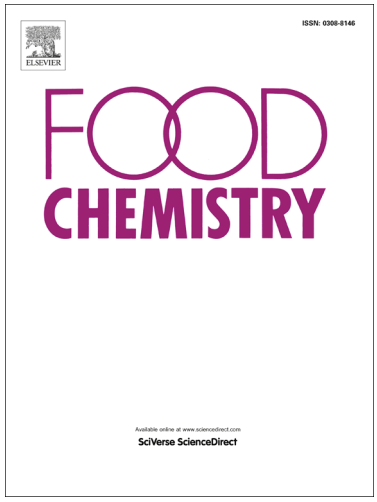

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Simultaneous Detection of Aflatoxin B1, Ochratoxin A, Zearalenone and Deoxynivalenol in Corn and Wheat Using Surface Plasmon Resonance

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Notes

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ABSTRACT

Mycotoxins are toxic metabolites produced by fungi or molds, which may cause serious harm to human health through polluted cereal foods. In order to measure the typical mycotoxin contaminations in wheat and corn, a surface plasmon resonance (SPR) method was established using SPR sensor chip that was fabricated based on self-assembled monolayer. The minimum detection limit of aflatoxin B1, ochratoxin A, zearalenone and deoxynivalenol were identified as 0.59 ng/mL, 1.27 ng/mL, 7.07 ng/mL and 3.26 ng/mL, respectively. The cross-reactivity for all four mycotoxins were demonstrated to be low. Moreover, the test data were compared with HPLC-MS/MS confirmatory analysis results and good agreement was found between them. In conclusion, the SPR method for simultaneously detecting four mycotoxins has been developed with high sensitivity, good linearity and specificity, which can meet the detection requirements of cereal foods.

Keywords: Mycotoxins, Surface plasmon resonance, Competitive immunoassay, Biosensor

1. Introduction

Mycotoxins are toxic secondary metabolites produced by toxigenic fungi under suitable environmental conditions. They are known as mold toxins in the fields of food hygiene and feed hygiene (Adányi, et al., 2018). Mycotoxins are widely found in peanuts, beans, peas, corn, wheat and other foods (Jiang, et al., 2018). These compounds are chemically stable at high temperature and may not be degraded in the processing of agricultural products (Xing, et al., 2017). Food or feed contaminated by mycotoxins may cause acute and chronic poisoning, including hepatotoxicity, carcinogenic, teratogenic and mutagenicity (Kolpin, et al., 2014; Y. Xu, et al., 2018).

Aflatoxin B1 (AFB1), ochratoxin A (OTA), zearalenone (ZEN) and deoxynivalenol (DON) (chemical structures are shown in Fig. S1) are typical contaminants in corn and wheat. The maximum limits of these mycotoxins have been set in many countries and regions (Song, et al., 2014). In China, the maximum residue levels by regulation are: OTA (5 μg/kg), DON (1000 μg/kg) and ZEN (60 μg/kg) in cereals and their products (National Health and Family Planning Committee of China, 2017). For AFB1, the maximum residue level was 5 μg/kg in wheat, barley and their products, while 20 μg/kg in corn, cornmeal and corn products. Likewise, EU has regulated a maximum residue level of AFB1 (2 μg/kg), OTA (3 μg/kg), ZEN (75 μg/kg) and DON (750 μg/kg) in all cereals and all products derived from cereals. Given of the low permissible limit and wide existence of these mycotoxins, it is important to simultaneously and quantitatively detect these mycotoxins for food safety. Current analysis of mycotoxins mainly relies on high-pressure liquid chromatography (HPLC)

or gas chromatography(GC) in combination with UV-VIS spectroscopy or mass spectrometry(MS) (Brückner & Reinecke, 2015; Lattanzio, Ciasca, Powers, & Visconti, 2014; Malachová, Sulyok, Beltrán, Berthiller, & Krska, 2014). Rapid screening assays based on immunoassays also have been developed, which include enzyme-linked immunosorbent assays (ELISA) (Gross, Asam, & Rychlik, 2017), lateral flow devices (Anfossi, et al., 2018), fluorescence polarization immunoassays (X. Zhang, et al., 2017) and an optical waveguide biosensor (Nabok, Al-Jawdah, & Tsargorodska, 2017). Although a lot progress has been achieved for these instrumental or rapid methods, challenges remain in real application (supplemental Fig. 1). As an alternative method to the above methods, surface plasmon resonance (SPR) is a rapid label free detection method (Park, et al., 2014).

