www.amlett.com, www.vbripress.com/aml, DOI: 10.5185/amlett.2014.5562 Published online by the VBRI press in 2014

Amperometric immuno-sensor for detection of toxin aflatoxin B_1 based on polyaniline probe modified with Mc-IgGs-*a*-AFB_1 antibodies

Keisham Radhapyari[1,2], Raju Khan[1*]

[1]*Analytical Chemistry Division, CSIR-North East Institute of Science & Technology, Jorhat 785006, Assam, India*

[2]*Central Ground Water Board, North Eastern Region, Ministry of Water Resources, Government of India, Tarun Nagar, Guwahati 781005, Assam, India*

[*]Corresponding author. Tel: (+91) 376-2370806, Fax: (+91) 376–2370011; E-mail: khan.raju@gmail.com

Received: 26 December 2013, Revised: 29 January 2014 and Accepted: 23 February 2014

ABSTRACT

An amperometric sensor for detection of toxin aflatoxin B_1 from *aspergillus flavus* based on conducting polyaniline probe using monoclonal anti-aflatoxin B_1 (*Mc-IgGs-a-AFB*$_1$) antibodies after activation with 3% Bovine Serum Albumin (BSA) through electrochemical polymerization has been proposed. The electrode was fabricated by immobilizing Mc-IgGs-a-AFB_1 antibodies molecules onto electrode surface and characterized by cyclic voltammetry (CV), scanning electron microscopy (SEM) and fourier transform infrared spectroscopic (FT-IR) etc. The proposed amperometric immune-sensor has demonstrated excellent electro-analytical properties relative to Aflatoxin B_1 in a linear range from 0.20 to 1.30 *A*ngmL^{-1} with a relatively low detection limit of 0.059 *A*ngmL^{-1}. The present study will help in improving for quantitative determination of mycotoxins in food samples may provide significant improvements in quality control of food safety through a simple, rapid, and sensitive testing system for agricultural products monitoring. Copyright © 2014 VBRI press.

Keywords: Immuno-sensor; polyaniline; bovine serum albumin; environmental toxins; aflatoxin B_1; electrochemical impedance spectroscopy.

Keisham Radhapyari obtained her Ph.D degree in Chemistry in the year 2008 and post-graduation in Environmental Chemistry in 2002 from Jiwaji University, Gwalior, India. She worked at Analytical Chemistry Division, CSIR-NEIST, Jorhat as Young Scientist from November, 2009 to January, 2013. In May, 2013 she joined as Scientist in Central Ground Water Board, Ministry of Water Resources, Govt. of India. Her research interests include pharmaceutical analysis, biosensors, wastewater treatment and analysis, electro-analytical and environmental chemistry.

Raju Khan is working as a Senior Scientist at Analytical Chemistry Division, CSIR-North East Institute of Science & Technology, Jorhat, Assam, Govt. of India. He received his MSc degree in Inorganic Chemistry and PhD in Physical Chemistry from Jamia Millia Islamia, Central University, New Delhi, India, 2002 & 2005, respectively. Thereafter, he worked as a Postdoctoral Fellow at the "Sensor Research laboratory" Department of Chemistry, University of the Western Cape, Cape Town, South Africa, in the year 2005-06 and also worked as a Fast Track Young Scientist Scheme at CSIR-National Physical Laboratory, New Delhi Govt. of India. Dr Khan also worked in the Department of Chemistry; University of Texas at San-Antonio, UTSA, USA during 2010-11 under the awarded BOYSCAST fellowship, Department of Science and Technology, Govt. of India. Dr. Khan has also received the nomination for CSIR Young Scientist Award 2012. Dr. Khan is also engaged in National & International collaborative project, Prague (Czech Republic) & Moscow (Russia) etc. His main current interest in the development of electrochemically sensors based on nano-composites and on conducting polymers etc.

Introduction

Analysis for mycotoxin in foods is a very important practice to ensure food quality and safety and to eliminate and control the risk of consuming contaminated foods. Hence, being able to analyse and detect mycotoxins in foods and drinks is a priority to comply with the legislative limits set by food authorities worldwide. Mycotoxins (fungal toxins) come under the top ten environmental toxins which can cause a range of health problems with exposure to only a small amount. The presence of aflatoxins which are the most studied group of mycotoxins in many food products and the outbreak of serious aflatoxicosis in India in 1974 in which nearly 100 people died from acute aflatoxicosis **[1]** calls for fast and cost-effective analytical techniques to be used in extensive monitoring programs. Aflatoxins are carcinogenic, teratogenic and immunosuppressive secondary metabolites produced by

Aspergillus flavus and *Aspergillus parasiticus*. Among them aflatoxin B_1 (AFB_1) being the most toxic in terms of both acute and chronic toxicoses **[2].** Aflatoxins have been found in several human foods like baby food, peanut and corn products dietary products animal feed, imported wheat and barley, rice, black tea, alcoholic beverages, deep-red ground pepper, edible oils, eggs, milk, meat and meat products **[3-6]** etc. Further, humans are exposed to AFB_1 by ingestion **[7]**. Due to various acute and chronic toxicity of AFB_1 much emphasis has been on the control or elimination of these fungi and/or their toxic metabolites. However, the determination of toxins has not easy due to their being found in complex matrixes and should be detected in low concentrations. This has led to development of various new methodologies, which can significantly differ, from the conventional macro and semi-micro analytical approaches. Considering the severe toxic effects of AFB_1, it is of great importance to develop rapid and sensitive sensing platforms for AFB_1 monitoring to ensure food safety issues and avoid the risk of AFB_1 consumption.

