herein, we have demonstrated several strategies (for aptamer immobilization on to SPCE) for dramatically improving the analytical figures of merit of an electrochemical, aptamer-based sensor capable of detecting the OTA at physiologically relevant concentrations. Specifically, we optimized sensor performance by exploiting binding-induced changes in probe aptamer flexibility and conformation by choosing the optimal DPV generated current to employ when measuring E-AB sensor signaling of the OTA sensor. Among tested spacers, diazonium chemistry and adsorption approach, we have found out that coupling of MB-aptamer on to the SPCE is better with HMDA in terms of sensitivity. It is noteworthy that this is the first adaptation of such kind of attempt to detect OTA in food sample that would undergo a larger conformation change and, thus, create a larger signal change. The developed folding based aptasensor could detect OTA at the level 0.01 ng/ml with the broad dynamic range 0.01-5 ng/ml. The method was also applied to test the matrix feasibility for aptasensor and for those cocoa beans samples were analyzed for OTA screening. A good recovery was obtained in the range 83.79-85.98%. The method displayed a good reproducibility as well with RSD value of 3.75%. The set of parameters employed to improve the performance of electrochemical, aptamer-based sensors utilizing structure switching aptamers should be applicable to improving the performance of sensors employing any aptamer. Here we show that an electrochemical aptasensor directed

against the fungal toxin OTA achieves physiologically relevant sensitivity and specificity and that it realizes these attributes even in the contaminant-ridden cocoa samples. In addition, the E-AB sensor features a number of other potential advantages as a sensing platform such as rapidity, highly reproducible and direct detection of contaminants even in complex sample. The E-AB approach is also amenable to miniaturization and parallelization, potentially rendering it possible to simultaneously monitor multiple targets with a single-chip device. Last, because all of the E-AB components are strongly attached to the electrode surface, the approach is both single step and readily reusable. The E-AB approach thus appears well suited for convenient field monitoring of cocoa beans and small molecule analytes such as OTA.

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Legends of Table

Table 1: Recovery percentages of spiked OTA in cocoa samples using developed folding based aptasensor.

Spiked OTA (µg/kg)	Found OTA (µg/kg)	R.S.D.%	Recovery %	A.E.%
10	8.590	6.79	85.98	14.3

5	4.251	4.17	85.02	15.2
2	1.675	3.2	83.79	17.3

Legend for Figures

Scheme 1: The principle of the biosensing of OTA using methylene blue tagged aptamer: schematic representation.

