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Fast and sensitive detection of mycotoxins in wheat using microfluidics based Real-time Electrochemical Profiling

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ABSTRACT

The objective of the study has been the development of a new sensing platform, called Real-time Electrochemical Profiling (REP) that relies on real-time electrochemical immunoassay detection. The proposed REP platform consists of new electrode arrays that are easy to fabricate, has a small imprint allowing microfluidic system integration, enables multiplexed amperometric measurements and performs well in terms of electrochemical immunoassay detection as shown through the deoxynivalenol detection assays. The deoxynivalenol detection has been conducted according to an optimised REP assay protocol using deoxynivalenol standards at varying concentrations and a standard curve was obtained ($y=-20.33\ln(x)+124.06$; $R^2=0.97$) with a limit of detection of 6.25 ng/ml. As both ELISA and REP detection methods use horse radish peroxidase as the label and 3.3′,5.5′-Tetramethylbenzidine as the substrate, the performance of the REP platform as an ELISA reader has also been investigated and a perfect correlation between the deoxynivalenol concentration and the current response was obtained ($y=-14.56\ln(x)+101.02$; $R^2=0.99$). The calibration curves of both assays have been compared to conventional ELISA tests for confirmation. After assay optimisation using toxin spiked buffer, the deoxynivalenol detection assay has also been performed to detect toxins in wheat grain.

1. Introduction

Mycotoxins are toxic secondary metabolites produced by filamentous fungi growing on many plants and processed food, either in the field or during storage. Mycotoxins are carcinogenic, produced quickly in damp conditions and can be fatal in high doses; therefore, they have been considered as the most significant chronic dietary risk factor, even higher than synthetic contaminants, food additives or pesticide residues. Deoxynivalenol (DON), as one of the mycotoxins, is economically very important due to its prevalence and heat stability. DON persists in grains during storage and is unaffected by high temperature and pressure during processing (Wolf-Hall et al., 1999). To avoid consumers' health risk due to unacceptably high dietary intake of Fusarium toxins, the EC has established maximum levels for DON (1.25 ppm in cereals; 0.2 ppm in processed cereal-based foods and baby foods) (European Commission, 2006; Lancova et al., 2008). For this reason, DON testing has a great importance to prevent contaminated food to reach the consumer as well as to be able to import the food products by passing the regulations set by the countries.

Traditional mycotoxin tests utilise gas chromotography, mass spectrometry, liquid chromatography or enzyme-linked immunosorbent assay (ELISA) type assays that use expensive equipment, need a specialist and have time consuming procedures. These tests also require sample transportation from storage to the laboratories that cause increased waiting time and high costs. Therefore, there is a need to develop a detection system that is cheap, quick to process, uses low sample and reagent volume and easily operated. Recent advances in the area of sensor technology and lab-on-a-chip applications have enabled the miniaturisation of the devices and multiplex testing of a range of analytes. Therefore, biosensor technology has the potential for the manufacture of mycotoxin detection devices (Tothill, 2011; Vidal et al., 2013). Recent advances in the area of sensor technology have enabled the miniaturisation of the devices and multiplex testing of a range of analytes. Therefore, biosensor technology has the potential for the manufacture of testing devices for toxin analysis in crops as the global food safety testing market by contaminants is estimated to

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grow at an annual growth rate of 10.46% to $2.5 billion in 2015 (MarketPublishers, 2011).

Recently, mycotoxin testing has been performed using various biosensing techniques from simpler immunodipstick type tests to the label free quartz crystal microbalance (QCM) (Vidal et al., 2009) and surface plasmon resonance (SPR) (Li et al., 2012; Choi et al., 2011), and electrochemical tests (Khan et al., 2011; Hervas et al., 2011; Rene Perrotta et al., 2011; Vidal et al., 2012). In a recent paper, Lattanzio et al. (2012) described a multiplex dipstick immunoassay that provided simultaneous detection of T-2 and HT-2 toxins, DON and fumonisins in crops. The results obtained from this study indicated that the optimised immunoassay was able to detect target mycotoxins at cut-off levels equal to 80% of EU maximum permitted levels. Kolosova et al. (2008) developed a lateral-flow immunoassay using a colloidal gold-labelled monoclonal antibody for the qualitative rapid determination of DON. While immunodipstick tests do not require an expert for use and they provide rapid, inexpensive testing, their main short-coming is their limited sensitivity and in most cases they can be used as a qualitative screening tool complementary to more accurate techniques. Commercial strip-tests are currently available for DON and aflatoxin detection in grain that can be used either as semi-quantitative or quantitative tests.