The most commonly used analytical methods for the determination of AFB_1 includes High performance liquid chromatography (HPLC) with various detectors and couple with different cleanup procedures are HPLC-Fluorescence detection **[8-9]** HPLC-UV **[10],** HPLC-FID **[11],** UHPLC-FLD **[12]** HPLC with post-column photochemical derivatization and fluorescence detection **[13],** HPLC-photo diode array detector **[14],** micellar electrokinetic capillary chromatography **[15].** Liquid chromatography couples with tandem mass spectrometry methods (LC-MS) were employed to detect and quantify AFB_1 in various matrixs such as medicinal herbs, human serum, pharmaceuticals, hazelnuts, cereals, lotus seeds, wines, urine **[16-17].** Other various methods adopted for AFB_1 also includes Enzymatic Spectrophotometric Method **[18]**, Conductometric immune-biosensor **[19]**,) and Thin layer chromatography **[20].** Recently, various electrochemical sensors were also given special attention due to their fast, simple, and low-cost detection capabilities for biological binding events **[21-23].** Further, the requirements of both time and costs in most traditional and commonly used analytical methods *viz.* chromatographic methods often constitute an important obstacle for their application on regular basis.

To overcome the above limitations, alternatives to conducting polymer polyaniline modified with antibodies IgGs based immuno-sensors for analytical applications are an area of active research. The design of immuno-sensors is probably one of the most alternative approaches to solve some problems concerning sensitive, fast and cheap measurements. There is considerable interest towards the application of conducting polymers to immuno and biosensors. This has been attributed to their many interesting properties such as biocompatibility, redox characteristics and the possibility of direct electron transfer between electrode and active sites of biomolecules [**24-27**]. Among the various conducting polymers, polyaniline has attracted much attention due to ease of preparation, high conductivity and good stability in environment. However, efforts are being made to find solutions to the problems relating to processability and poor thermal stability of PANI for application of this electrically conducting polymer in aflatoxin B_1 immuno-sensor. In this regard, immuno-sensors detection of AFB_1 based conducting PANI reveals to be a suitable alternative or complementary analytical tool. In the present study deals with the development of AFB_1 immuno-sensor based on electrochemically polymerized PANI modified with monoclonal anti-aflatoxin B_1 (Mc-IgGs-a-AFB_1) antibodies by a one-step immune reaction between the immobilized Aflatoxin B_1. In this first-hand approach, the validated developed methods are simple, selective, and sensitive with effective testing procedures which could provide an affordable and practical means of rapidly monitoring toxicity in agricultural products that will help in improving the quality of life and this is closely related to a better control of diseases, quality and safety of food, and last but not the least, the quality of our environment.

Experimental

Materials

Aflatoxin B1 (AFB_1) from aspergillus flavus and monoclonal anti-aflatoxin B_1 (Mc-IgGs-a-AFB_1) antibodies were procured from Sigma-Aldrich, USA. Aniline (99.5%) and hydrochloric acid (32%) of analytical grade were procured from Sigma-Aldrich, Germany. Bovine serum albumin (BSA; 98% purity) was procured Merck, India. Sodium azide (NaN_3, 99.5% purity) was procured from Sigma-Aldrich, USA. Sodium phosphate dibasic and sodium phosphate mono basic (Na_2HPO_4, 99.95% and $NaH_2PO_4.H_2O$, 98%) were procured Sigma-Aldrich, USA. All the reagents used in the present study were of analytical and molecular biology grade and obtained from Sigma Aldrich.

Preparation of solution

Mc-IgGs-a-AFB_1 solution was prepared in 0.1M phosphate buffer solution (PBS) at pH 7.01 and a 0.15M NaN_3 was used as a preservative. 3% Bovine serum albumin (BSA; 98%) dissolved in PBS was used as the blocking agent. AFB_1 solution was prepared by dissolving in PBS with 10% methanol. Phosphate buffer solution of 200 mL capacity with ionic strength 0.1 in the pH range 2 - 12 were prepared in deionized water by adding appropriately measured amounts of 85% H_3PO_4, KH_2PO_4, Na_2HPO_4, and Na_3PO_4 and used as supporting electrolyte.

Instrumentation

Electrochemical measurements were performed using a Potentiostat/Galvanostat/ZRA (Gamry Reference 3000, United States of America) with Gamry Echem Analyst Software. Platinum electrode, Ag/AgCl (3 M KCl) and a platinum wire were used as working, reference and auxiliary electrode, respectively. Film surface areas for sensor on platinum (Pt) electrode were 3 mm in diameter. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were carried out in a 20 mL Dr Bob's electrochemical cell stand. Alumina micropolish and polishing pads were used for electrode polishing. The FT-IR spectrum of solid complex was recorded using KBr pellets on an IR, spectrophotometer (Spectrum 100 with software version CPU32). The surface morphology of films

was studied using Scanning Electron Microscopy (JEOL-JSM-6390LV).