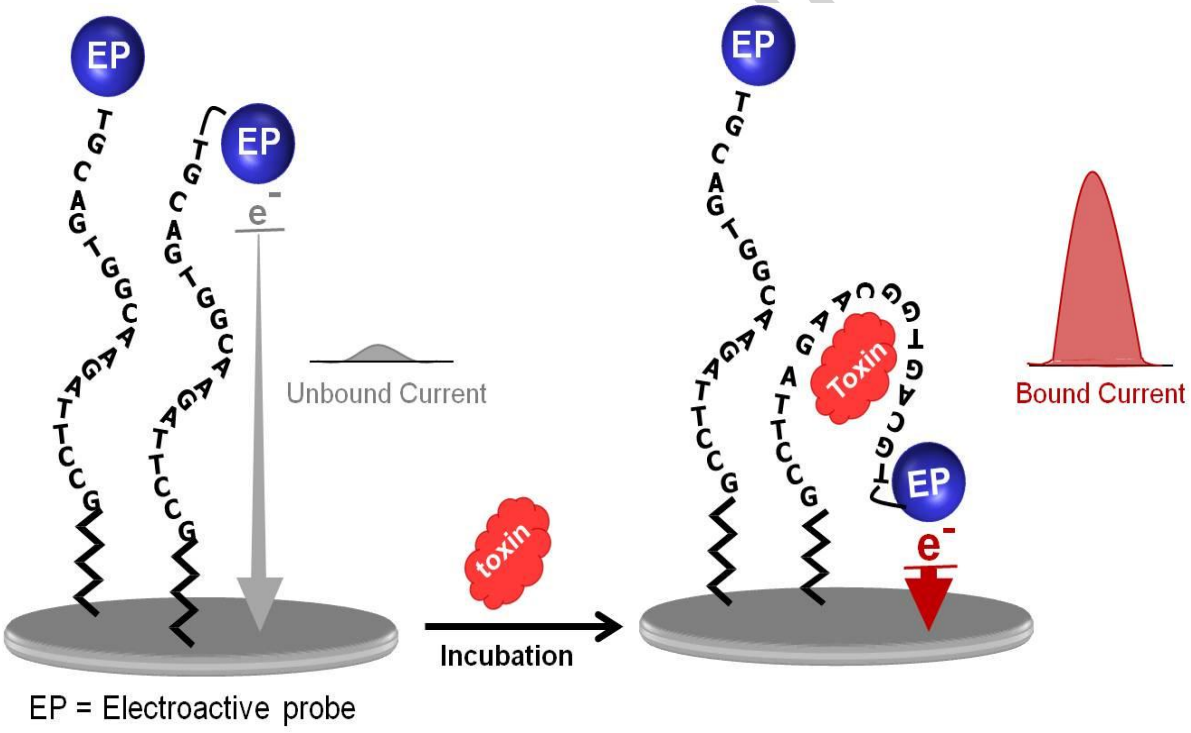


Figure 1: Electrochemical oxidation of amines using different spacer used to couple methylene blue modified aptamers on SPE surface.

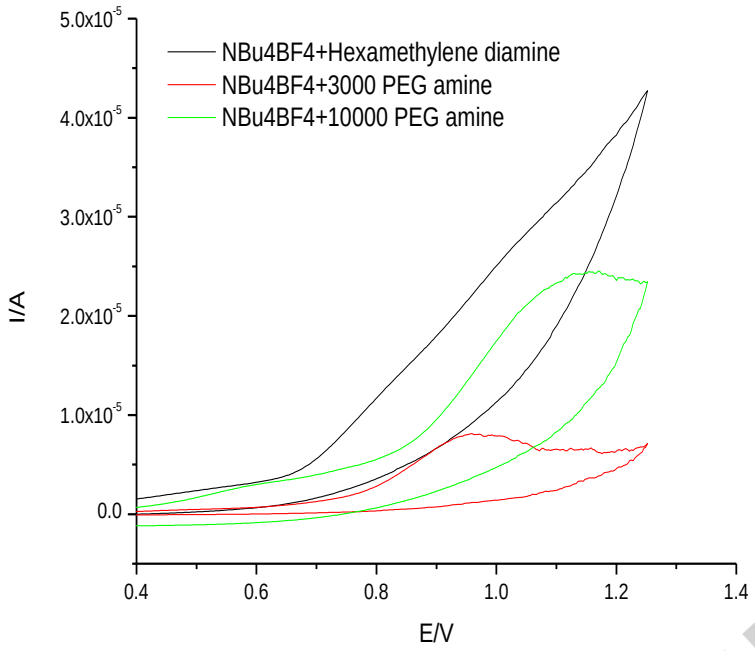


Figure 2a: Optimization of various redox probes labelled aptamer concentrations such as 0.15, 0.3, 0.5, 1, 2 and 3 µM. The original current response was presented as inset.

