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Lab-on-a-chip based biosensor for the real-time detection of aflatoxin

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ABSTRACT

Polymers were synthesized and utilized for aflatoxin detection coupled with a novel lab-on-a-chip biosensor: MiSens and high performance liquid chromatography (HPLC). Non-imprinted polymers (NIPs) were preferred to be designed and used due to the toxic nature of aflatoxin template and also to avoid difficult clean-up protocols. Towards an innovative miniaturized automated system, a novel biochip has been designed that consists of 6 working electrodes (1 mm diameter) with shared reference and counter electrodes. The aflatoxin detection has been achieved by a competition immunoassay that has been performed using the new biochips and the automated MiSens electrochemical biosensor device. For the assay, aflatoxin antibody has been captured on the Protein A immobilized electrode. Subsequently the sample and the enzyme-aflatoxin conjugate mixture has been injected to the electrode surfaces. The final injection of the enzyme substrate results in an amperometric signal. The sensor assays for aflatoxin B1 (AFB1) in different matrices were also performed using enzyme link immunosorbent assay (ELISA) and HPLC for confirmation. High recovery was successfully achieved in spiked wheat samples using NIP coupled HPLC and NIP coupled MiSens biosensor [2 ppb of aflatoxin was determined as 1.86 ppb (93% recovery), 1.73 ppb (86.5% recovery), 1.96 ppb (98% recovery) and 1.88 ppb (94.0% recovery) for immunoaffinity column (IAC)-HPLC, NIP-HPLC, Supel™ Tox SPE Cartridges (SUP)-HPLC and NIP-MiSens, respectively]. Aflatoxin detection in fig samples were also investigated with MiSens biosensor and the results were compared with HPLC method. The new biosensor allows real-time and on-site detection of AFB1 in foods with a rapid, sensitive, fully automated and miniaturized system and expected to have an immense economic impact for food industry.

1. Introduction

Mycotoxins are secondary metabolites mainly produced by filamentous fungi on various food and feedstuffs. Among mycotoxins, aflatoxins (AFs) are considered to be the most toxigenic fungal metabolites with carcinogenic, mutagenic, teratogenic and immunosuppressive effects. Aflatoxin is one of the most common mycotoxins in grains. The occurrence of AFs is influenced by certain environmental factors; hence, the extent of contamination varies with geographic location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during preharvest, storage, and/or processing periods [1,2]. Four types of AFs including B1, B2, G1, and G2 commonly occur in the natural environment even though 20 different AFs have been identified [3]. Aflatoxin B1 was classified as Group 1 carcinogen (carcinogenic to humans) by the International Agency for Research on Cancer (IARC) [4]. Aflatoxins have been found in grains and herb medicines in humid conditions [2]. European Commission has established maximum limits for AFs, due to their frequent presence in food and foodstuff and to minimize their toxic and health hazards effect in animals and humans. The permissible limits for AFB1 and total AFs are permitted 2 $\mu g\,kg^{-1}$ and 4 $\mu g\,kg^{-1}$, respectively in all cereals and products derived from cereals, including processed cereal products [5,6]. With the increase of regulations for acceptable levels of AFs in place, modern analytical methods have become highly sophisticated with high

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precision and accuracy, suitable for regulatory laboratories and for post-harvest sample testing in developed countries.

Solid-phase extraction (SPE) has appeared as a simple alternative to be coupled with a variety of methods for aflatoxin detection to provide easy and cost effective techniques [7]. The applications of SPE can be widely seen also in imprinting technology as it offers simple and successful clean up procedures. Molecularly imprinted polymers (MIPs) have increasingly been introduced in many analytical procedures, mainly due to their high selectivity in comparison to other stationary phases. MIPs have attracted significant attention as substitutes for natural receptors in chromatography, sensors and assays [8,9]. The reason for this is coming from the fact that molecular imprinting is arguably the most generic and cost effective technique for preparing synthetic receptors. Imprinted polymers have several important advantages as compared to natural receptor molecules: (i) polymers can be prepared for practically any compound; (ii) they have similar affinity to natural biomolecules but often have better specificity; (iii) polymers can work in organic solvents and they are stable at low/high pHs, pressure and temperature [7].

Most of the current methods for quantitative AFs determination include chromatographic methods such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and recently liquid chromatography tandem mass spectrometry (LC-MS/MS), suitable for use in regulatory laboratories [10]. Several immunology based semi-quantitative and qualitative methods including enzyme linked immunosorbent assays (ELISAs) were also developed. The novel AFs detection systems for rapid screening and detection in field and laboratory applications are dip-stick kits [11], optical-based sensing methods (e.g. hyperspectral imaging and electronic noses) [12], and biosensors [13]. Detection for regulatory purposes requires the development of validated official analytical methods. High-performance liquid chromatography (HPLC) [14], thin-layer chromatography (TLC) [15], surface plasmon resonance (SPR) and enzyme-linked immunosorbent assay (ELISA) [16] are some of the current methods for the confirmatory and quantitative determination of AFs in contaminated samples. However, these techniques require qualified personnel, time consuming sample pre-treatment and assay procedure and expensive laboratory equipments. These tests also

