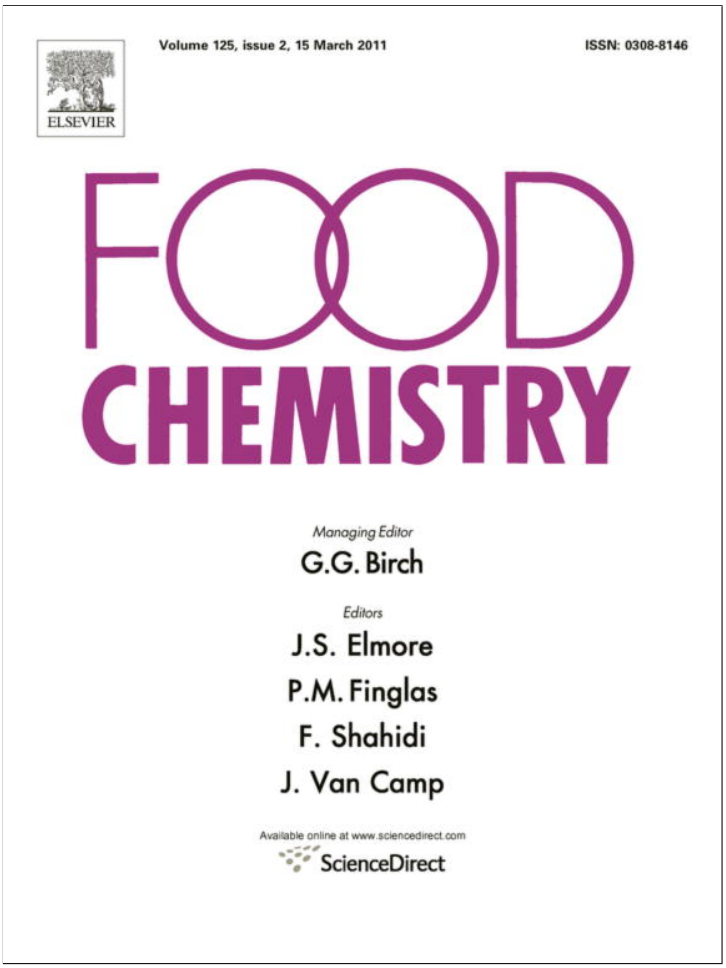

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Analytical Methods

Modified paramagnetic beads in a microfluidic system for the determination of zearalenone in feedstuffs samples

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ABSTRACT

In this work, we have developed and characterised a novel microfluidic immunoassay methodology for rapid and sensitive quantification of ZEA in feedstuffs samples. The detection of ZEA was carried out using a competitive direct immunoassay method based on the use of anti-ZEA monoclonal antibodies immobilized on magnetic microspheres 3-aminopropyl-modified manipulated for an external remobilize magnets. The ZEA in feedstuffs sample is allowed to compete with ZEA-horseradish peroxidase (HPR) conjugated for the immobilized anti-ZEA antibody. The HPR, in the presence of hydrogen peroxide (H_2O_2) catalyses the oxidation of 4-*tert*-butylcatechol (4-TBC) whose back electrochemical reduction was detected on gold electrode at 0.0 V. The calculated detection limits for electrochemical detection and ELISA procedure were 0.41 and 2.56 μg kg^{-1} respectively, the intra and inter-assay coefficients of variation were below 6.5% and the total assay time was 30 min. The microfluidic immunosensor showed higher sensitivity and lower detection limits than the standard ELISA method, which shows potential for detecting ZEA in foods and feeds diagnosis.

1. Introduction

Mycotoxins are toxic secondary metabolites produced by different species of fungi as *Aspergillus*, *Fusarium* and *Penicillium* that colonise crops in field or post-harvest and thus pose a potential threat to human and animal health. It is important, both for consumer's health and the economic point of view, to prevent mould growth and subsequent mycotoxin production in food products (Pardo, Marín, & Ramos, 2006).

