Malaysian Journal of Microbiology, Vol 12(5) Special Issue 2016, pp. 339-344

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Development of ochratoxin A detection based on electrochemical sensor using Au-ball labels

Suttiporn Pinijsuwan[1]*, Patsamon Rijiravanich[2] and Werasak Surareungchai[3]

[1]Food Technology, School of Agro-Industry, Mae Fah Luang University, 333 Moo 1 Thasud Muang, Chiang Rai, Thailand.
[2]National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park Phahonyothib Road Klong 1 Klong Luang, Pathumthani, Thailand.
[3]School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, 83 Moo 8 Thakham Bangkhuntein, Bangkok, Thailand.
Email: suttiporn.pin@mfu.ac.th

ABSTRACT

Aims: The present study was aimed to develop a highly sensitive and rapid method for ochratoxin A (OTA) detection.
Methodology and results: In this study, an electrochemical peptidesensor for OTA detection was developed using numerous number of Au particles coated on the surface of silica particle (Au-ball). Moreover, this assay was performed in 384 well plate, so the multiple detections was done. The synthesized silica particle was spherical in shape and size was 275±17 nm. After coated with Au layer, size of Au-ball was 280±14 nm. Au particles loaded can be taken up resulting in approx. 1×10^7 Au^{3+} molecules per silica particle. Moreover, the optimization conditions were studied. The limit of detection of this assay showed as low as 2 ppb.
Conclusion, significance and impact study: This platform showed rapid, sensitive and specific to ochratoxin A detection. In spite of these advantages, this Au-ball based peptidesensors is suitable to use in the detection of ochratoxin A contaminated in coffee or seed industry.

Keywords: Au-ball labels, Biosensor, coffee, ochratoxin A, peptide

INTRODUCTION

Ochratoxin A (OTA) is a mycotoxin produced by various *Aspergillus* and *Penicillium* strains. It can be contaminate in a widely foods such as cereal grains, dried fruits, roasted coffee and coffee products, as a result of fungal infection in crops, in field during growth, at harvest and in the storage. It has been identified as a carcinogenic, hepatotoxic, teratogenic and immunotixic toxin. This toxin represents a risk for human and animal health when ingested through contaminated food. Recently, there are increasing international attention to the problem of ochratoxin A (OTA) contamination in coffee. Due to the finding many countries have established maximum limits on OTA levels of 5 ppb in coffee and 2 ppb in coffee products (FAO). Therefore, it is necessary to develop a very sensitive analytical method for level of OTA detection to ensure the safety of coffee. Currently, the main techniques that used for determined OTA are high performance liquid chromatography (HPLC), gas chromatography. However, these method have some disadvantages such as laborious, time consuming and expensive.

The develop HPLC method adding with enzyme-linked immunesorbent assays (ELISA) and fluorescence polarization immunoassay (FPIA) were studied (Nakajima *et al.*, 1990). Since the antigen-antibody assay can be used as the selective material for OTA detection, others biomolecules that have immunoaffinity with OTA such as aptamer (Zhang *et al.*, 2012; Rhouati *et al.*, 2013; Zhang *et al.*, 2013) and peptide (Lai *et al.*, 2009; Bazin *et al.*, 2013) can be used as probe for OTA detection. Biosensor for OTA detection was developed using screen-printed electrodes (SPEs) (Alarcon *et al.*, 2004; Radi *et al.*, 2009) modified the direct and indirect competitive immunoassay via the electrochemical method showed the good result lower than 60 ng/mL. Among the different methods that applied in biosensor have been described, immobilization of the biorecognition elements on the supermagnetic particles may have a greater interest. Moreover, in the electrochemical methods using the metal nanoparticles labels is attractive.

In this communication we would like to report the development of an peptidesensor for the detection of OTA that combine the advantages of gold coated onto the surface of silica particles (Au-ball) and the electrochemical detection with rapid assay and specific to OTA detection.