High sensitivity, selectivity, rapid analysis, ability to operate in turbid solutions and the possibility of miniaturisation enabled electrochemical biosensors to become the most commonly used biosensors over other technologies (Shah and Wilkins, 2003). For example, Heurich et al. (2011) developed a disposable electrochemical immunosensor using screen printed electrodes for ochratoxin A detection in wine and obtained a limit of detection of 0.05 μg/L with a linear dynamic detection range of 0.01–100 μg/L. Hervas et al. (2009) have improved the electrochemical immunoassay response by means of magnetic nanoparticles and obtained a detection limit (LOD) of 0.011 μg/L for the zearalenone in baby food samples. In the literature several other studies can be found related with mycotoxin detection using electrochemical techniques (Bone et al., 2010; Asuncion Alonso-Lomillo et al., 2011; Barthelmebs et al., 2011; Panini et al., 2011) ; however, most of these publications describe the results of the tests that are performed using bench top potentiostats with off the shelf electrodes and require hands on work. This shows that most of the publications describe only the results for proof of principle of the toxin detection using biosensors and the number of studies performed using integrated systems with the necessary fluidics is quite limited. In addition mostly, the assay procedures are long, labourous and not suitable for automation. In response to that, we have developed a new detection technology, which we term Real-time Electrochemical Profiling (REP™). While this technology relies on the fundamental basics of electrochemical immunosensing, in particular amperometry, it has several key features including a new electrode array, microfluidics based assay and real-time amperometric measurements during the flow of enzyme substrate. For the common flow injection electrochemical immunoassay platforms, initially in a separate container or fluidic system the enzyme immunoassay is performed, and later the reaction solution is sent to (usually) a bare electrode for the detection at a certain flow rate. However with the REP system, we were able to combine both steps together on one electrode surface. That distinguishes our sensing platform from other flow injection based electrochemical immunoassays. In addition, whole assay is performed during the reagents flow; therefore, there is no need to stop the flow either during the analyte binding or enzymatic reaction; hence the sensing can be achieved reasonably fast. In an earlier study, a new electrode array and integrated microfluidics have been designed and characterised in order to create a sensor chip that is not only easy, rapid and cheaper to produce but also have a smaller imprint and good electrochemical sensing properties (Uludag et al., 2014). In the current study, this new electrode array, comprising Au quasi-reference electrode and shared reference/counter-electrodes with the integrated microfluidics, has been used for the detection of DON with REP platform.

2. Materials and instrumentation

Phosphate buffered saline (PBS, 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4) tablets, 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), 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). Deoxynivalenol (DON) was purchased from Santa Cruz Biotechnology (Heidelberg, Germany). DON–HRP conjugate and AgraQuant deoxynivalenol test kit were obtained from Romer Labs (Tulln, Austria). DON antibody was purchased from Lifespan Biosciences (Seattle, USA). Potassium chloride (KCl) and biotin were 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).

2.1. Safety awareness

Due to the toxic properties of DON, safety precautions were applied, such as wearing gloves, protection glasses and laboratory coat at all times and a facial mask when handling powder DON. The toxin was stored in a locked fridge specified for toxic reagents according to safety instructions.