In several countries, many individuals are chronically exposed to high levels of mycotoxin in their diet (Wu, 2006). Mycotoxins can enter into the human food chain directly through foods of plant origin and indirectly through foods of animal origin (Kovacs, 2004). ZEA is a nosteroidal estrogenic mycotoxin biosynthesized through a polyketide pathway by a variety of *Fusarium* fungi, including *Fusarium graminearum* (*Gibberella zeae*), *Fusarium culmorum*, *Fusarium cerealis*, *Fusarium equiseti*, *Fusarium crookwellense* and *Fusarium semitectum*, which are common soil fungi, in temperate and warm countries, and are regular contaminants of cereal crops worldwide (Bennett & Klich, 2003).

ZEA is a resorcyclic acid lactone, chemically described as 6-[10-hydroxy-6-oxo-trans-1-undecenyl]-β-resorcyclic acid lactone (Zinedine, Soriano, Moltó, & Mañes, 2007). In humans, ZEA and its products such as the zearalenol have estrogenic and anabolic effects, with comparable properties to the dietilestilbestrol, although his chemical structure is not steroidal (D'Mello et al., 1999; Richardson, Hagler, & Mirocha, 1985; Shier, Shier, Xie, & Mirocha, 2001). In animal of production, ZEA affected mainly reproductive system, causing big economic losses.

Fungi-producing ZEA contaminate corn and also colonise, to a lesser extent, barley, oats, wheat, sorghum, millet and rice. In addition, the toxin has been detected in cereals products like flour, malt, soybeans and beer. Toxin production mainly takes place before harvesting, but may also occurs post-harvest if the crop is not handled and dried properly (CCFAC, 2000). The ZEA derivatives (α-zearalenol (α-ZEA), β-zearalenol (β-ZEA), α-zearalanol (α-ZAL), β-zearalanol (β-ZAL), zearalanone (ZEN)) can be detected in corn stems infected with *Fusarium* in the field (Bottalico, Visconti, Logrieco, Solfrizzo, & Mirocha, 1985) and in rice culture (Richardson et al., 1985). Recently, (Schollenberger et al., 2006) have reported the occurrence of α-ZEA and β-ZEA in corn by-products, corn silage and soya meal at low levels.

Many countries have already laws that legislate the maximum levels of micotoxins in food of human and animal consumption. In Argentina, ZEA was found in grain (Lopez & Tapia, 1980), wheat (Quiroga et al., 1995), corn-based foods (Resnik et al., 1996) and poultry feeds (Dalcero, Magnoli, Chiacchiera, Palacios, & Reynoso, 1997; Dalcero et al., 1998). New data reported the contamination of cow feeding stuffs from Argentina with ZEA at levels from 1.2 to 3.06 mg/kg (Cavaglieri et al., 2005). Therefore, efficient analytical tools for qualitative analysis and quantitation of ZEA in food are required.

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Commonly the quantitative methods of analysis for most *Fusarium* toxins use immunoaffinity clean-up with high performance liquid chromatography (HPLC) or gas chromatography (GC) and enzyme-linked immunosorbent assay (ELISA). These laboratory techniques, unfortunately, require highly qualified personnel, tedious assay time, or sophisticated instrumentation. Therefore, development of a new method with reliability, velocity, high sensitivity and specificity for direct detection of ZEA for in field- application is necessary.

One possible solution involves the use of microbiochips that employ microfluidics. These kinds of devices that use micro electromechanical systems (MEMS) technology have been developed in the last decade, and include lab-on-a-chip (Cho, Han, Paek, Cho, & Paek, 2006), biosensors (Lange, Blaess, Voigt, Gotzen, & Rapp, 2006; Zaytseva, Goral, Montagna, & Baeumner, 2005), and a cell handling system (King et al., 2007). In addition, they have high speed of response, precise, accurate, lower cost and require less operator intervention.