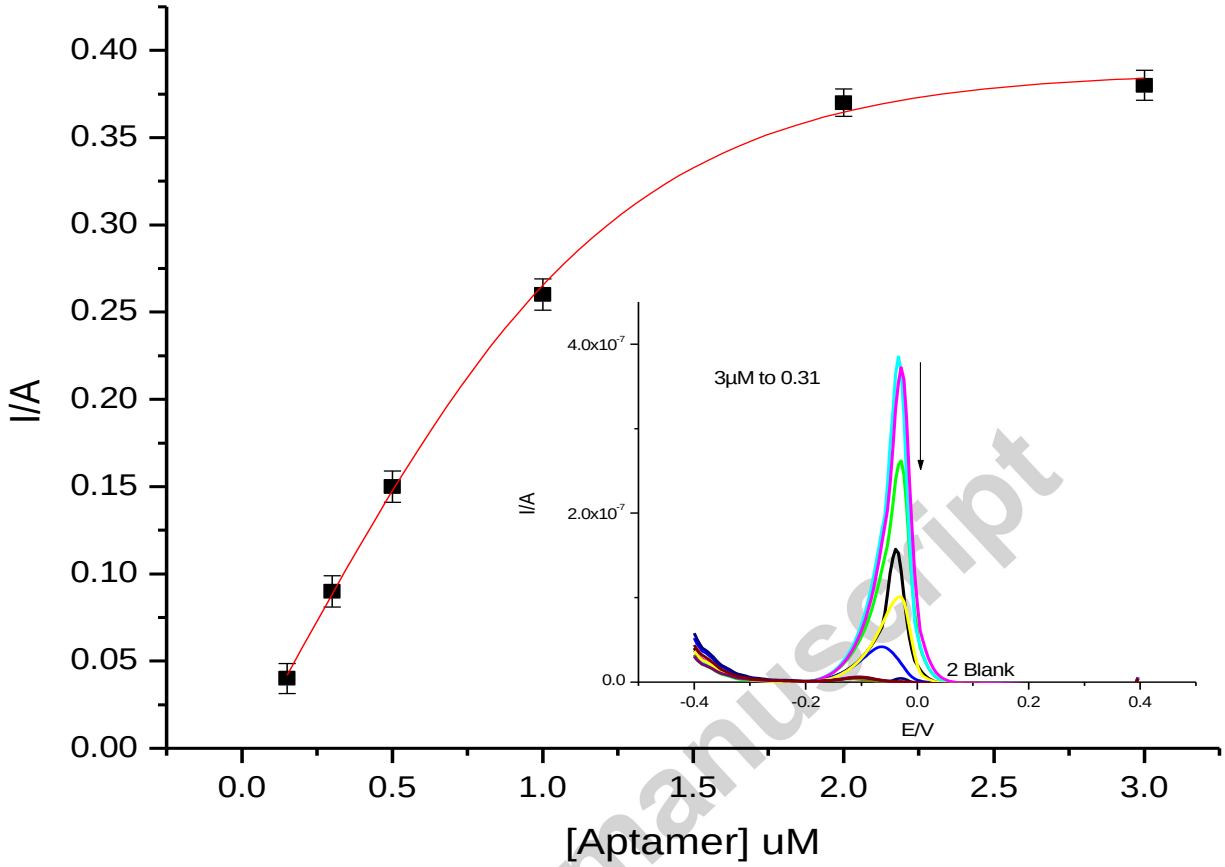


Figure 2b: Optimization of incubation time for OTA analysis, methylene blue modified aptamers and OTA incubation on SPE surface using spacers.