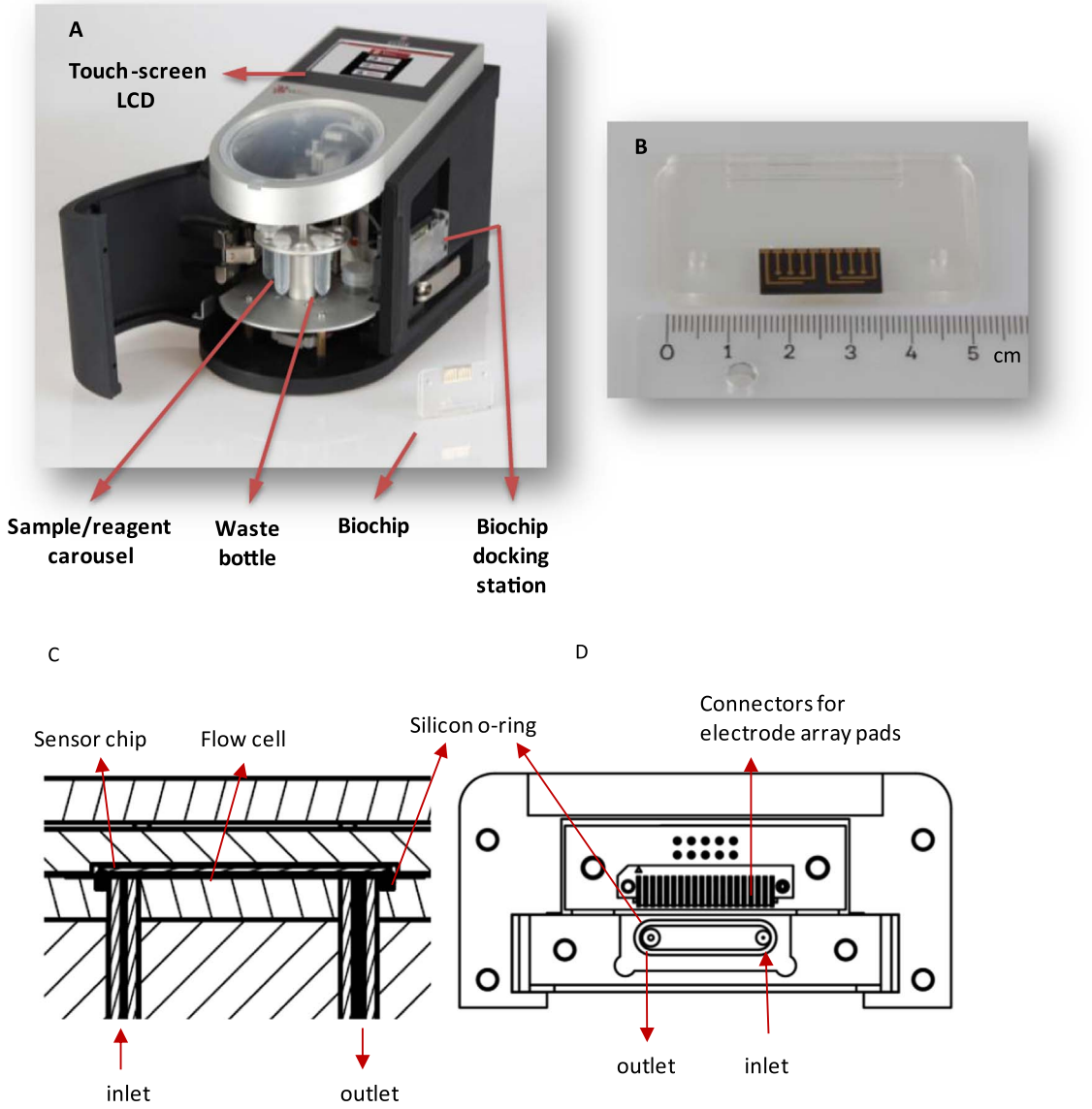

Fig. 1. A novel biosensor (A) and its sensor chip (B) designed and manufactured in-house for rapid and reliable detection of aflatoxin. The placement of the biochip to the docking station creates a microfluidic channel on electrodes and provides the electrode connection to the device (C-D).

entail sample transportation from storage to the laboratories that cause increased waiting time and high costs. Besides all the conventional techniques, sensor technologies and lab-on-a-chip applications have brought the miniaturization of the devices and multiplex testing in the field of chemical analysis. Thus, biosensor technology has the potential to be used for mycotoxin detection in crops. As the global food safety testing market by contaminants is estimated to grow at an annual growth rate of 10.46% to $2.5 billion in 2015, the need for easy and cost-effective testing solutions increase [17]. Here we introduce a new biosensor platform for the detection of AFB1 based on real time electrochemical profiling (REP™) assay method (Fig. 1) coupled with the use of natural and artificial antibodies (polymers) as affinity ligands. Sample clean-up was performed with aflatoxin-targeted polymer and the samples were then analyzed using natural antibody-based MiSens biosensor assay.