3. Methods

3.1. Transducer fabrication

A sensor chip has been designed and fabricated on silicon dioxide wafer that consists of 2 sets of electrode arrays; each set consists of 3 working electrodes (d=1 mm) with shared Au counter and quasi-reference electrodes (Fig. 1A). The design of the electrodes was formed on the silicon dioxide wafer by means of a Fine Metal Mask made of a laser cut patterned stainless steel, and Au metal was deposited on the wafer using an electron beam evaporator. Before the application of Au (200 nm), a 20 nm Ti layer is applied on to the silicon dioxide wafer as an intermediary adhesive layer to increase the adhesion between the Au and silicon dioxide wafer. After metal evaporation, the silicon wafer has been cut into 10×20 mm^2 pieces that form the sensor chips with two electrode arrays (Fig. 1A). A sensor cassette has been designed and fabricated from poly(methyl methacrylate) (PMMA) and a double sided sticky tape formed a microfluidic channel ($\sim$ 10 μL) on the electrode arrays (Fig. 1A and B). A potentiostat (MicroStat 8000; Dropsens, Astuias, Spain), a syringe pump (Mitos Duo XS; Dolomite, Royston, UK), an injection valve and a sensor chip docking station (Fig. 1C) have been set up for the experimental work.

3.2. Electrochemical analysis

Cyclic voltammetry and amperometric measurements were performed with a MicroStat 8000 Electrochemical Analyzer with

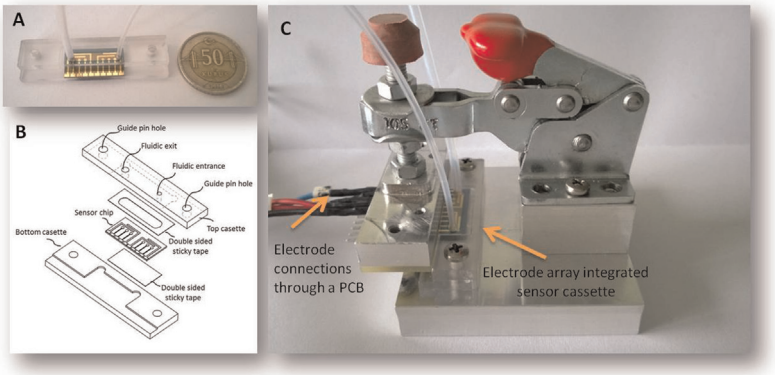

Fig. 1. (A) Electrode array integrated sensor cassette. (B) A flow cell is formed and the electrode array is fixed to the sensor cassette by means of a double sided sticky tape. (C) For the laboratory prototype, a printed circuit board (PCB) attached clamp is used to establish electronic connections between the electrode arrays and the potentiostat device.

the general purpose electrochemical software Dropview 1.4 (Dropsens, Oviedo, Spain). The electrochemical analyzer and the purpose built shielded cables enable simultaneous electrochemical measurements of 8 electrodes. Cyclic voltammetry (CV) tests were performed using 1 mM potassium ferrocyanide solution in 1 M KCl.

3.3. Surface modification

Surface modification of the electrode arrays with a self-assembled monolayer (SAM) has been achieved by plasma cleaning of the arrays and later immersing in ethanolic solution of 2 mM MUDA (80%) and 2 mM mercaptoethanol (20%) mixture for overnight. Later the electrode arrays were rinsed with ethanol and water. After drying with nitrogen stream, the arrays were stored at +4 °C till use. SAM coated electrode arrays and the PMMA cassette were assembled together by means of a double sided sticky tape to form the sensor chip with a flow cell, and placed to the docking station to establish the electronic and fluidic connections. The sensor surfaces were prepared by immobilising protein A on the electrode arrays using conventional amine coupling chemistry. The running buffer used for immobilisation was degassed Dulbecco's modified phosphate buffered saline (PBS, pH 7.4) and this buffer continuously flowed over the sensor surfaces between the injections. The flow rate of the buffer/reagents for the assay was 50 μL/min unless written otherwise. Sensor surfaces were first activated with a 1:1 mixture of 400 mM EDC and 100 mM NHS, and mixed immediately prior to use (final concentrations; 200 mM EDC and 50 mM NHS). First 250 μL EDC–NHS, later 250 μL protein A (30 μg/mL in NaAc pH 4.5 buffer, 30 μL/min) were injected across sensor surfaces. Non-reacted NHS esters were capped with 1 M ethanolamine, pH 8.5 (250 μL).