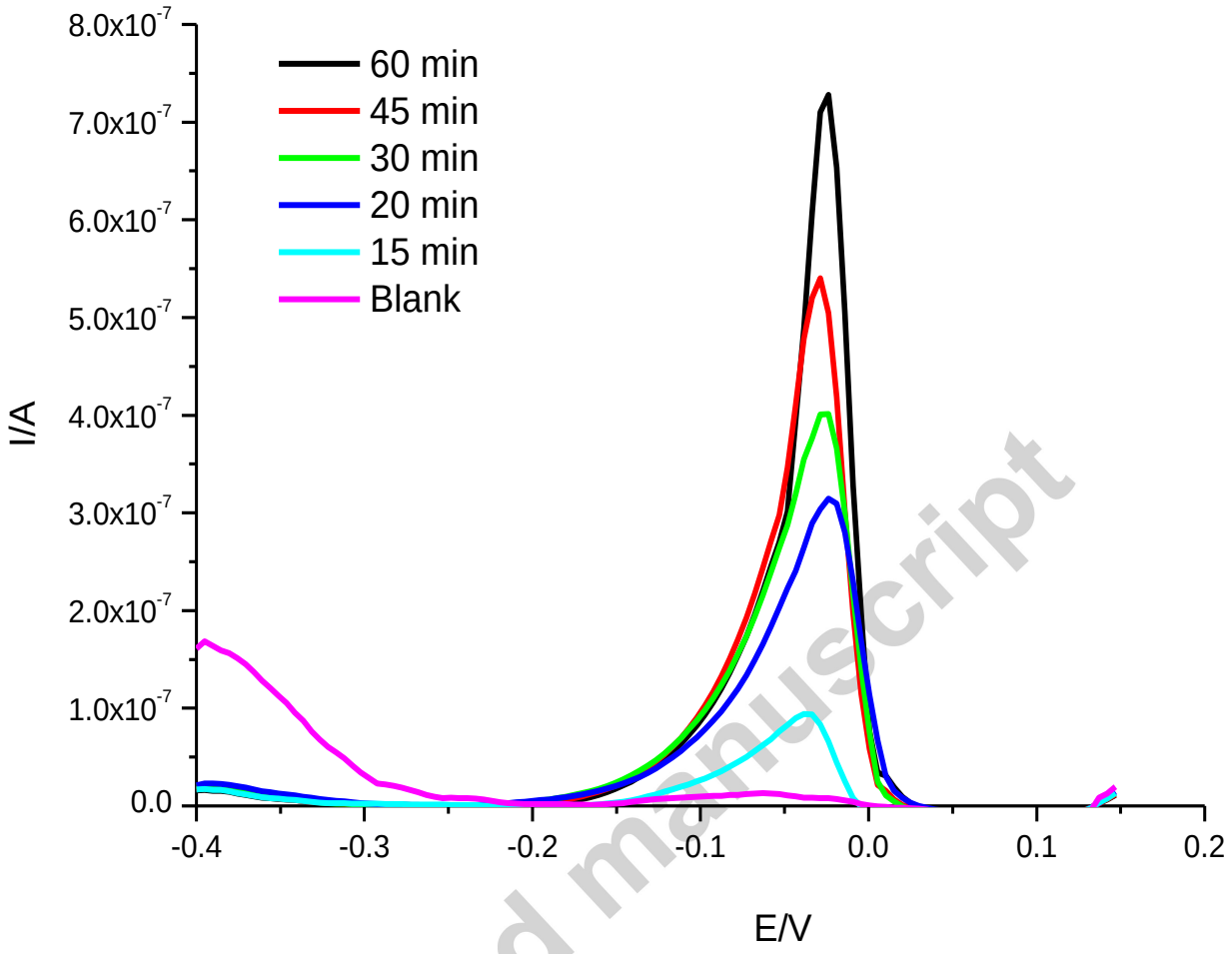


Figure 3: OTA calibration curve using methylene blue modified aptamer biosensors using spacer.