Recently, these microfluidic systems have been integrated with biosensing devices to perform ELISA (Wang & Lee, 2005), electrochemical sensing (Lin et al., 2006), DNA detection (Liang & Mu, 2006; Liao, Lee, Liu, Hsieh, & Luo, 2005), cell detection (Lin & Lee, 2003), and many procedures. The reduction of the amount of solvents required in sample pre-treatment; and the reduction in the amount and the toxicity of solvents and reagents employed in the measurement step, especially by automation and miniaturization have reduced the adverse environmental impact of analytical methodologies (Armenta, Garrigues, & de la Guardia, 2008; McGlennen, 2001, De la Guardia, 1999; Ruzicka & Marshall, 1990).

Heterogeneous enzyme immunoassays, coupled with a flow injection (FIA) system and electrochemical detection, represent a powerful analytical tool for the determination of low levels of many analytes such as antibodies, hormones, drugs, tumour markers, and viruses (Gübitz & Shellum, 1993) because they combine the high specificity of traditional immunoassay methods with the low detection limits and low expense of electrochemical measurement systems (Shen & He, 2007).

Magnetic particles are especially designed for concentration, separation, purification, and identification of molecules and specific cells (Hassen et al., 2008) and they are used as mobile substrates in microfluidic systems because the particles can be selectively functionalized to attach different biomolecules as a pre-treatment procedure to purify and enrich of sample and they can be precisely manipulated using external magnetic field gradients. Such magnetic micro-particles are typically made of a magnetic iron oxide core surrounded by a non-magnetic polymer that can be functionalised with biomolecules of interest (Gijs, 2004).

In this work, we coupled a microfluidic immunosensor to a gold electrode for rapid and sensitive quantification of ZEA in feedstuffs samples. Detection of ZEA was carried out using a competitive direct immunoassay method based on the use of anti-ZEA monoclonal antibodies immobilized on magnetic microspheres 3-aminopropyl-modified. The magnetic microspheres was injected into of microchannels devices and manipulated for external remobilize magnets. The ZEA in feedstuffs sample is allowed to compete immunologically with ZEA bind to horseradish peroxidase (HPR) for the immobilized anti-ZEA specific to ZEA. After washing, enzyme HPR, in the presence of hydrogen peroxide (H_2O_2) catalyses the oxidation of 4-*tert*-butylcatechol (4-TBC) (Ruan & Li, 2001), whose back electrochemical reduction was detected on gold electrode at 0.0 V. The response current obtained from the product of enzymatic reaction is inversely proportional to the activity of the enzyme and, consequently, to the amount of ZEA bound to the surface of the immunomicrofluidic of interest.

2. Materials and methods

2.1. Reagents and solutions

All reagents used were of analytical reagent grade. Mouse monoclonal anti-Zearalenone body (ZER-70) was supplied by Sigma Chemical Company, St. Louis, MO, USA Glutaraldehyde (25% aqueous solution) and H_2O_2 were purchased from Merck, Darmstadt. The micro-particles, magnetic, amino functionalized (53572) were purchased by Fluka, Buchs/Schweiz, USA. Methanol (MeOH) 99%, 98% was purchased by Biopack, Bs As, Argentina. The feedstuffs samples were obtained in feedlots of San Luis City, Argentina. 4-*tert*-Butylcatechol was purchased from Sigma Chemical Co., St. Louis and all other reagents employed were of analytical grade and used without further purifications. Aqueous solutions were prepared using purified water from a Milli-Q system. Veratox® for Zearalenone ELISA Test Kit was purchased from Neogen® Corporation, USA/Canada and was used in accordance with the manufacturer's instructions (Veratox® for ZEA ELISA, 2007).

2.2. Sampling

The feedstuff samples were collected in different feedlot located in San Luis Province, Argentina. Nineteen feedstuff samples of 0.5 kg were collected from the ending composite of feedlot cattle, which were obtained from the output of verticals mixers after a balanced mixture of the food ingredients. For each sample, aliquots of 25 g were used for ZEA detection.

2.3. Flow-through reactor/detector unit

The main body of the sensor was made of Plexiglas. Fig. 1 illustrates the design of the flow-through chamber containing the microfluidic immunosensor and the detector system. The gold layer electrode of 80 nm thickness was deposited at central channel (CC) by sputtering (SPI-Module Sputter Coater with Etch mode, Structure probe Inc., West Chester, PA) and the gold thickness electrode was measured using a Quartz Crystal Thickness Monitor model 12161 (Structure probe Inc., West Chester, PA) (Caruso et al.,1996; Lee, Anandan, & Zhang, 2008).