3.4. Real-time Electrochemical Profiling™ assay

First, the DON antibody (250 μL, 5 μg/mL in PBS buffer, 30 μL/min) was captured on protein A immobilised surface. Later 125 μL DON samples/standards were mixed with 125 μL DON–HRP conjugate, and injected over the DON antibody immobilised sensor surface (30 μL/min) (Fig. 2A). To obtain an electrochemical signal, a Real-time Electrochemical Profiling™ (REP™) assay has been performed by applying −0.1 V potential to the electrode array and the current was measured continuously. Initially the current measurements were taken during PBS buffer injection and this signal was recorded as a baseline. The subsequent injection of 250 μL TMB (50 μL/min) incurred a current change (due to the surface bound HRP) and the subtraction between the current obtained during TMB and buffer was used as sensor response (Fig. 2B). Regeneration of the sensor surface has been achieved with the injection of 250 μL 0.1 M HCl (100 μL/min) twice. The regeneration solution

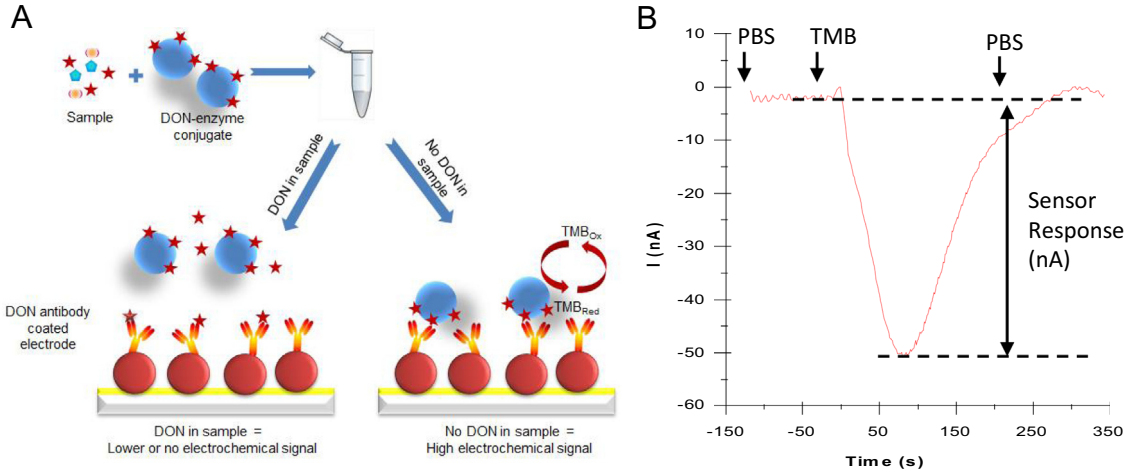

Fig. 2. (A) The schematics of the competitive mycotoxin detection assay on electrodes. (B) The current response of the electrodes against −0.1 V potential is measured continuously during the sequential injections of buffer and TMB substrate (50 μL/min) to the enzyme bound electrode to obtain a sensorgram. The amplitude of the obtained current is proportional to the amount of surface bound HRP enzyme.

removes the DON antibody from the protein A immobilised sensor. Therefore, after each regeneration fresh DON antibody has been injected on to the sensor surfaces to create the oriented DON antibody coated sensor surface ready for the assay. The efficiency of the regeneration protocol has been tested by performing the detection and regeneration assay cycle three times subsequently for the same DON concentration. In addition, for the each used sensor chip, the same DON concentration has been used for testing at the beginning and end of the assay to asses if there are any changes to the sensor chip activity. In general, to avoid any activity loss, each sensor chip has been used not more than 8 binding/regeneration cycles.

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.

3.5. ELISA assays

The ELISA assays were performed according to the manufacturers protocol (AgraQuant deoxynivalenol test kit, Romer Labs.). Briefly, the extracted sample and enzyme-conjugated DON are mixed and added to the antibody-coated microwell. DON in samples and control standards are allowed to compete with enzyme-conjugated DON for the antibody binding sites. After a washing step, an enzyme substrate is added and blue colour develops. The intensity of the colour is inversely proportional to the concentration of DON in the sample or standard. A stop solution is then added which changes the colour from blue to yellow. The microwells are measured optically using a microwell reader with an absorbance filter of 450 nm (OD450).