The sensor has abilities such as low cost unique sensor chip, simple instrumentation, automatization, high sensitivity, rapidity and applicability to a wide range of analytes. It relies on the fundamental basics of electrochemical immunosensing, in particular amperometry. With a new electrode array and a novel microfluidic channel design, all the steps of the assay including the immobilization, binding and amperometric measurements have been performed during the fluid flow. Compared to the conventional immunoassay platforms such as ELISA that takes 2–3 h, this technique provides automated and fast assay period in 25 min.

2. Materials and methods

2.1. Materials and reagents

Phosphate buffered saline (PBS, 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4) tablets and AFB1 standard were purchased from R-Biopharm Rhone Ltd. (Glasgow, UK). Mercaptoethanol, mercaptoundecanoic acid (MUDA), ethanolamine, spectrophotometric grade ethanol, horse radish peroxidase (HRP), 3,3′,5,5′-Tetramethylbenzidine (TMB) ready to use reagent (contains H_2O_2), hydrochloric acid (HCl), N-hydroxysuccinimide (NHS), HPLC grade acetonitrile (ACN), methanol (MeOH), N,N-dimethylformamide (DMF), dichloromethane (DCM), ethylene glycol dimethacrylate (EGDMA), itaconic acid (IA), N,N-methylenebisacrylamide (MBAA), Methacrylic acid (MAA), 1,1-azo-bis(cyclohexane carbonitrile) (ACCN), N,N′-bis(acryloyl)cystamine (BAC), benzyl diethyldithiocarbamate (BDDC) and potassium ferrocyanide were purchased from Sigma-Aldrich (Poole, UK). 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and Protein A was purchased from Thermo Scientific (Istanbul, Turkey). AFL-HRP conjugate and AgraQuant aflatoxin test kit were obtained from Romer Labs (Tulln, Austria). Potassium chloride (KCl) was purchased from Fisher Scientific (Loughborough, UK). Oxygen free argon was purchased from Habas (Istanbul, Turkey). Ultrapure water (18 MΩ cm^{-1}) was obtained from a Milli-Q water system (Millipore Corp., Tokyo, Japan).

2.2. Apparatus and equipment

During the polymer production process the incubator (Thermo Scientific, Istanbul, Turkey), UV device (Philips, LSI/Lightech), test sieves (63 μm and 125 μm) (Retsch, Haan, Germany), and Soxhlet extraction apparatus (Isolab, Istanbul, Turkey) were used. For MIP extraction 12-vial vacuum manifold and SPE cartridges with fritted poly-ethylene diskettes at the bottom and top (Agilent VacElut 12, Agilent technologies, CA, USA) were used. The HPLC analysis was performed on a 10AVP HPLC system (Shi-madzu, Milan, Italy). The reversed phase HPLC analysis was performed on a 10AVP HPLC system equipped with a RF-10AXL fluorescence detector set for wavelengths 360 nm excitation and 420 nm emission (Shimadzu, Milan, Italy) using post-column derivatization (Kobra Cell, 100 μA) (R-Biopharm Rhone Ltd., Glasgow, UK) with an analytical column (ACE, C18 250 × 4.6 mm, 5 μm, 100 A). MicroStat 8000 electrochemical analyzer was employed for cyclic voltammetry (CV) and amperometric measurements with the general purpose electrochemical software Dropview 1.4 (Dropsens, Oviedo, Spain). This electrochemical analyzer enables simultaneous electrochemical measurements of 6 electrodes.

2.3. Synthesis of NIPs and cartridge preparation

The polymers were manufactured according to the procedure reported by Piletska [18] with minor modifications and in the absence of template molecule. This novel non-imprinted polymer (NIP) designed for aflatoxin was prepared as following: N,N-methylenebisacrylamide (MBAA, 331.5 mg, 2.15 mmol) was dissolved in 3287 μl DMF, and mixed with EDGMA (6300.4 mg, 15.9 mmol) and 1,1-azo-bis(cyclohexane) (46.08 mg). After ultra-sonication for 5 min, the monomer mixture was purged with Argon for 7 min and sealed. It was polymerized by UV for 45 min and then was incubated at 75 °C in the oven 24 h for polymerization. The polymer monoliths were ground using a manual mortar and then sieved. Polymer fraction with size 63–125 μm was collected. The particles were washed using Soxhlet extraction.

2.4. Preparation of the food samples

Wheat: Artificially contaminated 50 g wheat sample was prepared by spiking of 0.1 ml and 0.02 ml AFB1 stock standard solution (1 μg ml^{-1}) in benzol to obtain final concentrations 2 ng g^{-1} and 0.4 ng g^{-1} in wheat, respectively. It was allowed to dry overnight at ambient temperature in the fume hood prior to extraction. *Dried fig:* Naturally contaminated dried fig samples were used for sample preparation. AFB1 concentrations of the samples were in the range of 5.7–8.1 ng g^{-1} according to reference IAC-HPLC method.