The diameter of the CC and the accessory channels was 100 μm. The electrode was cleaned and preconditioned using cyclic voltammetry in 0.5 M sulphuric acid by 3-fold cycling in the potential range between −300 and 1200 mV at 100 mVs^{-1} scan rate.

All solutions and reagent temperatures were conditioned before the experiment using a Vicking Masson II laboratory water bath (Vicking SRL, Buenos Aires, Argentina). Amperometric detection

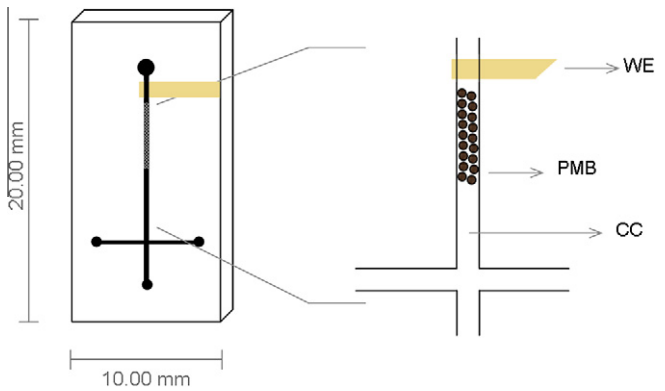

Fig. 1. Schematic representation of microfluidic immunosensorsensor. WE: Gold working electrode, PMB: Paramagnetic beads CC: Central channel. All measurements are given in millimeters.

Table 1
Sequences required for the ZEA immunoassay.

Sequence	Condition	Time
Pre-treatment of samples	Capture antibody coated microbeads + sample	10 min
Washing buffer	PBS buffer, pH 7.2	3 min
Injection of magnetic beads	20 μL of modified magnetic beads 5 μL min^{-1}	4 min
Washing buffer	Flow rate: 5 μL min^{-1} (PBS, pH 7.2)	3 min
Enzyme conjugated	ZEA-HRP conjugated (0.15 μg/ml) 5 μL min^{-1}	5 min
Washing buffer	Flow rate: 5 μL min^{-1} (PBS, pH 7.2)	3 min
Substrate	(5 μL of 0.1 M phosphate-citrate buffer, pH 5.0 containing 1 mM of H_2O_2 and 1 mM of 4-TBC)	1 min
Signal analysis	LC-4C amperometric detector, 0.0 V	1 min

was performed using the BAS LC-4 C (Bioanalytical Systems, West Lafayette, IN, USA). The BAS 100B electrochemical analyzer Bioanalytical Systems) was used for cyclic voltammetric analysis. The potential applied to the gold electrode was 0.0 V versus the Ag/AgCl wire pseudo-reference electrode and a Pt wire was the counter-electrode. At this potential, a catalytic current was well established. Pumps (Baby Bee Syringe Pump, Bioanalytical Systems) were used for pumping, sample introduction, and stopping flow.

All pH measurements were made with an Orion Expandable Ion Analyzer (Orion Research Inc., Cambridge, MA, USA) Model EA 940 equipped with a glass combination electrode (Orion Research Inc.). Absorbance was determined with a Bio-Rad Benchmark microplate reader (Japan) and Beckman DU 520 General UV/VIS spectrophotometer.

2.4. Sample preparation and extraction

Extraction of ZEA from feedstuffs samples is achieved with a mixture of organic solvents, e.g., ethyl acetate, MeOH, chloroform and especially ACN and water. (Krska, Welzig, & Boudra, 2007). The samples were extracted according to Neogen's Mycotoxin Extraction Kit (Veratox® for ZEA ELISA, 2007).

Representative samples of feedstuffs were grinded until that at least 75% of the groun material passes through a 20 mesh sieve, the particle size of a fine instant coffee. 25 g of feedstuffs samples were blended with 125 mL of 70% MeOH (v/v) and were shacked vigorously for 3 min. The extract was filtered through a Whatman #1 filter and collected. Then the filtrate was diluted four fold with purified water and used as sample.