Electrochemical polymerization of PANI film

Electrochemical polymerization of aniline on platinum (Pt) electrode was carried out by adding 0.2M aniline into 1M HCl solution prepared in 10 mL of de-ionized water (TKA Millipore water system). The active electrode surface area for immono-sensor was 3 mm, which was controlled by placing a physical mask during thin film formation. Prior to the electro-polymerization, the Pt electrode was polished on 1, 0.3 and 0.05 µm alumina slurries (make Buehler-Gamma Micropolish) and then thoroughly rinsed with de-ionized water after each polishing step. Polymerization was achieved in a potentiodynamic mode in 0.2 M aniline per 1 M HCl solution following standard methodology of Olsson and Ogren [**28**]. Based on the optimum increasing trend (8 cycles) of amperometric immune-sensor response the potential was cycled between -0.2 V to 1.1 V at a scan rate of 50 mVs^{-1}.

Immobilization of Mc-IgGs-a-AFB_1 on PANI film

Mc-IgGs-a-AFB_1 solution in 0.1M phosphate buffer solution (PBS) at pH 7.01 was directly immobilized with PANI electrode by adsorption technique (overnight dipping in a special assembled cell to allow the uniform distribution of IgGs on the surface of Pt/PANI matrix). The conditions for the immobilization of the bio-molecules were selected based on prior studies [**29-31**]. The IgGs immobilized electrodes were washed with PBS to remove unbounded sites. Bovine serum albumin (BSA, 98% purity) dissolved in PBS was immobilized for 6 h at 4 ^{o}C to prevent nonspecific interactions of AFB_1 at the surface.

Electrochemical measurements

The detection of the AFB_1, IgGs immobilized working electrodes was placed in 10 mL PBS (0.1 M, pH 7.01 and 0.9% NaCl) and 10 µL volume with different concentrations of AFB_1 was added by stirring for 30s before the measurements. Platinum wire and Ag/AgCl were used as counter and reference electrodes; respectively between the frequency ranges 0.01 and 105 Hz at constant 5mV amplitude and 250 mV initial potential for all the measurements. Cyclic Voltammograms were performed at a scan rate of 5 mVs^{-1} at concentration range of studied AFB_1 ranging from 0.2 to 1.3 $ngmL^{-1}$.

Results and discussion

CV Studies

Fig. 1 described the electro-polymerization of PANI on to the Pt electrode in 0.2 M aniline with 1M HCl solution for eight cycle process at potential window of -0.2 V to +1.1 V with a scan rate of 50 mVs^{-1}. The voltametric peak current and number of cycles as a measure of immuno-sensor response have shown positive relationship up to eight electro-polymerizations and beyond that it demonstrates negativity.

The reversibility in reduction process was investigated by using CV. The cyclic voltammogram behavior of the PANI film in **Fig. 2a** was studied at different scan rate (5 to 40 mVs^{-1}) in PBS (0.1 M, pH 7.01 and 0.9% NaCl). Plot of i_p against $\upsilon^{1/2}$ (**Fig. 2b**) have evidenced significant straight line relationship of Randles-Sevcik nature with positive correlation coefficient of 0.992 supporting mass transport as a means of diffusion [**32**].

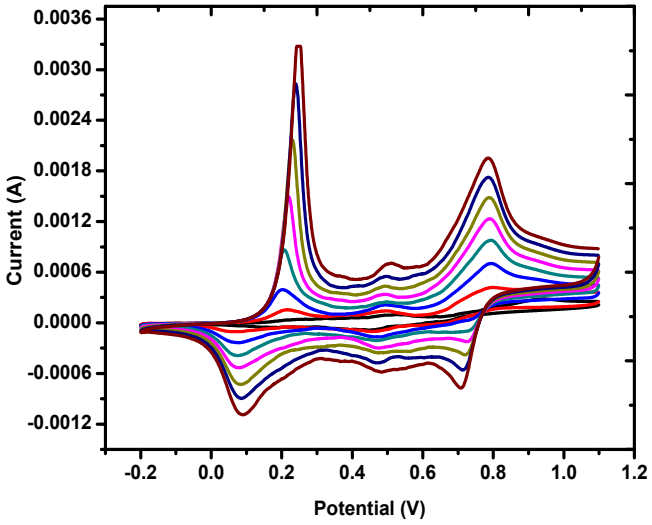

Fig. 1. Electrochemical polymerization of PANI film in 1M HCl on platinum disk electrode at a scan rate of 50 mV s^{-1}.