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

MATERIALS AND METHODS

Materials

Tetraethyl orthosilicate (TEOS), ammonium hydroxide, tin (II) chloride dehydrate ($SnCl_2$), gold (III) chloride trihydrate ($HAuCl_4$), Tween-20, ochratoxin A (OTA) and ochratoxin B (OTB) were purchased from Sigma-aldrich. Sodium di-hydrogen phosphate, di-potassium hydrogen phosphate, sodium chloride, potassium chloride were from Merck. Zearalenone (ZEA) was from Fluka. Aflatoxin B2 (AFL) was from Supelco. Bromine was from Panreac and Hydrobromic acid was from Ajax Finechem. Peptide (biotin-GPAGIDGPAGIRC) was from Proteogenix. Monomer avidin magnetic beads (A-beads) with 1 µm diameter was purchased from Bioclone Inc, USA.

Apparatus

Transmission electron microscope (TEM) was carried out with a JEOL model JM-2100. UV visible spectra was recorded using Ocean Optics model USB4000 UV-Vis. An electrochemical experiments were performed using an Autolab PGSTAT 10 computer-controlled potentiostat (Eco Chemic) with GPES software and portable potentiostat of Palm sense Instruments BV, PSTrace 2.5.2.0. The electrode was screen printed carbon with Ag/AgCl track (C-Ag/AgCl SPE) and used for differential pulse anodic stripping voltammetry (DPASV) technique.

Preparation of Sn-Functionalized silica particle (Sn-SiO_2)

The white colloidal silica particle were synthesized using Stober's method by mixed 10 mL of ammonium hydroxide, 7 mL of water, 31 mL of ethanol and 1.7 mL of TEOS (Stober *et al.*, 1968). The solution was stirred overnight. The silica particles solution was added to 500 mL of 2.5 mM $SiCl_2$ and adjust pH to 2.8 with 0.1 M HCl. Sn-SiO_2 particles were then centrifuged and washed eight times to remove excess residuals. The solution was adjusted and controlled the pH to 6 by 0.1 M NH_4OH.

Preparation of gold nanoparticles coated on surface of Sn-SiO_2 (Au-ball)

Briefly, 1.5 mL of 25 mM $HAuCl_4$ was added to 100 mL of water solution contain 25 mg potassium carbonate and incubated in the dark for 1 night. Four different concentration ratios of Sn-SiO_2 and gold particle solution from 1:4 to 1:19 were prepared to control the thickness of gold shell. A 200 µL of 2% (volume in water) formaldehyde solution was added to the mixture solution and incubated for 6 h. The solution was filtered to separate the Au-ball by using 0.45 µm filter paper, washed 2 times. Put the filtrate into the auto desiccator cabinet for 1 night to get the purple powder of Au-ball particles.

Preparation of A-beads/peptide conjugate

Two hundred microliter of A-beads (10 mg/mL) was washed one time with water and twice with phosphate buffer solution. 400 µL of 2 mM biotin was added to the A-beads solution and incubated for 5 min. The solution was washed with regeneration buffer (0.1 M glycine/HCl pH 2.8) and phosphate buffer solution. 30 µL of peptide (0.5 mg/mL) and 4 µL TCEP (1 mM) were added to the A-beads solution and incubated at room temperature for 30-60 min, wash 5 times. Add 100 µL of 2 mM biotin for 5-10 min, washed 2 times. Finally, the soft sediment of peptide-beads were then re-suspended to 10 mg/mL in phosphate buffer solution and kept in 4 °C.