2.5. ELISA for determination of ZEA

A ZEA standard was supplied with the Veratox® for Zearalenone ELISA Test Kit. A standard curve for the spectrophotometric procedure was produced by following the manufacturer's protocol with a range of detection of 0 to 500 pg mL^{-1}. Concentrations of ZEA were detected spectrophotometrically by measuring absorbance changes at 655 nm.

2.6. Immobilization of mouse monoclonal anti-ZEA on magnetic, amine polystyrene beads

Mouse monoclonal anti-ZEA antibody was immobilizing on magnetic microbeads modified with amino groups in an Eppendorf tube.

100 μL of magnetic beads modified amino functionalized were washed with 1.0 mL of phosphate buffer saline (PBS) pH 7.2 for three times. The pellet was suspended in 1.0 mL of an aqueous solution of 5% (w/w) glutaraldehyde at pH 10.00 (0.20 M carbonate) with continuous mixing for 2 hs at room temperature. After three washes with PBS buffer pH 7.2 to remove the excess of glutaraldehyde, 250 μL of antibody preparation (10 μg mL^{-1} 0.01 M PBS, pH 7.2) was coupled to the residual aldehyde groups with continuous mixing for 12 hs at 5 °C. The immobilized antibodies preparation was finally washed with PBS (pH 7.2) and resuspended in 250 μL of the same buffer at 5 °C. Immobilized antibody preparations were perfectly stable for at least 1 month.

2.7. Amperometric analysis of ZEA in feedstuffs samples

This method was applied to determine ZEA in 19 feedstuffs samples. Initially, the immobilized antibodies preparations on magnetic microbeads were conditioned with desorption buffer (0.1 M glycine–HCl, pH 2). The nonspecific binding was blocked by 10 min treatment at 37 °C with 3% skim milk in a 0.01 M phosphate buffer saline (PBS), pH 7.2 and finally washed with 0.01 M PBS buffer (pH 7.2) and stored in 250 μL of the same buffer. After that, 10 μL of modified magnetic microbeads were mixed with 1000 μL of sample filtrate and shaker for 10 min at room temperature. ZEA present in the sample was allowed to react immunological with the modified magnetic beads so they feature a large binding surface area per volume and consequently a large number of analytes molecules can be bound in a small final volume, allowing a sensitive detection (Pamme, 2006; Verpoorte, 2003). Then, the magnetic microbeads were washed three times with 0.01 M PBS buffer (pH 7.2) to remove the excess of sample and resuspended in 250 μL of 0.01 M PBS (pH 7.2).

The microfluidic device was prepared by injection of 20 μL magnetic microbeads in the flow system by a micro pump at a flow rate of 5 μL min^{-1} for 4 min. A permanent magnet was used to attract the beads at specific area of the channel, near of the gold electrode plate. The magnet was not moved during the experiment to keep the beads into the channel and they were not carried away by the continuing flow.

The carrier buffer was 0.01 M PBS, pH 7.2. A solution of ZEA-HPR conjugate at a concentration of 0.15 μg/mL (0.01 M PBS, pH 7.2) was injected into the PBS carrier stream at a flow rate of 5 μL min^{-1} for 5 min at 25 °C. The immunosensor was washed with 0.1 M citrate buffer, and 5 μL of substrate solution (1 mM H_2O_2 and 1 mM 4-TBC in 0.1 M phosphate-citrate buffer, pH 5) was injected into the carrier stream and the enzymatic product (4-terbuthyl-o-quinone) was measured on the surface of a gold electrode.

A standard curve for the amperometric procedure was produced by following our protocol with a sequence of standard dilutions that covered the range 0–500 μg $kg^{-1}$of ZEA, supplied with the Veratox® for Zearalenone ELISA Test Kit. Amperometric measurements were performed at 0.0 V at room temperature in 0.1 M phosphate-citrate buffer, pH 5, and the resulting cathodic current was displayed on the x–y digital recorder. The stock solution of 4-*tert*-butylcatechol was prepared freshly before the experiment and stored in the dark for the duration of the experiment.