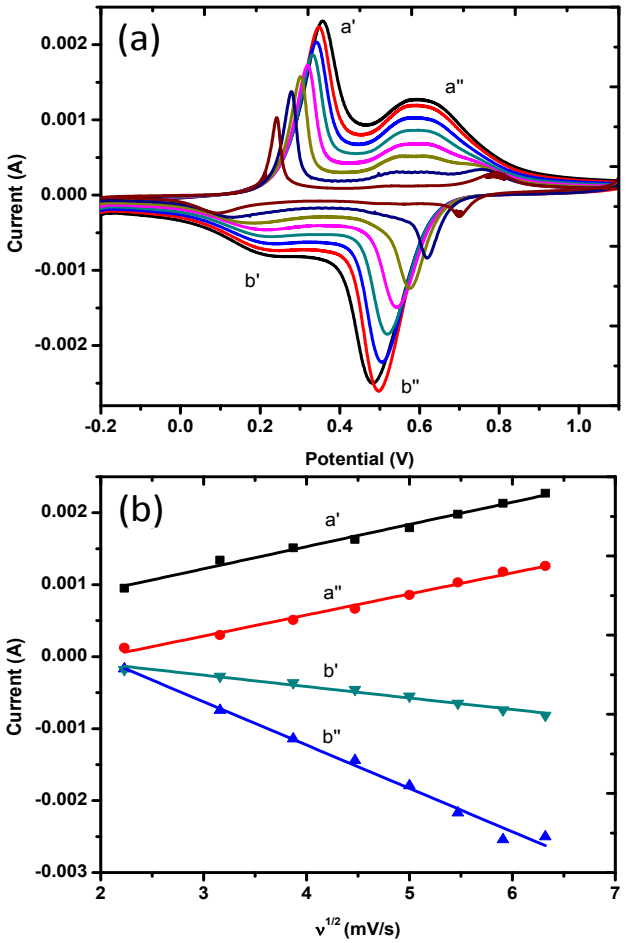

Fig. 2. (a) Cyclic voltammograms of the Pt/PANI in PBS (0.1 M, pH 7.01 and 0.9% NaCl) at different scan rate of 5-40 mV s^{-1} (b) Effect of peak current reduction a', a" and peak current oxidation b', b" scan rates of the 5-40 mV s^{-1}.

Fig. 3 also shows the relative variation in CV (a) Pt/PANI (b) Mc-IgGs-a-AFB$_1$-PANI/Pt electrodes at constant scan rate 5 mVs^{-1} in PBS (0.1 M, pH 7.01 and 0.9% NaCl). During the bio-electrochemical reaction, decrease in peak current in curve b is due to slow redox process and also supported in EIS of Nyquist plot increases the R_{CT} values after immobilization of IgGs on Pt-PANI. Further, peak potential and peak current increases with increasing scan rate suggest diffusion controlled nature of the electrode process. Thus it can clearly be attributed that the polymerization is electro-active in nature and mechanism of diffusion took place through the polymer chain. This has further demonstrated a rapid reversible electron transfer undergoing on the thin film surface of the conducting electro active polymer.

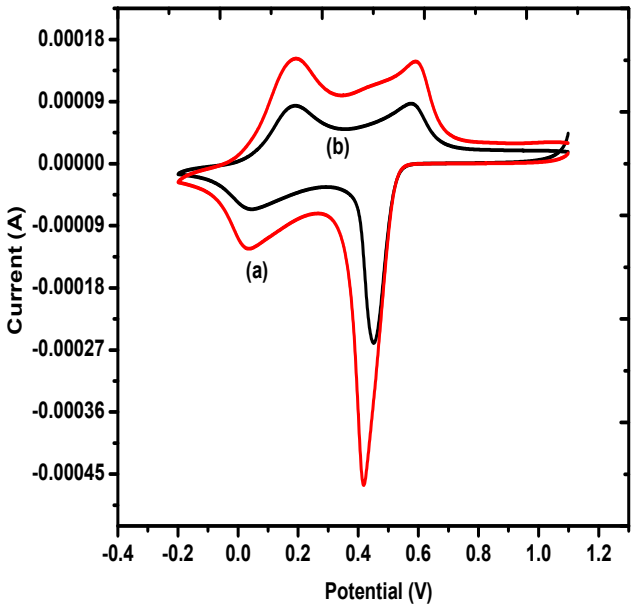

Fig. 3. Cyclic voltammogram in 0.1 M phosphate buffer solution (PBS) containing 0.9% NaCl at a scan rate of 5 mV s^{-1} (a) at Pt/PANI electrode and (b) at Mc-IgGs-a-AFB$_1$-PANI/Pt.

FT-IR studies

FT-IR spectra of electrochemically polymerized PANI films were obtained and characterized (**Fig. 4a**). The absorption bands at 629 cm^{-1} and 790 cm^{-1} are attributed to the C-H vibrations in the benzene ring. The in-plane vibration of C-H bending mode in N=Q=N, Q-N$^+$H-B or B-N$^+$H-B (where Q = quinoid and B = benzenoid) is observed at 1105 cm^{-1} in the FT-IR spectra. The presence of this absorption band is due to the polymerization of PANI i.e., polar structure of the conducting protonated form. In the spectra, bands between 1236 and 1391 cm^{-1} are associated with C-N stretching in aromatic amines and 1568 cm^{-1} due to C=C stretching of benzenoid rings and quinoid rings. Strong peak at 2238 cm^{-1} is associated with -N≡N in diazonium salts. IR band at 2922-3446 cm^{-1} corresponds to N-H stretching with hydrogen bonded amino groups and free O-H stretching vibration. However, the presence of 1627 cm^{-1} peak in the curve (b) of Mc-IgGs-a-AFB$_1$-PANI/Pt immuno-electrode corresponds to amide II band of IgGs exhibiting immobilization of IgGs on electrode **[32].**