2.5. Extraction procedure for food samples

Wheat: 50 g samples were mixed with distilled water (100 ml), MeOH (150 ml) and sodium chloride (5 g), and blended at high speed for 3 min. The slurry were transferred to 500 ml erlenmeyer flask containing 250 ml distilled water and mixed with magnetic stirrer for 1 min. After mixing, the slurry was filtered through filter paper (Whatman 4). *Dried fig:* 50 g samples were mixed with 60 ml of distilled water, 240 ml MeOH and 5 g of sodium chloride and blended at high speed for 3 min. After mixing, the slurry was filtered through filter paper (Whatman 4). The crude extracts were filtered (syringe filter 0.44 μm, Millipore MF membrane) and later used for the biosensor detection assay.

2.6. Clean-up sample with solid phase extraction (SPE)

MBAA polymer particles (100 mg) were packed into 1 ml SPE cartridges with fritted polyethylene diskettes at the bottom and top and they were placed in a 12-vial vacuum manifold (Agilent VacElut 12, Agilent technologies) connected to a vacuum pump. SPE columns containing 100 mg of blank IA-based polymer were conditioned with 5 ml H_2O and 1 ml of the filtered extract was then cleaned up on the SPE column at a flow rate of 0.5 ml min^{-1}. The column was washed with 2 ml of MeOH: H_2O (2:8) with a flow rate of 0.5 ml min^{-1}. AFB1 was then eluted with 1 ml MeOH: Dichloromethane (1:1). The eluted extract was then analyzed by HPLC and electrochemical sensor.

2.7. Clean-up procedures for IAC and SUP

10 ml of clear wheat filtrate and 6.0 ml of clear fig filtrate were pipetted into reservoirs containing 40 ml and 44 ml phosphate buffered saline (PBS) solutions respectively and placed on immunoaffinity columns (Aflaprep, R-Biopharm Rhone Ltd, Glasgow, UK). The solution was passed through the immunoaffinity column at a flow rate of around 3 ml min^{-1}. Column was washed with 20 ml water and dried by applying little vacuum for 5–10 s or passing air through with a syringe for 10 s. Samples were eluted from the column by passing 1 ml of HPLC grade MeOH and then 1 ml of HPLC grade water by gravity to collect the eluate into a glass vial. 100 μl of the eluate was injected into the HPLC. In addition to IAC-HPLC, SUP-HPLC was conducted according to the manufacturer's recommendation.

2.8. MiSens transducer fabrication

The MiSens biochip design was repressed on silicon dioxide wafer. This new design consists of 2 sets of Au electrode arrays; each set consists of 3 working electrodes (d = 1 mm) with shared Au counter and quasi-reference electrodes [19].

2.9. MiSens biochip surface modification

To acquire a clean sensor surface and successful surface modification, plasma cleaning was applied on the electrode arrays [20] and a self-assembled monolayer (SAM) was then applied by immersing the sensor chips in ethanolic solution of 2 mM MUDA (80%) and 2 mM mercaptoethanol (20%) mixture for overnight. Later the electrode arrays were washed with ethanol and water. After drying in flow hood, the arrays were packed with oxygen barrier package and stored at +4 °C till use. SAM coated electrode arrays and the PMMA cassettes were combined by means of a double sided sticky tape. The sensor chip was then placed to the MiSens docking station to establish the electronic and fluidic connections to create a microfluidic channel (~7 μl). Cyclic voltammetry tests were performed using 1 mM potassium ferrocyanide solution in 1 M KCl for surface characterization prior to Protein A immobilization on the sensing areas via conventional amine coupling chemistry [21]. Dulbecco's modified phosphate buffered saline (PBS, pH 7.4) was used as immobilization running buffer and continuously flowed over the sensor surfaces between the injections. The flow rate of the buffer/reagents for the assay was 50 μl min^{-1} unless written otherwise. Sensor surfaces were first activated with a 1:1 mixture of 400 mM EDC and 100 mM NHS and later 250 μl protein A (30 μg ml^{-1} in NaAc buffer pH 4.5, 30 μl min^{-1}) were injected across sensor surfaces. Non-reacted NHS esters were capped with 1 M ethanolamine, pH 8.5 (300 μl).

2.10. MiSens detection assay

MiSens biosensor relies on the REP™ technology where all the steps of the assay including the immobilization, capturing and amperometric measurements are performed during the fluid flow. Using the automated MiSens biosensor, the aflatoxin antibody (300 μl, 5 μg ml^{-1} in PBS buffer) was bound on protein A immobilized surface via 30 μl min^{-1} fluid flow. Later 100 μl aflatoxin samples/standards (containing 70% methanol) were mixed with 200 μl aflatoxin-HRP conjugate, and injected to sensor surface (30 μl min^{-1}) (Fig. 2). The electrochemical measurements were applied at −0.1 V potential between reference and counter electrodes simultaneously with 50 μl min^{-1} fluid flow. The current measurements were taken during PBS buffer injection to obtain a baseline and then the subsequent injection of 240 μl TMB (50 μl min^{-1}) incurred a current change (due to the surface bound