3. Results and discussion

3.1. Electrochemical study of 4-TBC with the gold electrode

The electrochemical behaviour of enzyme substrate 4-*tert*-butylcatechol (4-TBC) was examined by cyclic voltammetry at the gold electrode. A cyclic voltammetric study of 1 mM solution of 4-TBC in an aqueous solution containing 0.1 M phosphate-citrate buffer, pH 5.0, was performed by scanning the potential from –200 to 550 mV versus Ag/AgCl. The cyclic voltammogram showed a well-defined anodic peak and a corresponding cathodic peak, which corresponds to the transformation of 4-TBC to

4-tertbutyl-o-benzoquinone (Q) and vice versa in a quasi-reversible two-electron process (Bard & Faulkner, 2001) (Fig. 2). A peak current ratio (Ip,a/Ip,c) of nearly unity, particularly during the recycling of potential, can be considered a criterion for the stability of Q produced at the surface of electrode under experimental conditions.

3.2. Optimum conditions for the immune reactions and the determination of enzymatic products

Bead-based immunoassays, which are very easy to use in conjunction with microchips, provide several advantages over conventional techniques, such as easy of manipulate and high reaction efficiencies when both molecules of various dimensions (DNA and protein) and cells are assayed (Bienvenue, Duncalf, Marchiarullo, Ferrance, & Landers, 2006; Choi et al., 2002; Lim & Zhang, 2007; Sato et al., 2002). Nevertheless, various factors that affect the biochemical reaction must be considered because the reaction conditions in the microbiochip are different than those of conventional microtubes or well plates.

It has been shown that the theoretical framework developed for a static ELISA system cannot be used to describe the kinetics of antibody–antigen interactions occurring in a continuous-flow immunoassay (Wemhoff et al., 1992). Furthermore, the use of protein-coated nano- or micro-particles offers additional advantages like an enhanced specific binding surface (Kawaguchi, 2000; Verpoorte, 2003), a high active surface per volume permitting to improve of sensitivity.

The proposed method manifolds follow enzyme immunoassay principles and the magnetic microbeads were used as solid supports for immunological reaction. The use of capture antibody binding to magnetic beads as a pre-treatment procedure allows purifying and enriching of ZEA from biosamples.

Microfluidic control systems are essential for the control of a flow rate of fluid because of their rapid and precise control features, therefore, in our microbiochip, all reactions and washing procedures were performed using a syringe pump. The flow rates of the sample and reagent have an effect on the reaction efficiencies of the antigen–antibody interactions, and unlike conventional immunoassays, samples and reagents in our system are continuously flowing through the microbiochips. Therefore, it is very important to consider flow rate when designing microfluidic biosensors (Maeng et al., 2008).

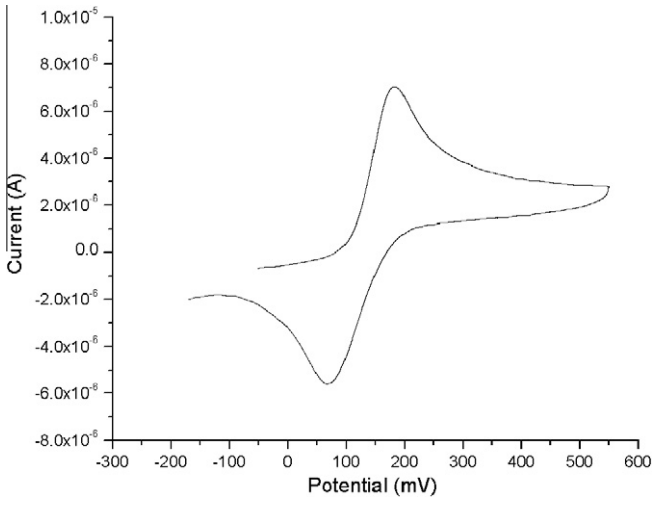

Fig. 2. Cyclic voltammogram of 4-TBC in aqueous solution containing 1 mM of 4-TBC in 0.10 M phosphate–citrate buffer, pH 5.0 with a gold electrode. Scan rate: 100 mV s^{-1}.