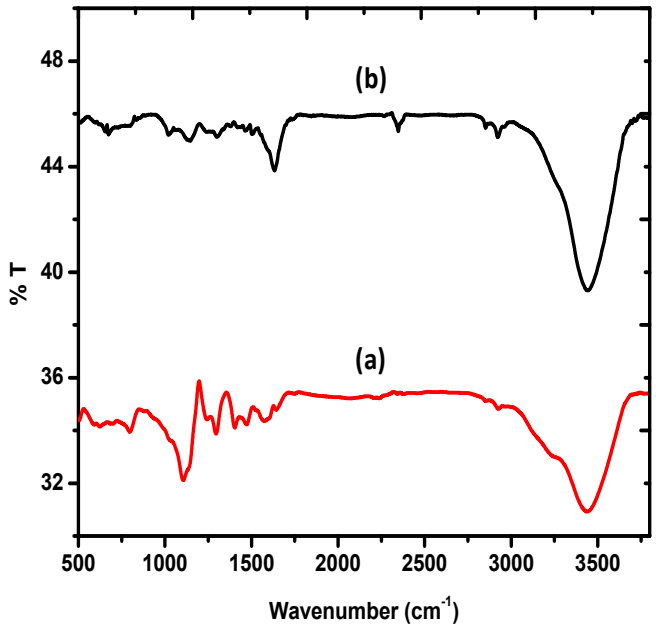

Fig. 4. FT-IR spectra of (a) PANI/Pt (b) Mc-IgGs-a-AFB$_1$-PANI/Pt.

SEM studies

The surface morphologies of PANI, and Mc-IgGs-a-AFB$_1$ film have been investigated using Scanning Electron Microscopy (SEM) (**Fig. 5a-b**), respectively. SEM of PANI film shows porous cage like, rough structure. After the immobilization of Mc-IgGs-a-AFB$_1$ cage like morphology of PANI has been changes into another regular form resulting due to incorporation of Mc-IgGs-a-AFB$_1$. It may be noted that the affinity of Mc-IgGs-a-AFB$_1$ is very strong with in PANI matrices may prevent the linkage of antibodies during the immuno-sensing. The rough surface morphology has added advantage and Mc-IgGs-a-AFB$_1$ are expected to incorporation strongly on the surface of PANI film. The observed change in the morphology of PANI by the incorporation of Mc-IgGs-a-AFB$_1$ may provide effective confirmation for the immobilization of antibodies.

EIS studies

Fig. 6 revealed that electrochemical impedance spectra (EIS) of (a) PANI/Pt and (b) Mc-IgGs-a-AFB$_1$-PANI/Pt electrode, respectively. The charge transfer process in Mc-IgGs-a-AFB$_1$-PANI/Pt immuno-electrode has been studied by monitoring charge transfer resistance (R_{CT}) at the electrode and electrolyte interface. The value of the electron transfer resistance (semicircle diameter) (R_{CT}) depends on the dielectric and insulating features at the electrode/electrolyte interface. The R_{CT} for the PANI/Pt, (0.329 kΩ), and Mc-IgGs-a-AFB$_1$-PANI/Pt (1.218 kΩ), immuno-electrode electrodes have been observed after immobilization of Mc-IgGs-a-AFB$_1$, R_{CT} drastically increased as Mc-IgGs-a-AFB$_1$-PANI/Pt immuno-electrode. The electron transfer of the redox couple is hindered by the presence of Mc-IgGs-a-AFB$_1$ on the electrode surface. The increased R_{CT} value Mc-IgGs-a-AFB$_1$-PANI/Pt immuno-electrode is due to the immobilization of antibodies on the PANI/Pt surface.