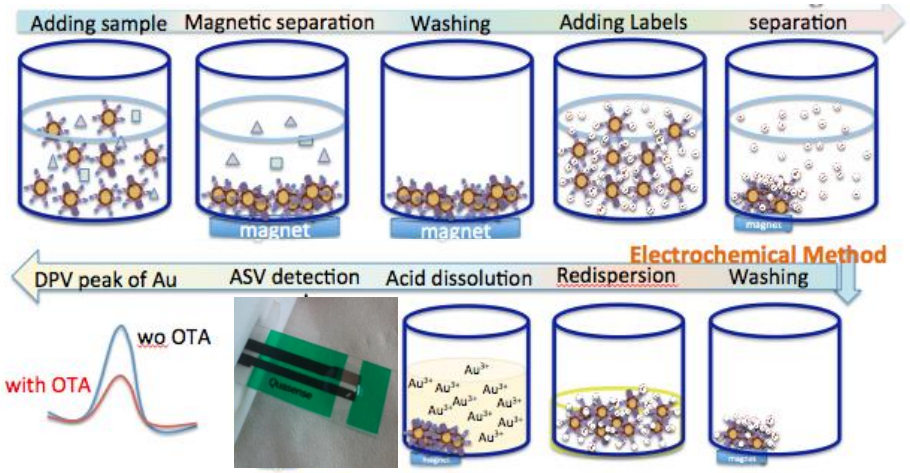

Figure 1: Schematic representation of the OTA assay into 384 well plate using peptide-beads and Au-ball labels. After the labels are attached to the peptide-beads, Au dissolution and quantification by anodic stripping using DPV.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Detection of OTA using Au-ball labels

The assay was performed in 384 well plate. First, 5 µL of peptide-beads (2 mg/mL) were added into the well and washed for 2 times. 50 µL of different concentration of OTA sample were added and incubated at 500 rpm, 35 °C for 1 h. Then, solution was washed the excessed residue using phosphate buffer solution for 3 times. The Au-ball was allowed to react at 500 rpm, 35 °C for 15 min and then again washed off for 5 times. Finally, the gold on Au-ball was dissolved using acid-dissolution/detection medium (1 M HBr/ 0.1 mM Br_2) and DPASV used to detect the released Au^{3+} ions (scan rate = 20 mV/s, t_{dep} = 30 sec, E_{dep} = −0.70 V). The procedure is illustrated in Figure 1.

RESULTS AND DISCUSSION

The Au-ball was synthesized by fabrication of Sn-SiO_2 and coated the surface seeding and shell-growing with gold particles. To monitor the Sn-SiO_2 and Au-ball, TEM was obtained the image results. As shown in Figure 2 (a), the TEM image of Sn-SiO_2 showed spherical in shape. The average size of Sn-SiO_2 particles were 245±17 nm, which was taken from many individual different TEM images. After the $SnSiO_2$ were dried in auto dry cabinet for 1 night, the white color of Sn-SiO_2 powder were observed (Figure 2 (b)). The different concentration ratios

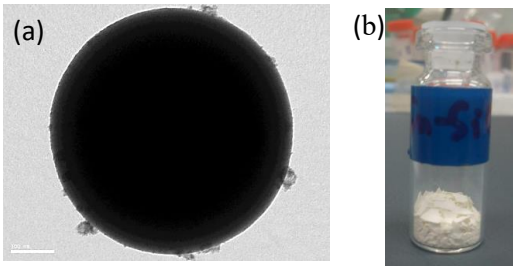

Figure 2: a, TEM image of Sn-SiO_2 and b, powder particle of Sn-SiO_2.