The optimal flow rate was determined by analyzing a standard of 150 μg kg^{-1} ZEA at different flow rates and evaluating the current generated during the immune reaction. Flow rates from 1 to 5 μL min^{-1} had little effect on antigen–antibody reaction. Conversely, when the flow rate exceeded 10 μL min^{-1}, the signal was dramatically reduced. Therefore, a flow rate of 5 μL min^{-1} was used for injections of reagents and washing buffer. The response current obtained from the product of enzymatic reaction is proportional to the activity of the enzyme conjugated and, consequently, conversely proportional to the amount of ZEA in feedstuffs samples bound to magnetic microbeads modified with ZEA-specific antibodies.

The volume of magnetic beads injected after the pre-treatment sample procedure was studied in the range of 5–20 μL. Sensitivity was almost tripled in the range 5–20 μL. Over 20 μL of magnetic beads dilutions, the central channel was obstructed. Finally a sample volume of 20 μL was used to evaluate other parameters.

The rate of enzymatic response under flow conditions was studied in the pH range 4–7 and reached a maximum at pH 5.0. The pH value used was 5.0 in phosphate-citrate buffer. The effect of varying 4-TBC concentration from 0.1 to 5 mM on the enzymatic response was evaluated. The enzymatic response was increased from 0.1 to 1 mM. Over 1 mM the signal did not increase. The optimum enzymatic response was obtained when we used 4-TBC 1 mM. This concentration was used to evaluate other parameters.

3.3. Quantitative test for the detection of ZEA in the microfluidic immunosensor

Under the selected conditions described above, the electrochemical response of the enzymatic product is conversely proportional to the concentration of ZEA in the feedstuffs sample. Table 1 summarises the complete analytical procedure required for the ZEA immunoassay using our system. A linear calibration curve to predict the concentration ZEA in feedstuffs sample was produced within the range 0–500 pg mL^{-1} using an ZEA standard supplied with the ELISA Test Kit. The linear regression equation was $i = 292.37 - 0.463^{*} C_{ZEA}$, with the linear regression coefficient $r = 0.998$. The coefficient of variation (VC) for the determination of 150 μg kg^{-1} de ZEA was below 4.1% (six replicates). These values demonstrate that our microfluidic immunosensor can be used to quantify the amount of ZEA in unknown samples. An ELISA was also carried out as described, absorbance changes were plotted against the corresponding ZEA concentration, and a calibration curve was constructed. The linear regression equation was $A = 0.860–0.0013^{*} C_{ZEA}$, with the linear regression coefficient $r = 0.995$, and the VC for the determination of 150 μg kg^{-1} ZEA was 6.86% (six replicates). The limit of detection (LOD) was considered to be the concentration that gives a signal three times the standard deviation (SD) of the blank. For electrochemical detection and EIA, the LODs were 0.41 and 2.56 μg kg^{-1}, respectively. This result shows that electrochemical detection was more sensitive than the spectrophotometric method. Sensitivity (S) is defined as

Table 2
Within-assay precision (five measurements in the same run for each control sample) and between-assay precision (five measurements for each control sample, repeated for three consecutive days).

[a]Control	Within-assay		Between-assay	
	Mean	VC%	Mean	VC%
25	280.93	3.61	281.61	4.91
150	214.47	2.74	212.04	5.62
500	63.75	4.06	67.13	6.41

[a] μg kg^{-1} ZEA.

Table 3
Determination of ZEA in original and spiked feedstuffs samples and controls.