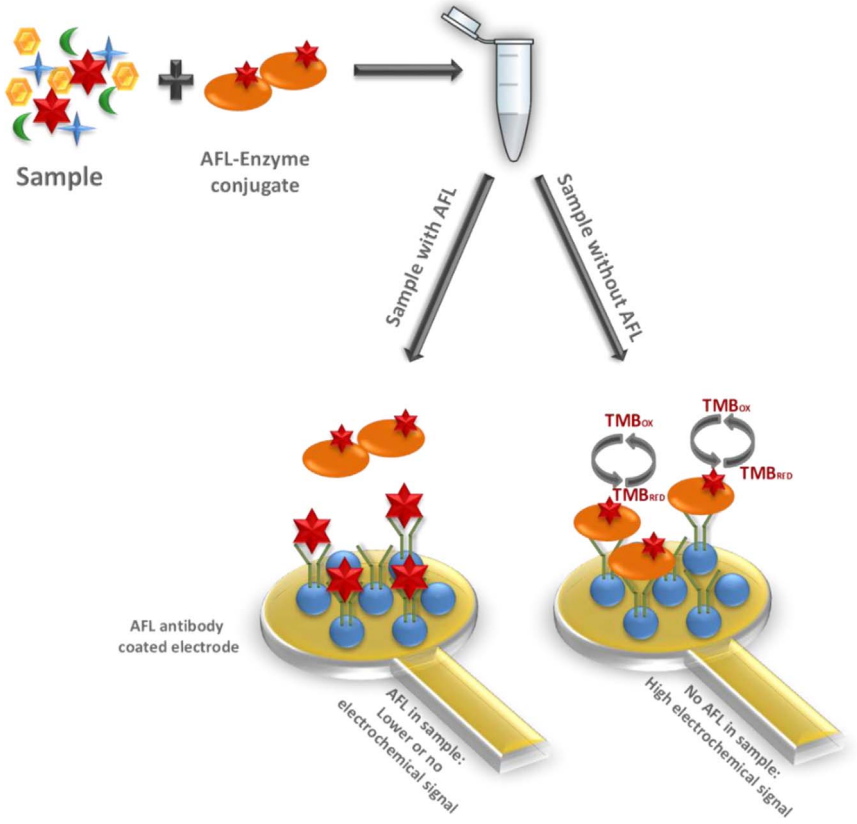

Fig. 2. The schematics of the aflatoxin detection assay on electrodes using the developed biosensor MiSens.

HRP). The distinctness between the current obtained during TMB and buffer was used as signal correspond to target concentration. By injecting 0.1 M HCl (150 μl, 100 μl min^{-1}) twice, the interaction between the aflatoxin antibody and protein A was disrupted hence antibody was removed from the surface; allowing surface regeneration. Therefore, after each regeneration step, the sensor surface was ready to test new sample/standard concentration with fresh capture antibody. Three data points were used to obtain the mean and standard deviation of the results. The limit of detection (LOD) was calculated as the signal obtained from the assay that is equivalent to the 3 times the standard deviation of the signals obtained from the blank standards.

3. Results and discussion

In this work AF standards and contaminated food matrixes (wheat and fig) were used and the developed NIP columns and the REP™ platform (MiSens biosensor) were compared with the reference methods. With this aim, MiSens biosensor and ELISA were employed for AFB1 detection directly. MiSens system was also utilized after extraction using NIP and the results were compared with IAC-HPLC and SUP-HPLC (Fig. 3). Towards an innovative miniaturized automated system, a novel electrode array has been designed and integrated to a microfluidic system. This new sensor chip consists of 6 working electrodes (1 mm diameter) with shared reference and counter electrodes, a sensor cassette made of PMMA was designed and a silicon o-ring has been used to create a microfluidic channel (~7 μl) on the electrode arrays. We have reported the use of similar electrode array together with a REP™ platform for deoxynivalenol (DON) detection in an earlier study [19] and here we successfully integrated the electrodes into a microfluidics system to accomplish a closed and fully automated sensor system MiSens (Fig. 1). The quality of the SAM was checked by cyclic voltammetry and the mycotoxin detection assays were then performed with the REP™ technique. The results were compared with other tools (ELISA and HPLC) for comparison and confirmation.