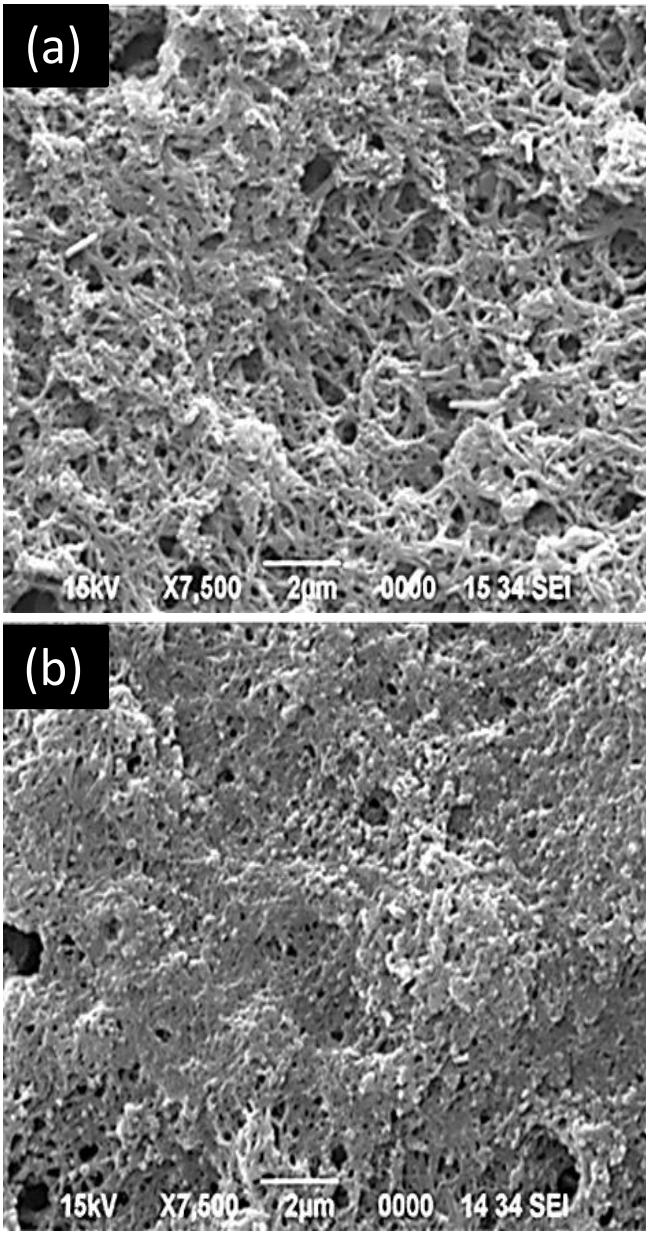

Fig. 5. SEM micrograph of (a) at Pt/PANI electrode and (b) at Mc-IgGs-a-AFB_1-PANI/Pt.

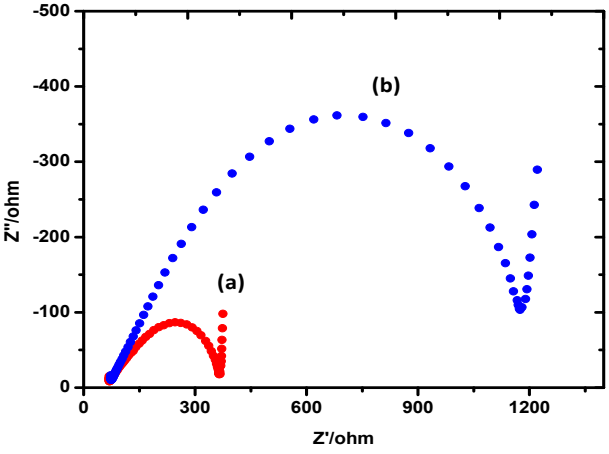

Fig. 6. Electrochemical impedance Nyquist plots of (a) at Pt/PANI electrode and (b) at Mc-IgGs-a-AFB_1-PANI/Pt in 0.1 M phosphate buffer solution (PBS) containing 0.9% NaCl at a scan rate of 5 mV s^{-1}.

Reproducibility and storage stability of the biosensor

In order to check the performance of the Mc-IgGs-a-AFB_1-PANI/Pt immuno-electrode based sensor for determination of AFB_1, reproducibility and storage stability of the immuno-sensor were carried out by keeping the immuno-sensor electrode in the dark at 4°C for 15 days and were analyzed at different times (every day). It has been seen that repeatable peak currents of AFB_1 (0.20 $ngmL^{-1}$) occurred up to 7 days and after that the peak current decreases. Reproducibility and storage stability were calculated in terms of residual standard deviation yielding a value of 1.05% (n = 5) which demonstrated better reproducibility for a comparatively longer period and storage stability at 4 °C.

Application of method for detection of Aflatoxin B_1

The developed amperometric immuno-sensor was successfully applied for detection of AFB_1 in aqueous medium. Voltammograms of AFB_1 in 0.1 M phosphate buffer solution of pH 7.01 containing 0.9% NaCl exhibit well defined cathodic peak at the potential range 0.40 ± 0.05 V. in **Fig. 7a**. The current is due to partial diffusion-adsorption controlled and proportional to the concentration over a convenient range.