In addition to the conventional ELISA test with optical measurements, a second ELISA test has been performed where the REP™ platform and the sensor chips have been used as an ELISA reader. The ELISA kit obtained from Romer Labs. contains both HRP conjugated DON and enzyme substrate (TMB), and this pair is used for the detection. For this study, after HRP–DON conjugate addition to the wells, the well is washed and excess HRP–DON is removed. Later TMB solution is added to the wells and incubated for 5 min. Then, the enzyme substrate (250 μL) is removed from the microwell with a pipette and immediately injected on to the electrode arrays (bare Au) for electrochemical measurement of the enzyme substrate reaction (HRP and TMB), and the current was measured continuously for a given −0.1 V potential.

3.6. Wheat sample preparation

Samples were extracted following the procedure reported by Maragos (2011) with minor modifications. Samples of wheat (20 g) were spiked with DON by addition of a DON spiking solution in 5:1 (w:v) ratio to the wheat samples. Spiking solution was prepared by dilution of the stock DON solution with acetonitrile. Then the samples were allowed to dry overnight at ambient temperature prior to extraction in the fume hood. The samples were extracted with 100 mL of distilled water, in 250 mL bottle, by shaking on a wrist-action shaker for 10 min at ambient temperature, having the ratio 1:5 (w:v) of sample to extraction solution. The crude extracts were filtered (syringe filter 0.44 μm, Millipore MF membrane) and later used for the biosensor detection assay.

4. Results and discussion

Towards the realisation of an electrochemical device for on-site mycotoxin testing, it is essential to miniaturise the electrode array and integrate with a microfluidic system. Therefore, a new sensor chip has been fabricated that consists of electrode arrays with 1 mm diameter working electrodes (Uludag et al., 2014). This new electrode array consists of shared reference and counter-electrodes to minimise the size of the sensor. In addition, the use of Au quasi-reference electrodes instead of traditional Ag/AgCl electrodes enabled a one-step sensor chip fabrication protocol that involves a fine metal mask and Au thin film evaporation. The characterisation of this new electrode array has been discussed in detail in an earlier study (Uludag et al., 2014); here we report the use of this electrode array together with a REP™ platform for DON detection. For the REP™ assay, a sensor cassette made of PMMA was designed and fabricated that created a microfluidic channel (capacity = ~10 μL) on the electrode arrays. Initially, the quality of the sensor surface chemistry has been checked by cyclic voltammetry, later the mycotoxin detection assays have been performed with the REP™ platform.

4.1. Sensor surface quality control

Application of SAM on a gold electrode surface insulates the electrode surface. Therefore, by comparing the cyclic voltammograms of a bare gold electrode and alkanethiol coated gold electrode, it is possible to investigate the coverage of the monolayer on the gold electrode. Cyclic voltammograms of bare Au and SAM coated electrode arrays were obtained using potassium ferrocyanide as the electroactive marker. As it could be seen from Fig. 3, the oxidation and reduction peaks of potassium ferrocyanide were visible and there was a larger redox area between positive and negative potential sweeps when cyclic voltammetry performed on bare gold sensor chip whereas the redox area between positive and negative potential sweeps of the SAM coated sensor chip was very small. This indicated the formation of the SAM on the electrode surfaces. The surface coverage (θ) of the self-assembled monolayer can be calculated by comparing the charge (Q) during reduction/oxidation of cyclic voltammetry at a monolayer covered electrode with respect to a bare gold electrode (Yang and Khoo, 2004):

$$\theta = 1 - \frac{Q_{SAM}}{Q_{bare\ gold}} \quad (1)$$

Using Eq. (1), the surface coverage of the SAM surface was calculated as 0.95 ± 0.03. This proves the quality of the SAM layer on electrodes for further protein immobilisation. The cyclic