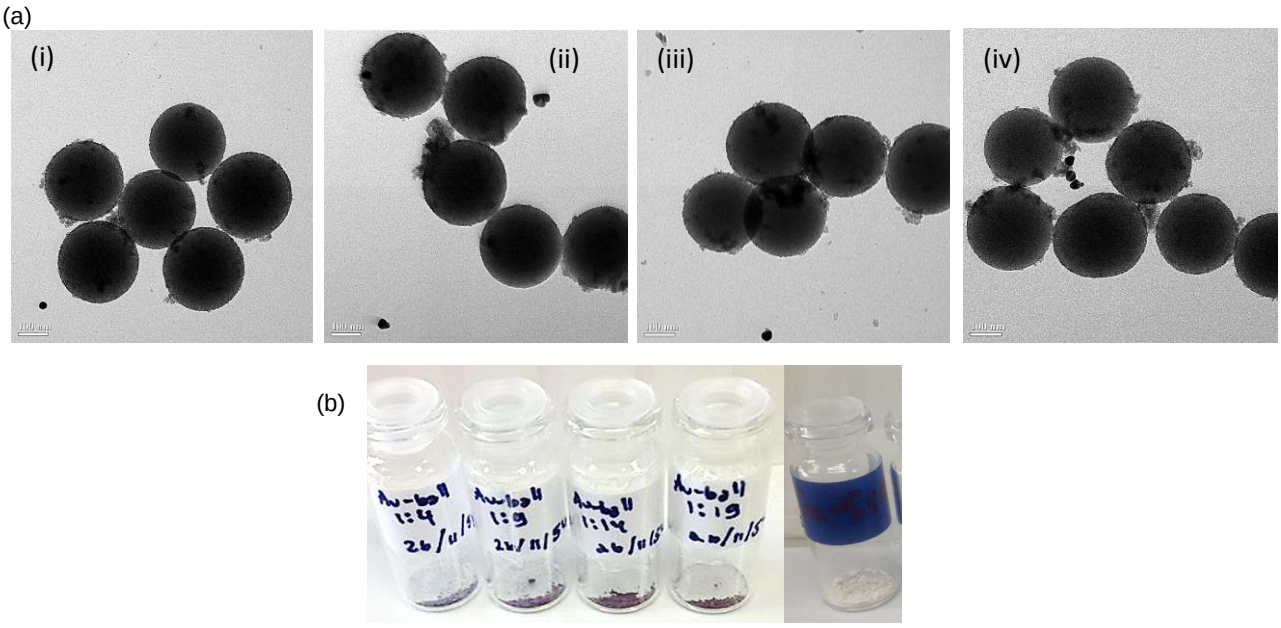

Figure 3: a, TEM image of (i) Au-ball 1:4 (Au-ball4), (ii) Au-ball 1:9 (Au-ball9), (iii) Au-ball 1:14 (Au-ball14) and (iv) Au-ball 1:19 (Au-ball19) and b, powder particle of Au-ball.

of Sn-SiO_2 and gold particle solution from 1:4, 1:9, 1:14 and 1:19 were incubated for 6 h, washed and dried. Then, the characteristic of four concentrations Au-ball were monitored as TEM images as shown in Figure 3a. There were obtained clearly elucidate the formation of the gold shells according to the ratio of Sn-SiO_2 to gold salt solution (Lim *et al.*, 2003). The surface of the Au-ball was roughly when compared with Sn-SiO_2. Moreover, the purple color of Au-ball powder were observed (Figure 3b). The biggest particles was Au-ball14 which showed the average diameter of 280±14 nm, with about 5 nm roughness. The analysis of dissolved Au-ball was performed by DPASV. The optimization of DPASV parameters were studied. The results found that the optimum electrodepositon potential (E_{dep}) was −0.70 V, electrodeposition time (t_{dep}) was 30 sec and scan rate was 20 mV/s (data not show). As shown in Figure 4, the peak current increased with higher ratio concentration of gold solution. The current result found that at Au-ball14 had the highest current. While, there was no current found in Sn-SiO_2. Therefore, the Au-ball14 labels were found to be the most sensitive, and they were used to examine the specificity of the technique.