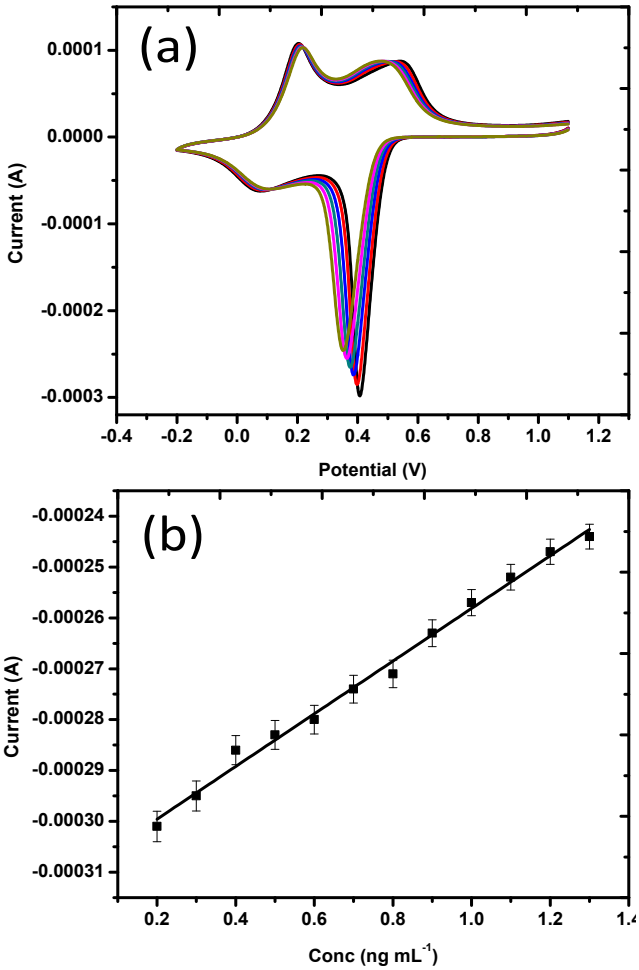

Fig. 7. (a) Immuno-sensor response with increases in concentration of aflatoxin B_1 in 0.1 M phosphate buffer solution (PBS) containing 0.9% NaCl at a scan rate of 5 mV s^{-1} and (b) calibration curve shows linearity for aflatoxin B_1 as 0.20 to 1.30 $ngmL^{-1}$.

In the **Fig. 7b**, a linear relationship has been found over the concentration range 0.20-1.30 ngmL^{-1} of AFB_1. The limit of detection is given by the expression LOD= 3 S.D/m, where S.D. is the standard deviation of replicate determination values and 'm' is the slope of the calibration curve. However, S.D. = 1.0211×10^{-6} and m= 5.1783×10^{-5}, hence LOD= 0.059 ngmL^{-1} with %RSD (1.03±0.05). The LOQ is defined as 10 S.D./m [33], and was found to be 0.197 ngmL^{-1}. Representative voltammograms and calibration curve are shown in (**Fig. 7b**). The obtained result demonstrated good precision of our experimental task.

Conclusion

This study shows that the developed electrochemical immune-sensor is a potentially very useful device that offers the unique possibility to detect and quantify one of the most potent environmental toxins AFB_1. The electro-activity of AFB_1 on antibody-based immuno-sensor on Pt/PANI electrode was developed and studied. The electrochemical behavior of AFB_1 under the conditions described in this work is an irreversible process controlled by diffusion. In spite of the simplicity, the resulting proposed sensor exhibited high sensitivity as 0.059 *A*ngmL^{-1} and good stability, indicating the composite film can retain antibodies activity which exhibited promising application prospect as sensor for AFB_1.

Acknowledgements
All authors are thankful to Director, CSIR-NEIST for his kind permission to publish this paper.