ZEA spiking level (μg kg^{-1})					
Sample N°	ZEA	25	Recovery (%)	150	Recovery (%)
S1	234.42 ± 3.14	259.96 ± 3.31	102	388.53 ± 4.22	103
S2	103.48 ± 2.19	127.97 ± 2.66	98	257.71 ± 3.52	102.8
S3	312.07 ± 2.98	338.38 ± 2.27	105.2	467.55 ± 3.48	103.6
25 (μg kg^{-1})	25.17 ± 1.51	51.16 ± 2.32	104	182.29 ± 3.47	104.7
150 (μg kg^{-1})	151.87 ± 3.52	177.24 ± 2.72	101.5	305.82 ± 2.77	102.6
500 (μg kg^{-1})	504.43 ± 4.01	528.51 ± 3.64	96.4	664.96 ± 3.83	107

Representative samples of feedstuffs (S) and ZEA controls solutions were spiked separately with 25 and 150 μg kg^{-1} of ZEA.

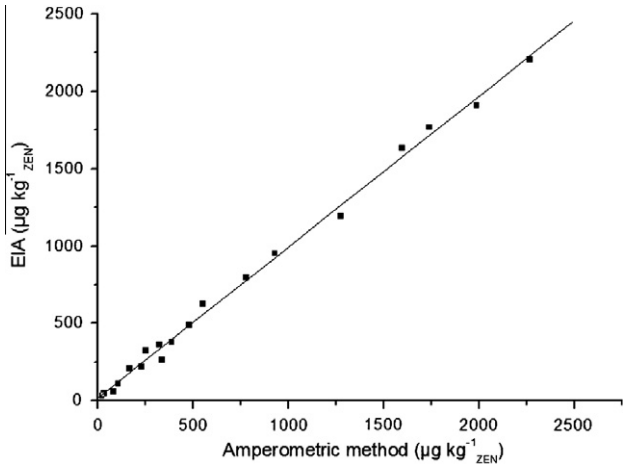

Fig. 3. Correlation between proposed method and commercial photometric assays.

the slope of the regression line of the signal-versus-concentration plot. For electrochemical detection and ELISA, *S* values were 0.463 nA/μg kg^{-1} and 0.0013 Abs/μg kg^{-1}, respectively.

The precision of the electrochemical assay configured VC was checked with control sample at ZEA concentrations of 25, 150, and 500 μg kg^{-1}. The within-assay precision was tested with five measurements in the same run for each serum. These series of analyses were repeated for 3 consecutive days to estimate between-assay precision. The results obtained are summarised in Table 2. The ZEA assay showed good precision; the VC within-assay values were below 4.1% and the between-assay values below 6.5%.

This method was applied to determine ZEA in 19 feedstuffs samples. The validation of the electrochemical assay was checked with control solutions at ZEA concentrations of 25, 150, and 500 μg kg^{-1} and three feedstuffs samples (Table 3). Each feedstuffs samples and spiked sample was extracted and treated as described above. All extracted samples and controls were analysed for ZEA. Concentration results were plotted against spike levels and interpolated using weighted linear regression (Table 3).

The electrochemical system was compared with a commercial spectrophotometric system for the quantification of ZEA in feedstuffs samples. The slopes obtained were reasonably close to 1, indicating good correspondence between the two methods (Fig. 3). Compared with ELISA, our method shows large enhancement in sensitivity and its sensitivity is high enough to determine ZEA in unknown samples with very low levels.

4. Conclusions

In this work, we have developed a microbiochip microfluidic immunosensor coupled with flow injection (FIA) system that can be used for the rapid sensitive and selective quantification of ZEA in feedstuffs samples using electrochemical detection. In this system, a gold electrode was used to measure an electrical signal and a permanent magnet was used to fix efficiently the microbeads into the central microchannel. Compared with ELISA commercial analysis, the use of magnetic beads, modified with a specific anti-ZEA antibody as a pre-treatment procedure to purify and enrich the sample, shown a large enhancement in sensitivity with no reduction on the selectivity, being these important advantages. Also minimises the waste of expensive reagents; shows physical and chemical stability, low background current, wide working potential range, and accuracy. We took advantage of the simplicity of the ELISA system to construct an immunosensor that was capable of measuring the same levels of ZEA in cereal samples as detected by the conventional methods while having the advantages of low detection limit, speed and simplicity. Owing to the wider applications in many fields, miniaturized immunosensors will make a significant contribution to faster, direct and secure analysis of chemicals, pathogens and biological molecules.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2010.09.035.

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