The analysis of OTA in this technique using peptidesensor. The peptide that specific to OTA was immobilized onto the surface of magnetic particle by

ISSN (print): 1823-8262, ISSN (online): 2231-7538

biotin-avidin conjugated. The detailed principle of our peptidesensor is illustrated in Figure 5. Briefly, peptide was binding with OTA sample in buffer solution and Au-ball labels in 384 well plate. Au particles was dissolved using dissolution/detection medium to release Au^{3+} ions and then determined the current with DPASV method. The results showed into 2 cases. First, in an absence of OTA samples, the structure of peptide could not be change. The binding between Au-ball labels and peptide was occurred via thiol group at the end of Cys of peptide. Since, there were a lot of numerous of Au-ball binding to peptide, the current signal was high. In another case which had OTA sample, the hair-pin binding of OTA-peptide was occurred at the peptide backbone (Heurich *et al.*, 2013). The Au-ball could not be bind to the peptide. Thus, the current signal was decreased. The current signal was inversely to the concentration of OTA. The amount of peptide loaded onto the magnetic beads was studied. The concentration of peptide was varied from 4.4 to 26.6 nmol/mg. The current signal with 25 ppb and without OTA were determined. As shown in Figure 6a, the response found to be optimum at 13.3 nmol/mg. Moreover, the graph plot from ratio of with/without OTA at 13.3 nmol/mg concentration of peptide show the lowest signal (Figure 6b). This optimum concentration of peptide was added and used for quantitative detection of OTA.

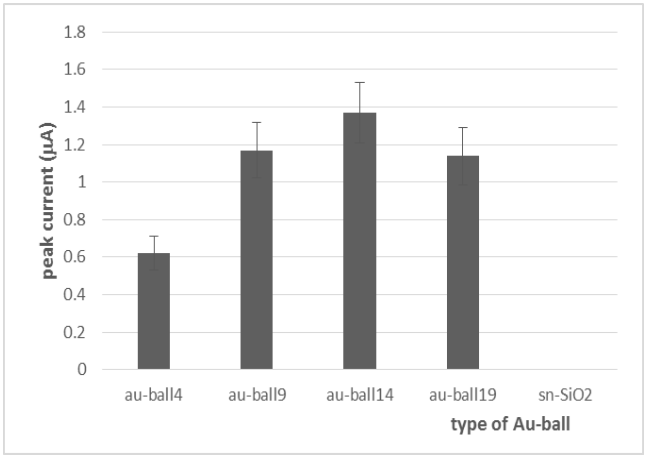

Figure 4: Current signal from DPASV measuring of Au-ball and Sn-SiO_2.

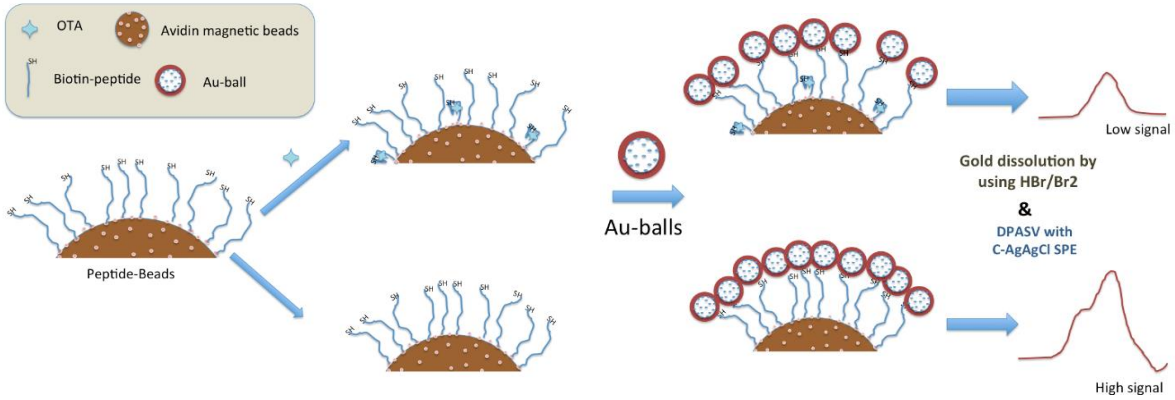

Figure 5: Schematic of peptide-beads used for OTA detection via Au-ball label and DPASV technique.