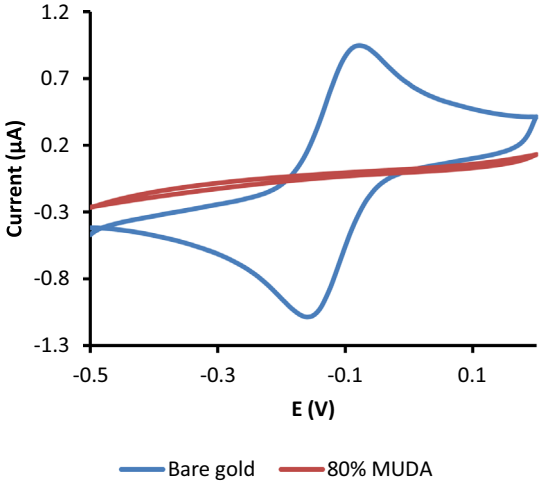

Fig. 3. Cyclic voltammetry (CV) has been performed with 1 mM $K_4[Fe(CN)_6]$/KCl at 0.1 V/s scan rate using bare Au and 80% MUDA-20% mercaptoethanol coated Au electrodes.

voltammetry test has been performed after each SAM coating procedure to the electrodes as a quality control procedure, and only the electrodes with >0.95 surface coverage has been utilised for the DON detection assay.

4.2. REP assay for DON detection

REP™ technology relies on the fundamental basics of electrochemical immunosensing, in particular chronoamperometry. The key features of this new platform include a new electrode array, microfluidics based assay and real-time amperometric measurements during the flow of enzyme substrate. The assay protocol has been described in detail in Section 3.4. Initially, we have optimised the REP™ assay with respect to assay flow rate, reagent concentrations (protein A, anti-DON and DON–HRP conjugate) and reagent volume (data not shown). To investigate the optimum flow rate of the assay 30–50–100–200–800 μL/min flow rates have been tested. Optimal flow rate for the reagents was found as 30 μL/min for protein A immobilisation and DON–HRP conjugate binding. For the regeneration of surfaces with 0.1 M HCl, to avoid from surface damage, faster flow rates were preferred and 100 μL/min was found optimal; for the rest of the assay 50 μL/min was found optimal to get the required sensitivity from the sensors. Optimal detection signals were achieved using 30 μg/mL Protein A (in 0.01 M PBS, pH 7.4) for immobilisation, 5 μg/mL DON antibody to capture on sensor (in 0.01 M PBS, pH 7.4) and 125 μL of DON–HRP conjugate to be mixed with 125 μL sample/standards. The DON detection was based on a direct competitive immunoassay format with HRP used as enzyme label and TMB/H_2O_2 as the substrate system. Initially protein A has been immobilised on sensor electrodes, that followed by the oriented capture of DON antibody. Later the mixture of DON and DON–HRP conjugate has been injected over electrode surfaces that resulted in the binding of DON–HRP conjugate to DON antibody after a competition between DON and DON–HRP conjugate. The subsequent injection of 250 μL TMB (50 μL/min) incurred a current change (due to the surface bound DON–HRP conjugate). As the reaction of HRP and TMB/H_2O_2 is very fast, as soon as the TMB/H_2O_2 solution reaches DON–HRP conjugate bound sensor surface with a flow rate of 50 μL/min, with the applied -0.1 V potential, a current change has been observed (Fig. 4A). Although it is possible to enhance the signal further by stopping the flow and allowing the accumulation of HRP–TMB reaction products to obtain a higher current, as the response obtained during the TMB flow provided enough response to detect down to 6.25 ng/ml of DON, a shorter assay protocol with continuous flow has been preferred. In accordance with the competition immunoassay format, the higher the DON in the sample, the lower the signal achieved from the amperometric measurements (Fig. 4A). The DON assay has been conducted according to the optimised assay protocol using DON standards