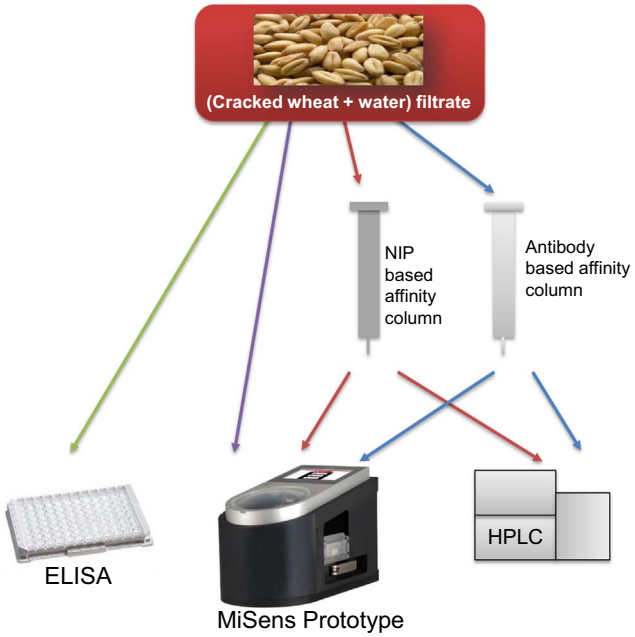

Fig. 3. Comparative work on aflatoxin detection using natural and artificial receptors coupled with three different detection tools (ELISA, MiSens biosensor and HPLC).

3.1. NIP synthesis for SPE column

Owing to high selectivity, MIPs are extensively used in SPE [22–24] and sensor applications [25,26]. Performance of MIPs is evaluated through comparing to their counterparts, non-imprinted polymers. Based on the imprinting strategy for an instance solid phase synthesis, MIP can have considerably better affinity than non-imprinted polymer. Otherwise, NIP also shows high level of binding against a target of interest due to the hydrophobic interaction characteristics of the polymer. This is particularly the case in bulk imprinting which was used in the current research. In cases the templates are found unavailable, very expensive or/and toxic, it is therefore practical to use NIPs. These polymers have capacity to be used in sample clean up procedure due to the binding affinity between aflatoxin and NIP in SPE cartridges, particularly when acrylic monomers such as MAA are used during polymer synthesis. This realty was also stated in a previous work [27]. NIP for SPE column is a very good alternative to the IAC-based SPE columns in the case of highly toxic targets since it is not subjected to the requirement of very limited amounts of organic solvents as well as certain operation conditions such as well-controlled pH, matrix components' concentration levels, ionic strength and water-based media. All these conditions have to be fulfilled for IAC-based systems which necessitate much longer experimental time and also significantly higher cost. The polymers are needed for simply cleaning up the sample before very selective detection systems, such as HPLC or immunoassays. In such cases, it is proposed to use non-imprinted polymer (NIP) rather than imprinted, and the NIP can be prepared using the procedures applied for the design of MIPs [28,29]. In this work, we need adsorbent for clean-up of mycotoxin samples prior to electrochemical immunosensing. To be safe and cost-effective, we avoided to make MIPs, which use the toxins as template. NIPs can eliminate the false positive result brought by template leaching and extensive washing in MIPs. Therefore, we prepared NIP-SPE for the clean-up of wheat samples in the system.

The choice of solvent in SPE is important since the analyte and polymer interactions depend on the solvent. To simplify the procedure and lessen the time required for sample preparation, it is required to load the extract of wheat sample into SPE column without solvent reconstitution. Here, NIP-SPE column exhibited high binding capacity in the solvent (80% methanol). Full retention of the analyte was achieved after direct loading of the wheat extract. Washing the column with 2 ml PBS solution at pH 9.2 assured the analyte remaining on the column and removing some interference from the column. Complete elution was accomplished using 1 ml methanol and the eluent was analyzed both by HPLC and REP assay. The performance of NIP-SPE column was evaluated by HPLC comparing to the commercial columns such as affinity column (IAC) and bond elute column (SUP). To prove the SPE column extraction success, we measured the AFB1 standard solution with sensor chip before and after the extractions in different concentrations. The recovery values were found to be 96%, 108% and 89% for 10 ppb, 20 ppb and 40 ppb concentrations, respectively. Recently, graphene oxide adsorbent for AF was synthesized and selected to be applied as an effective adsorbent for determination and quantification of aflatoxins in peanuts by HPLC. The limit of detection of this method ranges from 0.08 to 0.65 ppb. The recoveries of the four aflatoxins are in the range from 85.1% to 100.8% with the relative standard deviations between 2.1% and 7.9% [30]. Therefore, a better recovery was achieved in our work.

3.2. NIP performance tests with HPLC

The obtained polymers were packed into SPE columns and evaluated under similar chromatographic conditions. The column

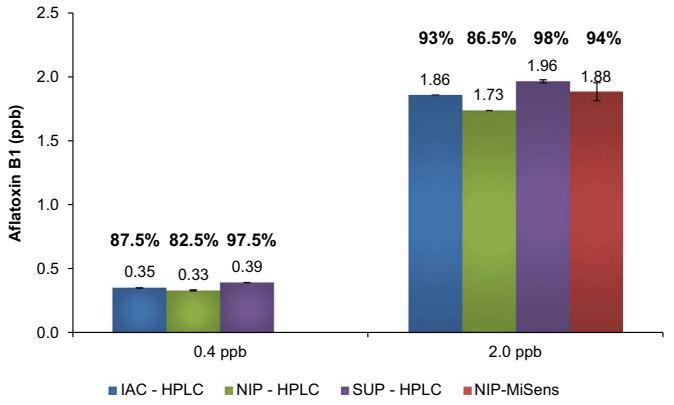

Fig. 4. HPLC and MiSens results of the different column extraction and comparison of the extraction recoveries.