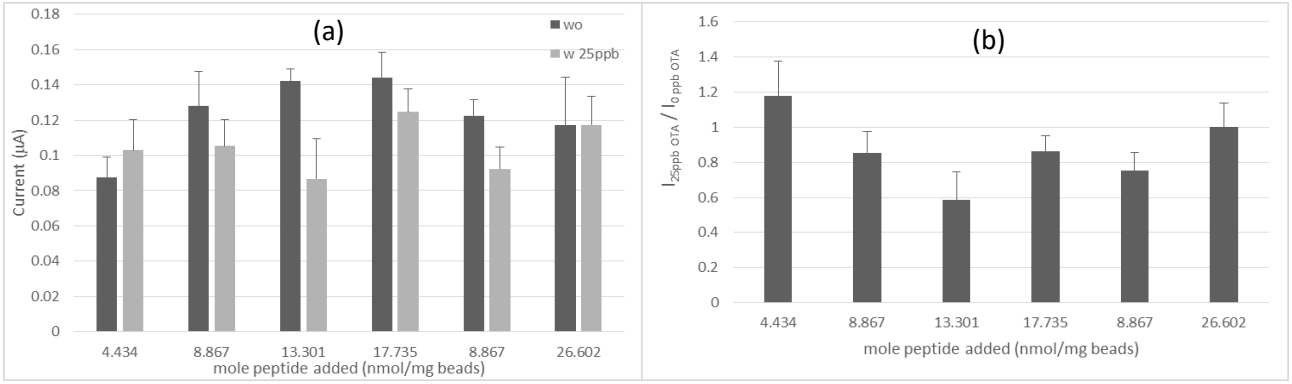

Figure 6: a, comparison of peak current and b, the ratio current between with 25 ppb and without OTA by varied concentration of peptide-beads.

Quantification of the OTA detection was performed by varying the concentration of OTA sample from 0 to 50 ppb in well plate while keeping the concentration of peptide-beads and Au-ball labels constant. The resulting

ISSN (print): 1823-8262, ISSN (online): 2231-7538

calibrations are shown in Figure 7a. The dynamic range of Au-ball label was 2 to 25 ppb. The calibration equation obtained from this curve was y = -0.0163x + 1.0254 with a correlation coefficient of 0.97516 (inset in Figure 7a). Figure 7b showed voltammogram of OTA detection using DPASV technique. The selectivity is another important parameter for the developed peptidesensor In order to evaluate the selectivity of OTA detection, the change of current signal was compared by three other mycotoxins; ochratoxin B (OTB), afratoxin B1 (AFL) and zearalenone (ZEA). As shown in Figure 8, at the same concentration of mycotoxins, the signals were significantly different to the OTA. Therefore, due to the high regcognition ability of OTA peptide, the developer of peptidesensor presented in high selectivity, which was sufficient for the practical application.