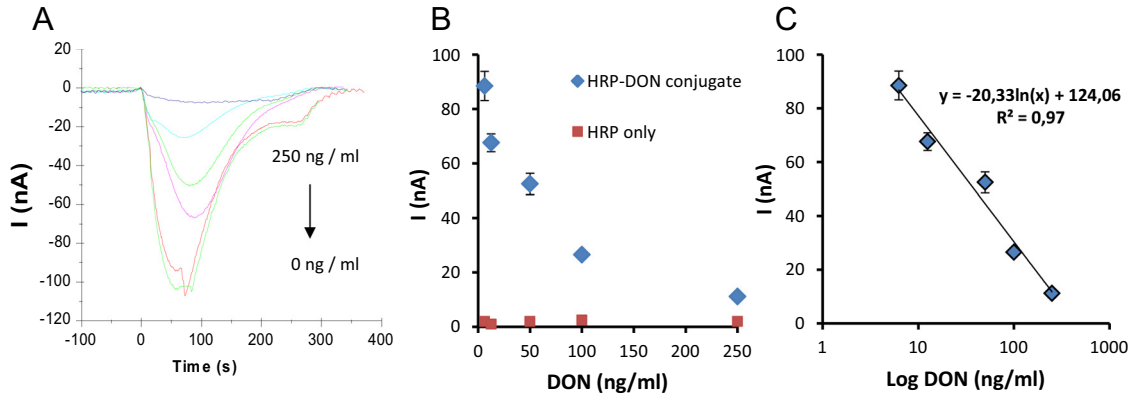

Fig. 4. (A) Raw data of the REP™ assay for DON detection, where the amperometric measurement was taken in real time during the injection of TMB substrate (50 μL/min). The obtained current is proportional to the amount of the surface bound DON–HRP conjugate and inversely proportional to the DON concentration in sample. (B) Linear and (C) logarithmic calibration curves for the DON detection assay.

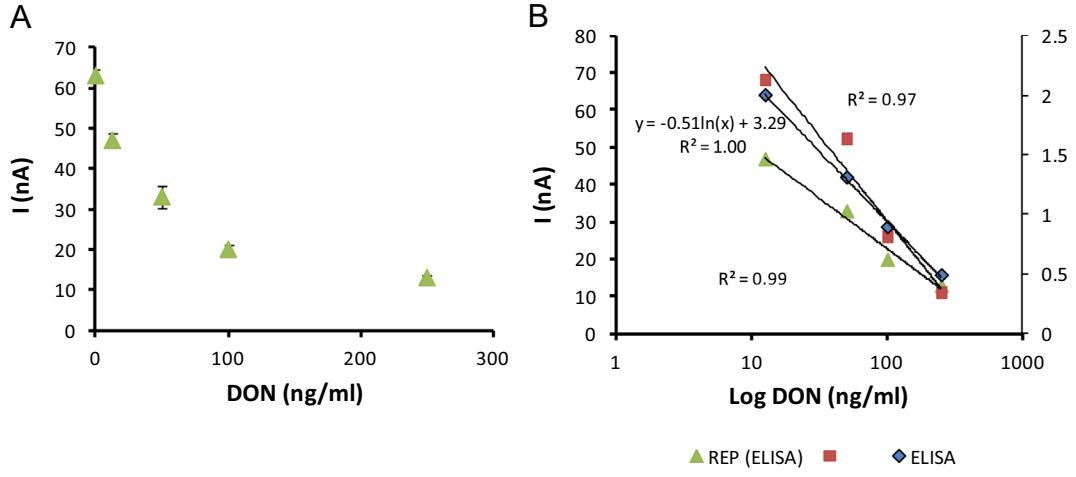

Fig. 5. (A) The REP-ELISA assay has been performed in microwells. After 5 min incubation of the microwells with TMB, the solution (250 μL) was removed with a pipette and immediately injected on to the electrode arrays (bare Au) for electrochemical measurement and the current was measured continuously for a given -0.1 V potential, the plot shows the concentration versus the current obtained for the assay. (B) The comparison of the REP-ELISA and REP assay results with respect to the conventional ELISA readings for DON detection.

at varying concentrations and the range of quantitation has been found as 6.25–250 ng/ml with and LOD of 6.25 ng/ml (Fig. 4B and C) (R^2=0.97). Conducting the DON assay in a microfluidic environment enabled faster assays (ca. 15 min) with respect to conventional chronoamperometric measurements as well as ELISA format assays (ca. 30 min).