Reference

1. Krishnamachari, K.A.V.R.; Bhat R.B.; Nagarajon V.; Tilek T.B.G. *Lancet*, 1975, 1061
DOI: 10.1016/S0140-6736(75)91829-2
2. Asao, T.; Buchi, G.; Abdel-Kader, M.M.; Chang, S.B.; Wick, E.L.; Wogan, G.N.; *J. Am. Chem. Soc*. **1963,** *85*, 1706.
DOI: 10.1021/ja00894a050
3. Razzazi-Fazeli, E.; Noviandi, C.T.; Porasuphatana, S.; Agus, A.; *Mycotoxin Research*, Vol. 20. **2004.**
DOI: 10.1007/BF02946735
4. Viswanath, Prema.; Nanjegowda, D.K.; Govindegowda, H.; Dattatreya A.M.; Siddappa, V. *Journal Of Food Safety*, **2012,** *32*, 13.
DOI: 10.1111/j.1745-4565.2011.00339.x
5. Yousif, Idris M.A.; Abdalbasit, A.; Mariod, Elnour I.A.; Mohamed, A.A, *Food and Chemical Toxicology*, **2010,** *48*, 2539.
DOI: 10.1016/j.fct.2010.05.021
6. Saqer, Herzallah, M.; *Food Chemistry*, **2009,** *114*, 1141.
DOI:10.1016/j.foodchem.2008.10.077
7. Miraglia, M.; Brera, C.; Colatosti, M.; *Microchem J.* **1996,** *54*, 472.
DOI:10.1006/mchj.1996.0124
8. Sheijooni-Fumani, N.; Hassan J.; Seyed, R. Yousefi, *J Sep Sci* **2011,** *34*, 1333.
DOI: 10.1002/jssc.201000882
9. Afsah-Hejri, L.; Jinap S.; Arzandeh S.; Mirhosseini, H.; *Food Control*, **2011,** *22*, 381.
DOI: 10.1016/j.foodcont.2010.09.007
10. Curticapean, A.; Toma, Felicia.; Tarcea, M.; Curticapean.; Samarghitan, V.; Pop, IA.; Gulea A, *Croat. Chem. Acta*, **2011,** *84*, 413.
DOI: 10.5562/cca1788
11. Jinap, S.; De Rijk, T.C.; Arzandeh, S.; Kleijnen, H.C.H.; Zomer, P.; Van der Weg, G.; Mol, J.G.J. *Food Control*, **2012,** *26*, 42.
DOI: 10.1016/j.foodcont.2011.12.007
12. Ibanez-Vea, M.; Corcuera, L.A.; Remiro, R.; Murillo-Arbizu, M.T.; Gonzalez-Peias, E.; Lizarraga, E. *Food Chemistry*, **2011,** *127*, 351.
DOI: 10.1016/j.foodchem.2010.12.157
13. Quinto, M.; Spadaccino, G.; Palermo, C.; Centonze, D. *Journal of Chromatography A*, **2009,** *1216*, 8636.
DOI: 10.1016/j.chroma.2009.10.031
14. Eshaghi, Z.; Sorayaei, H.; Samadi, F.; Masrournia, M.; Bakherad, Z.; *Journal of Chromatography B*, **2011,** *879*, 3034.
DOI: 10.1016/j.jchromb.2011.08.042
15. Arroyo-Manzanares, N.; Gamiz-Gracia, L.; Garcia-Campan, A.M.; Soto-Chinchilla, J.J.; Garcia-Ayuso, L.E. *Electrophoresis*, **2010,** *31*, 2180.
DOI: 10.1002/elps.201000062
16. Tamura, M.; Uyama, Atsuo.; Mochizuki N, *Anal. Sci.* **2011,** *27*, 629.
DOI: 10.2116/analsci.27.629
17. Perez-Ortega, P.; Gilbert-Lopez, B.; Garcia-Reyes, J.F. Ramos-Martos, N.; Molina-Diaz, A. *Journal of Chromatography A*, **2012,** *1249*, 32.
DOI: 10.1016/j.chroma.2012.06.020
18. Arduini, F.; Errico, I.; Amine, A.; Micheli, L.; Palleschi, G.; Moscone, D.; *Anal. Chem.* **2007,** *79*, 3409.
DOI: 10.1021/ac061819j
19. Liu, Y.; Qin, Z.; Wu, X. Jiang, H. *Biochem. Eng. J.* **2006,** *32*, 211.
DOI: 10.1016/j.bej.2006.10.003
20. Jarvis, B.B.; Miller, J.D.; *Appl. Microbiol. Biotechnol.* **2005,** *66*, 367.
DOI: 10.1007/s00253-004-1753-9
21. Sun, A.L.; Qi, Q.A.; Dong, Z.L.; Liang, Ke Z. *Sens. & Instrumen. Food Qual*. **2008,** *2*, 43.
DOI: 10.1007/s11694-008-9040-6
22. Nagwa, H.S.; Ammida, L.M.; Palleschi, G. *Anal. Chimica Acta*, **2004,** *520*, 159.
DOI: 10.1016/j.aca.2004.04.024
23. Delmulle, B.S.; De Saeger, S.M.D.G.; Siba, L.; Barna-Vetro, I.; Van Peteghem, C.H.J. *Agric. Food Chem.* **2005,** *53*, 3364.
DOI: 10.1021/jf0404804
24. Karimian, N.; Zavar, M. H. A.; Chamsaz, M.; Turner, A.P.F.; Tiwari, A. *Electrochem. Commun*. **2013,** *36*, 92.
DOI: 10.1016/j.elecom.2013.09.014
Parlak, O.; Turner, A.P.F.; Tiwari, A. *Advanced Materials*, **2014**, *26*, 482.
Parlak, O.; Tiwari, A.; Turner, A.P.F.; Tiwari, A. *Biosensors and Bioelectronics*, **2013**, *49*, 53.
25. Kamyabi, M.A.; Hajari, N.; Turner, A.P.F.; Tiwari, A. *Talanta*, **2013,** *116*, 801.
DOI: 10.1016/j.talanta.2013.07.068
Tiwari, A.; Singh, S.P. *Journal of Applied Polymer Science*, **2008**, *108*, 1169.
26. Soylemez, S.; Kanik, F.E.; Ileri, M.; Hacioglu, S.O.; Toppare, L. *Talanta* **2014,** *118*, 84.
DOI: 10.1016/j.talanta.2013.10.007
27. Foxx, D.; Kalu, E.E.; *Electrochem. Commun*. **2007,** *9*, 584.
DOI: 10.1016/j.elecom.2006.10.021
28. Olsson, B.; Ogren, L. *Anal. Chim. Acta* **1983,** *145*, 87.
DOI: 10.1016/0003-2670(83)80050-6
29. Khan, R.; Dhayal, M. *Biosens. Bioelectron*. **2009,** *24*, 1700.
DOI: 10.1016/j.bios.2008.08.046
30. Khan, R.; Dey, N.C.; Hazarika, A.K.; Saini, K.K.; Dhayal, M. *Anal. Biochem*, **2011,** *410*, 185.
DOI: 10.1016/j.ab.2010.11.013
31. Khan, R.; Dhayal, M. *Electrochem. Commun*. **2008,** *10*, 263.
DOI: 10.1016/j.elecom.2007.12.001
32. Giacomelli, C.E.; Mremer, M.G.E.G.; Norde, W. *J. Colloid Interf. Sci.* **1999,** *220*, 13.
DOI: 10.1006/jcis.1999.6479
33. Alonso-Lomillo, M.A.; Kauffmann, J.M.; Arcos Martinez, M.J. *Biosens. Bioelectron*. **2003,** *18*, 1165.
DOI: 10.1016/S0956-5663(02)00251-8