was washed with MeOH:H_2O (2:8) with a flow rate of 0.5 ml min^{-1}. AFB1 was then eluted with MeOH:Dichloromethane (1:1). The eluted extract was then analyzed by HPLC and electrochemical sensor. 0.4 ppb of AFB1 in spiked wheat samples was found as 0.35 ppb (87.5% recovery), 0.33 ppb (82.5% recovery) and 0.39 ppb (97.5% recovery) for IAC-HPLC, NIP-HPLC and SUP-HPLC, respectively. 2 ppb of AFB1 in spiked wheat samples was determined as 1.86 ppb (93% recovery), 1.73 ppb (86.5% recovery), 1.96 ppb (98% recovery and 1.88 ppb (94.0% recovery for IAC-HPLC, NIP-HPLC, SUP-HPLC and NIP-MiSens, respectively (Fig. 4))). These results clearly indicate that the produced NIP column works as effective as immunoaffinity columns used in the study. In addition, the aflatoxin detection performance of the NIP coupled MiSens assay is comparable to immunoaffinity column coupled HPLC results; hence, can be utilized as an alternative on-site aflatoxin detection method.

3.3. MiSens assay for aflatoxin detection and comparison with HPLC for real samples

MiSens biosensor relies on the REP™ technology where all the steps of the assay including the immobilization of the capture protein, binding of the conjugate protein and amperometric measurements with substrate have been performed during the fluid flow. For all steps of the assay, flow rate and quantity are the key factors. The REP™ assay with respect to assay flow rate, reagent concentrations (protein A, anti-AFL and AFL-HRP conjugate) and reagent volume was optimized. At first, the AFB1 standard solution was used to determine the calibration curve and an LOD of 2 ppb was obtained. The tests were also performed by utilizing ELISA and the results of two systems were compared (Fig. 5).

For real sample analysis, fig samples were comparatively investigated with MiSens biosensor and HPLC using the optimized conditions. For this, 6 ppb aflatoxin (*r*-biopharm standard) spiked dry fig samples were used to compare the aflatoxin detection capacity of the MiSens device with respect to the conventional HPLC. To determine the AFB1 amount in dry fig samples, the obtained biosensor responses were converted into the concentrations using the equation obtained from the calibration curve reported in Fig. 5. The testing of the nine samples that are spiked with 6 ppb aflatoxin, resulted in 5.50 ± 0.73 ppb and 6.51 ± 0.58 ppb concentrations using the MiSens and HPLC devices, respectively (Fig. 6), indicating the potential of the MiSens biosensor as a fast and an on-site aflatoxin pre-screening device with comparable results to HPLC.

Many other methods relying on immunoassay principle can be found from the literature. Strip or dipstick-based lateral flow devices have currently offered the easiest and most rapid detection principle although they can only provide qualitative testing [30]. The currently available analytical tools have provided the detection limits of 0.01–5 ng ml^{-1} for mycotoxins [12,31]. These