4.3. REP-ELISA assay for DON detection

Both ELISA and REP™ detection methods use HRP as the label and TMB as the substrate. For ELISA, the colour formation due to the HRP and TMB reaction provides an optical signal, whereas the same reaction results in current change during the REP™ assay. Therefore, the performance of the REP™ platform as an ELISA reader also has been investigated. In this format, TMB is incubated only 5 min in the microwells, and instead of adding a solution to stop the reaction (as done with conventional ELISA prior to optical measurement), it is collected with a pipette (250 μL) and injected on to a bare Au electrode array (200 μL/min) for the amperometric measurement. As seen from Fig. 5A, a perfect correlation between the DON concentration and current response was obtained. This result indicates that REP™ platform can also be utilised as a very convenient, fast and accurate ELISA reader.

Both the REP™ and REP-ELISA assay results for DON detection have been compared to responses obtained from conventional ELISA tests, and as seen from Fig. 5B, a very good correlation between the results was obtained with R^2 values 0.97 and 0.99, respectively. These results indicate that the REP™ platform could be used for DON detection assays instead of conventional ELISA tests, as a faster and more user friendly system that has the potential to be automated for on-site testing.

To develop a portable, flexible, rapid and user friendly sensor it is essential to miniaturise the biosensor device by means of microfluidics tools (Trietsch et al., 2011). Miniaturisation also requires smaller amounts of reagents for the analysis, which leads to lower costs. Furthermore, utilisation of microfluidic tools allows parallel measurements of several toxins. Advanced micro-fabrication techniques have facilitated integration of microfluidics with sensing functionalities on the same chip making system automation more convenient (Gervais et al., 2011). For example, in a study, Panini et al. (2010) describe an immunosensor that utilises multiwall carbon nanotubes and a continuous-flow system for the rapid and sensitive quantification of zearalenone in corn silage samples. The limit of zearalenone detection was found as 0.77 ppb using the developed electrochemical detection system that showed higher sensitivity and lower detection limit than the standard ELISA method (limit of detection, 2.56 ppb). Hervas et al. (2009) utilised a “lab-on-a-chip” strategy integrating an electrokinetic magnetic bead-based electrochemical immunoassay on a microfluidic chip and obtained a limit of zearalenone detection of 0.4 μg/l in infant food samples. Compared to these results, the REP™ platform with a shorter assay time and with the possibility of automation has a potential to achieve fast and on-site mycotoxin testing. In addition, the reproducibility of the REP™ assays (although performed using the first laboratory prototype) is comparable to conventional ELISA (Table 1).

Table 1
The comparison of the coefficient variations for the ELISA, REP™ and REP-ELISA tests for DON detection.

DON (ppm)	ELISA % CV	REP™ (as ELISA reader) % CV	REP™ % CV
0.25	2.5	3.6	7.4
1.00	2.7	8.2	6.6
2.00	4.7	5.0	7.2
5.00	7.7	5.2	14.7

4.4. DON detection in wheat

After assay optimisation using toxin spiked buffer, the DON detection assay has been performed to detect toxins in spiked wheat grain. The obtained biosensor responses have been converted into the concentrations using the equation obtained from the calibration curve at Fig. 4C. The calculated concentration of DON in spiked wheat samples has been found as 209 ng/ml (104.5% recovery) and 13.4 ng/ml (106.9% recovery).

5. Conclusion

The possible out of the laboratory, on-site detection of toxins would provide frequent and on-time detection of toxins before it spreads to whole silos and hence will prevent disposal of vast amounts of crops and provide a huge economic impact for the agricultural industry. In addition, would provide an on-site, fast and cost-effective solution for testing the imported/exported crops. Therefore, the objective of the study has been the development of a new sensing platform (REP™) for rapid, accurate and on-site detection of mycotoxins in crops. The advantages of the proposed toxin detection platform with respect to existing technologies are its ease of use, sensitivity and reliability. The proposed REP™ platform consists of novel electrode arrays that are easy to fabricate, has a small imprint allowing fluidic system integration, enables multiplexed measurements and performs well in terms of electrochemical detection as shown through the DON detection assays. Therefore, it could provide a convenient tool to fabricate portable, convenient, fast, sensitive, low cost and automated sensing devices for a variety of applications. Our further work involves the integration of the developed electrode array and its fluidics cassette to an electrochemical analyser to obtain an automated REP™ based detection platform for automated mycotoxin tests.

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