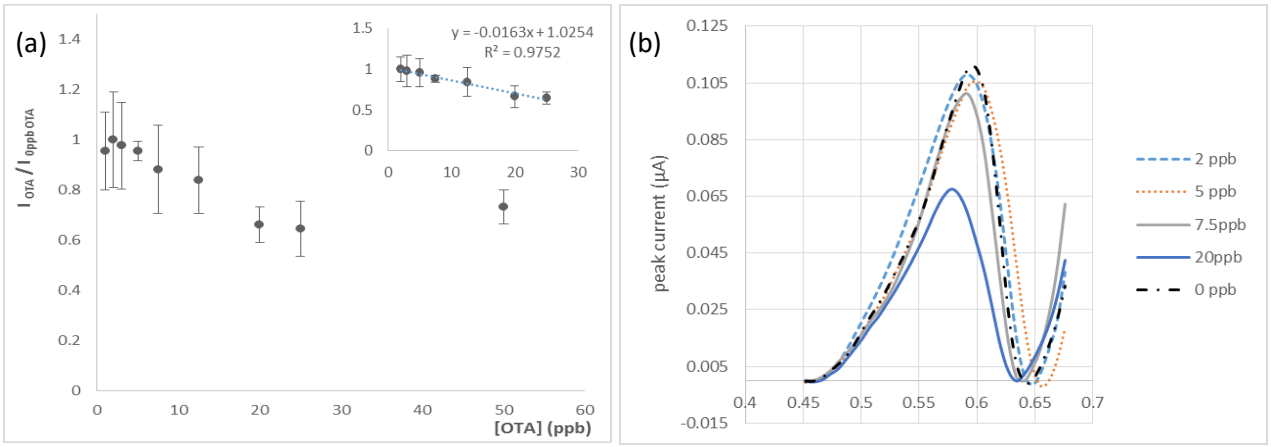

Figure 7: a, Calibration curve of OTA detection using DPASV conditions (scan rate = 20 mV/s, t_{dep} = 30 sec, E_{dep} = −0.70 V) and b, voltammogram of OTA detection by DPASV method.

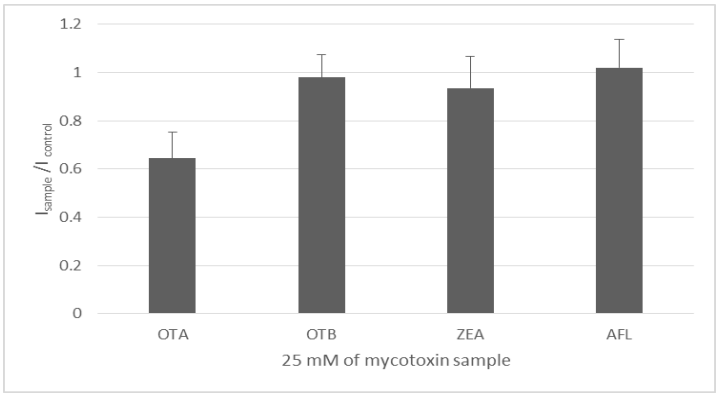

Figure 8: Selectivity of the peptidesensor toward OTA against other mycotoxins (OTB, AFL, ZEA) at the same concentrations (25 ppb).

Table 1: Previous method for detection of OTA.

Modification	Detection Method	Detection Time	Detection Limit	Ref.
Peptide-OTA using anti-mouse HRP and Anti-biotin HRP react with luminol substrate	Chemiluminescence	12 min (Prepared for 2 days)	0.22 μg/L	Sauceda-Friebe *et al.*, 2011
Anti-OTA using OTA-HRP and TMB substrate	Electrochmical detection	1 h	60 μg/L	Radi *et al.*, 2009
Anti-OTA mAb conjugated AuNPs	Lateral flow	n/a	10 μg/L	Lai *et al.*, 2009
Hairpin Anti-OTA aptamer tagged biotin and restriction endonuclease	DPV	1 h 10 min	0.4 μg/L	Zhang *et al.*, 2013
Peptide probe with Au-ball	DPASV	1.5 h	2 μg/L	This work

n/a, not available.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Previous method for detection of OTA are summarized in Table 1 with the relevant detection limit and detection time. It can be seen that the limit of detection achieved here is higher than the peptide-OTA (Sauceda-Friebe *et al.*, 2011) and Hairpin Anti-OTA aptamer (Barthelmebs *et al.*, 2011; Zhang *et al.*, 2013). It might be because this technique use the magnetic assay, some of the signal might be loss at washing step. Moreover, the detection time was 1.5 h. Although we used 384 well plate, we measured the current by potentiostat per one time. Therefore, if we used the multiple potentiostat, the possibility is currently being examined.

ACKNOWLEDGEMENT

The authors gratefully acknowledge a grant from Cluster and Program Management Office (CPMO), National Science and Technology Development Agency (NSTDA), Thailand. The authors would like to thanks Laboratory of sensor technology at BEC, King Mongkut's University of Technology Thonburi and Mae Fah Luang University and all staff for supporting this project.

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 ISSN (print): 1823-8262, ISSN (online): 2231-7538