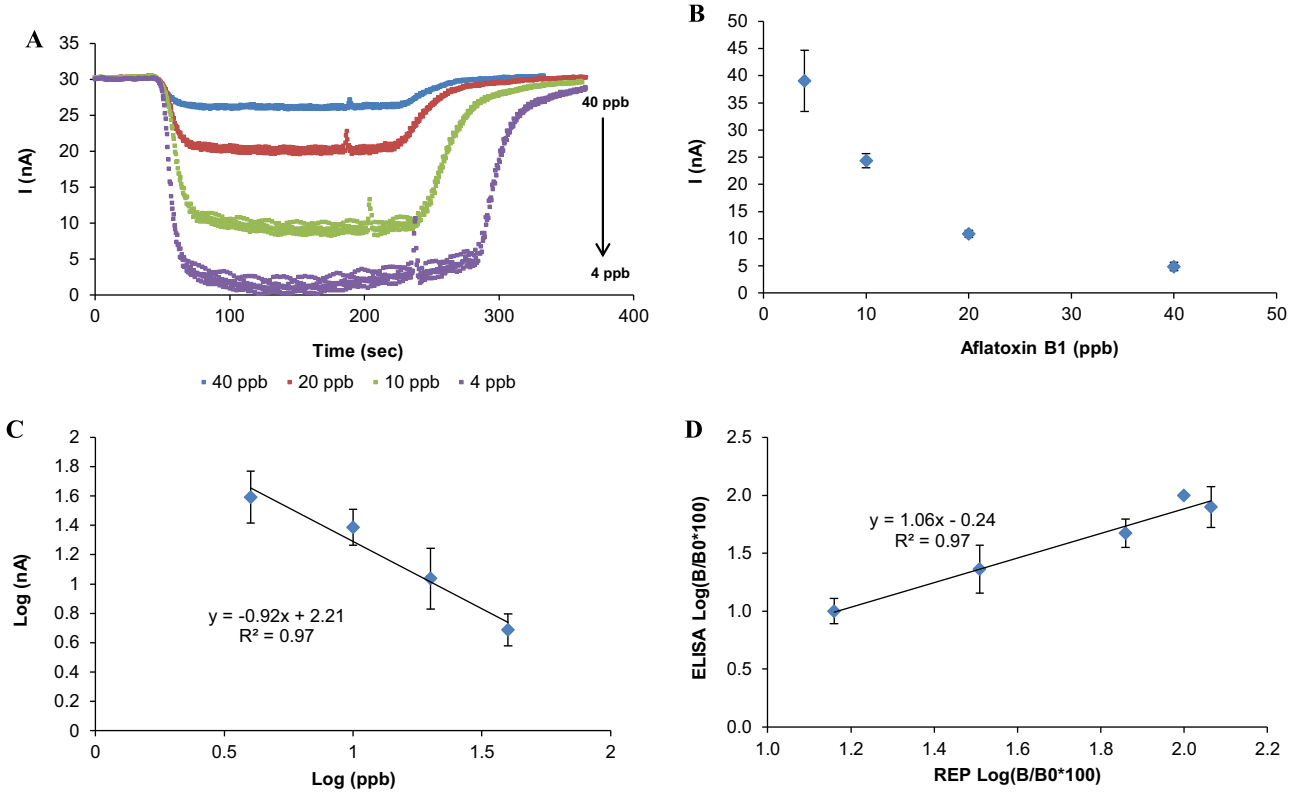

Fig. 5. Real amperometric measurement during the injection of 50 μl min^{-1} TMB substrate (A). Linear (B) and logarithmic (C) calibration curves for the aflatoxin detection assay using MiSens biosensor (n =6). Comparison of the aflatoxin ELISA and MiSens assay results (D).

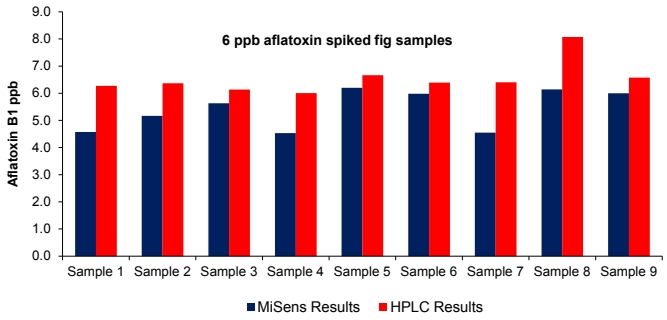

Fig. 6. Analysis of 6 ppm aflatoxin contaminated dry fig samples by employing Misens biosensor and comparison with HPLC.

techniques are commonly based on immunoaffinity or SPE columns including antibodies against mycotoxins as well as biosensing-based detection techniques. Some important investigations have been reported in the literature covering a variety of voltammetric detection principles for AFB1, AFB2, DON [32–34], amperometric detection techniques for ZEA and ZEA metabolites [32,34] as well as ELIME array based electrochemical detection of nivalenol and DON [35]. However, the techniques described in the literature mostly involve several steps that are performed manually by the researchers and lack the automation required for the fast food analysis [36].

4. Conclusions

Non-imprinted polymers (NIP) were manufactured for AF detection coupled with REP™ based novel MiSens biosensor and high performance liquid chromatography (HPLC). A miniaturized automated system was designed and engineered using a novel electrode array integrated to a microfluidic system. This new sensor consists of 6 working electrodes (1 mm diameter) with shared reference and counter electrodes, a sensor cassette made of poly(methyl methacrylate) (PMMA) was designed and a double sided sticky was used to create a microfluidic channel on the electrode arrays. The sensor surface and SAM formation were characterized by employing cyclic voltammetry prior to aflatoxin detection assays with the real-time electrochemical profiling (REP™) technique. The sensor results were compared with ELISA and a great correlation was achieved between the results with a R^2 value of 0.96. On the other hand, a very high recovery was achieved in spiked wheat samples: 0.4 ppb of AFB1 in spiked wheat samples was found as 0.35 ppb (87.5% recovery), 0.33 ppb (82.5% recovery) and 0.39 ppb (97.5% recovery) for IAC-HPLC, NIP-HPLC and SUP-HPLC, respectively. 2 ppb of aflatoxin was determined as 1.86 ppb (93% recovery), 1.73 ppb (86.5% recovery), 1.96 ppb (98% recovery) and 1.88 ppb (94.0% recovery) for IAC-HPLC, NIP-HPLC, SUP-HPLC and NIP-MiSens, respectively. Aflatoxin detection in fig samples were also investigated with MiSens biosensor and the results were compared with reference HPLC method. Although a very good output was triumphed, a small variation was observed between samples. However, this problem can easily be overcome by normalizing the sensor results. The new biosensor allows on-time detection of mycotoxins in real samples using NIPs and antibodies with a rapid, sensitive, fully automated and miniaturized system and will have an immense economic impact for food industry.

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