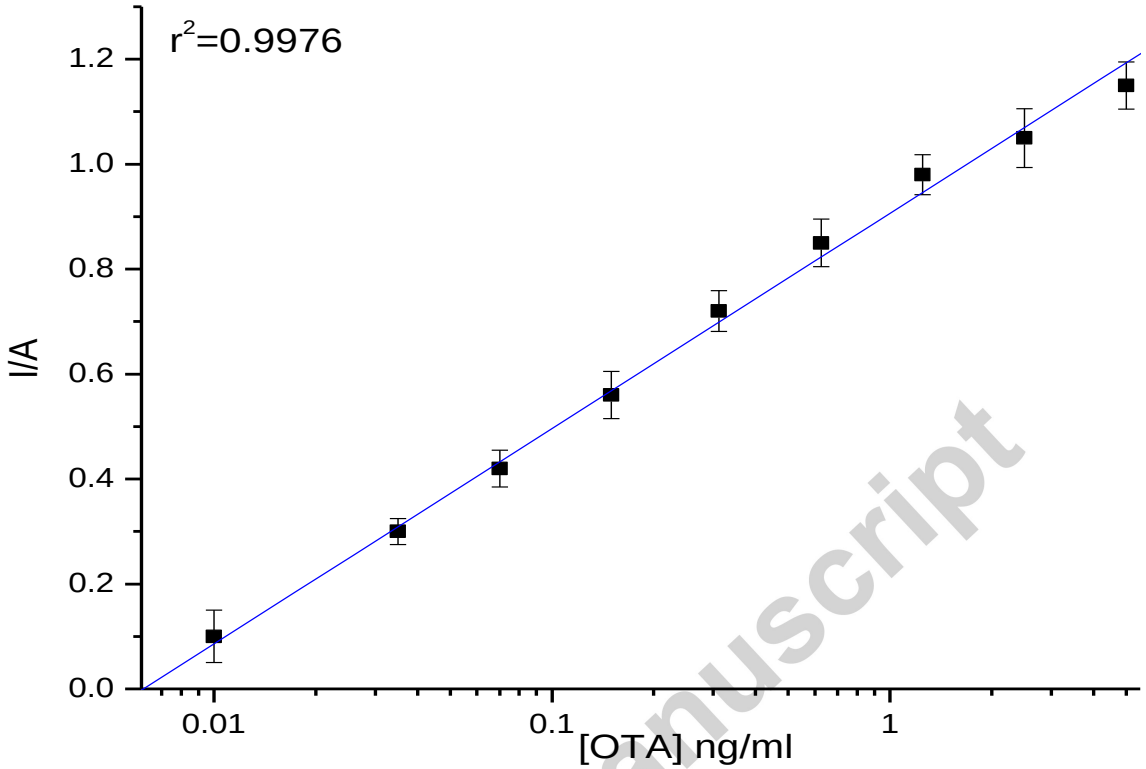


Figure 4: Original response (current changes) of conformational changes upon OTA biosensing using methylene blue modified aptamer.

Table 1: Recovery percentages of spiked OTA in cocoa samples using developed folding based aptasensor.

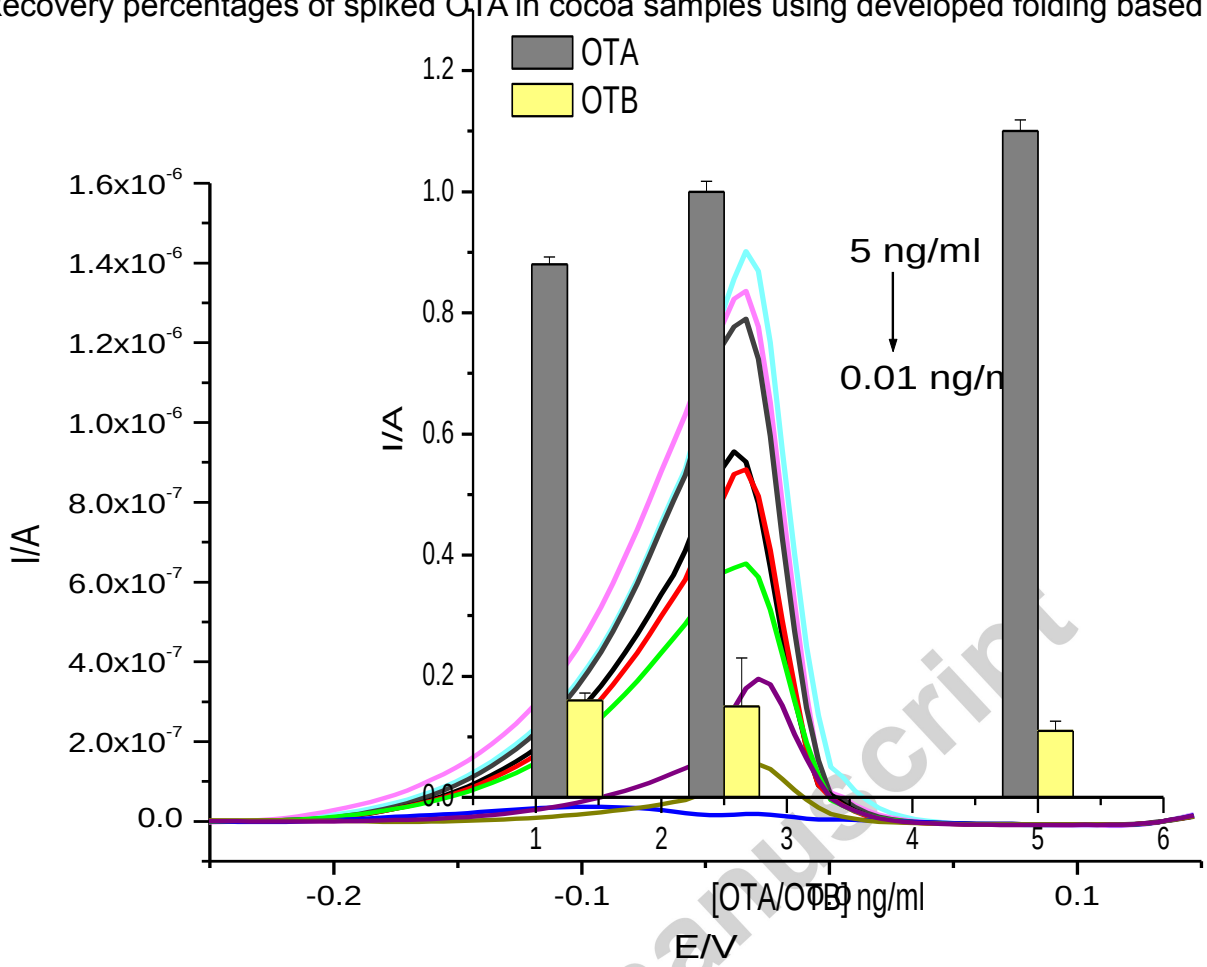


Figure 5: Specificity of the developed folding based aptasensor for OTA detection.

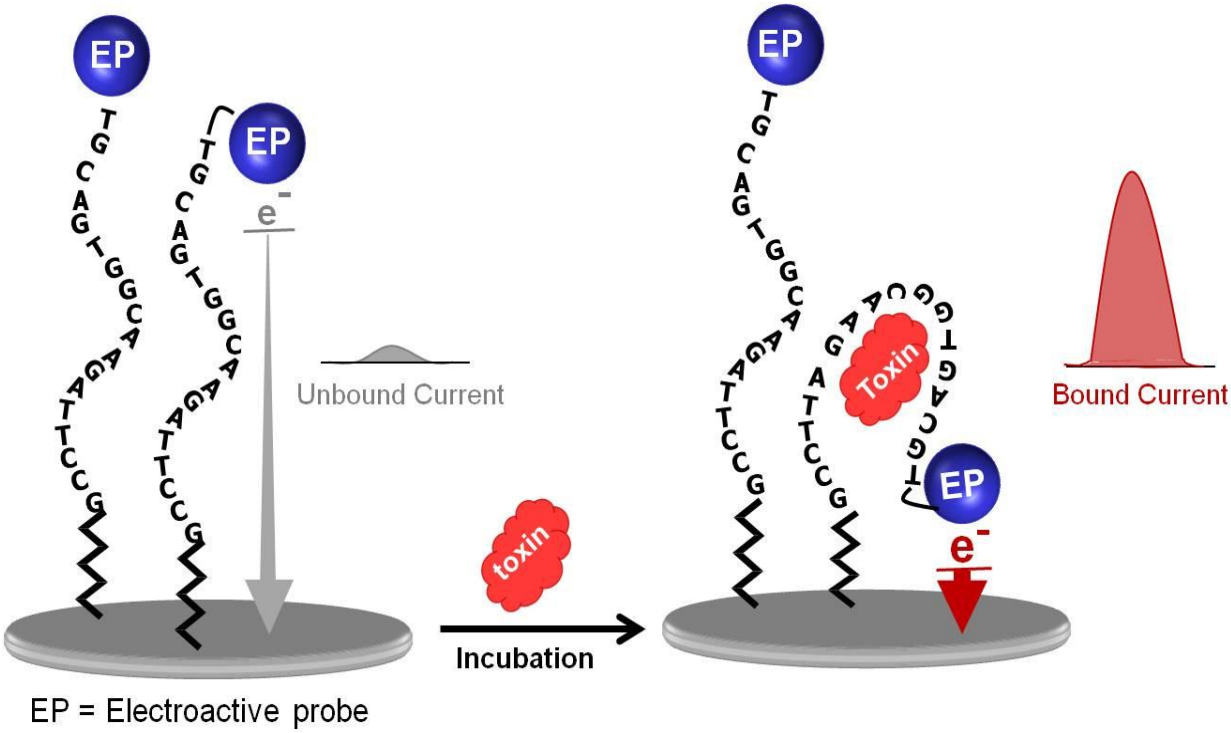
Highlights:-

- First time demonstrated a redox probe labeled aptamer coupling using spacers.
- Easy to perform using click chemistry
- First report of folding based Aptasensor for OTA in cocoa.

Spiked OTA (µg/kg)	Found OTA (µg/kg)	R.S.D.%	Recovery %	A.E.%
10	8.590	6.79	85.98	14.3
5	4.251	4.17	85.02	15.2
2	1.675	3.2	83.79	17.3

Graphical abstract

Graphical Abstract



EP = Electroactive probe