SPR technique have many advantages including high selectivity, high sensitivity, high throughput, real-time monitoring and low sample consumption (Li, Liu, & Lin, 2012; Patel, et al., 2017). The Nielen group established a competitive inhibition immunoassay using imaging SPR technique for the multiplex-detection of DON and ZEN in corn and wheat samples (Wu, et al., 2012). Lately, they detected ZEA, DON, OTA, T-2 and AFB1 in beer and barley samples employing gold nanoparticle (AuNP)-enhanced imaging SPR (iSPR) chip (Joshi, et al., 2016). The system can realize in-field or at-line detection of DON without pre-concentration. The limits of detection (LOD) DON and OTA in beer were 17 ng/mL and 7 ng/mL, respectively. The LODs of ZEA, DON, OTA, T-2 and AFB1in barley were 6 ng/g, 26 ng/g, 3 ng/g, 0.6 ng/g and 0.6 ng/g, respectively. This method achieved a rapid and semi-

quantitative screening for multiplex mycotoxins. Meanwhile, an sensitive iSPR method was established based on gold chip surface setting gold nanoparticles as signal amplification tags (Chen, Hu, Sun, Li, & Qin, 2016). The LODs of ZEN, AFB1 and OTA in adulterated peanut oil were 15 pg/mL, 8 pg/mL and 30 pg/mL, respectively. As mentioned above, SPR techniques exhibited potential application prospect in simultaneous detection of multiple mycotoxins.

SPR method has not been reported for simultaneous detection of four mycotoxins in corn and wheat. Therefore, the aim of this work is to shed light on a rapid and high-throughput detection of mycotoxins in corn and wheat samples, and to develop a SPR sensor chip based on self-assembled monolayer (SAM) in the form of hydrazone connection.

2. Materials and methods

2.1. Materials and reagents

Antigens of AFB1, OTA, ZEN, DON as well as antibodies of AFB1, OTA, ZEN, DON and SPR two-dimensional sensor chip were homemade by laboratory according to procedures described by Fig. 1. Mycotoxin standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Thiol-PEG-Carboxyl (SH-PEG-COOH) and Thiol-PEG-Hydroxyl (SH-PEG-OH) were purchased from Yare Biotech (Shanghai, China). Glycerol was purchased from Amresco (Ohio, USA). N-hydroxysuccinimide (NHS), 1-ethyl (3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), ethanolamine and other chemicals were purchased from Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China).

2.2. Apparatus

SPR analysis was carried out using SPR imager IID (GWC, USA). SPR sensor chip was cleaned using PDC-32G-2 plasma cleaner (Harrick Plasma, USA). Deionized water was obtained from nanopure water purification system (Millipore, USA).

2.3. Sensor chip modification

The antigen was immobilized on the surface of the sensor chip by SAM in the form of hydrazone connection. Carboxymethyl groups were modified when the mixed solution of NHS and EDC attached to N-hydroxysuccinic acid esters on the surface of the chip. Amine or other nucleophilic groups on the toxin structure were further covalently attached to N-hydroxysuccinicester, which is fixed on the sensor chip.

2.3.1 Sensor chip cleaning

In order to clean dust, the chip surface was rinsed by deionized water and ethanol successively. Then the chips were taken face up into plasma cleaner for 10 min before usage.

2.3.2 Coupling carboxyl and immobilizing antigen

Chips were immersed in PEG mixture and held for a minimum of 12 days at 25 °C in the dark. After washing with deionized water and absolute ethanol and drying with nitrogen gas, the chips were immersed in the activation solution and shaked for 30 min at 25 °C. Then the chips were rinsed with deionized water and dried with nitrogen gas. Finally, mycotoxin antigen (0.2 μL/dot) was labeled on the chip surface and held for 2 h at 25 °C. The spotted chips were held in a humid environment to avoid antigen inactivation.

2.4. SPR analysis

The antigen-loaded chip was loaded in SPR biosensor. Detection temperature was 25 °C. PBS solution was injected into SPR biosensor at a constant speed for 5 min. After the baseline was stabilized, the ethanolamine solution was introduced for 10 min to block the activated carboxyl groups of the unattached antigen. Then PBS solution was injected into SPR biosensor at a constant speed for 5 min. After the baseline was stabilized, a mixture of 1 mL sample and 5 μg mycotoxin antibody was introduced and adjusted to low flow rate. The electrical signal value was recorded.

2.5. Antigen-antibody binding optimization

Mycotoxin antigens were diluted according to concentration gradient. AFB1 antigen was diluted to 1600, 800, 400, 200, 100, 50, 25 and 12.5 ng/mL. OTA antigen

was diluted to 1400, 700, 350, 175, 87.5, 43.75 and 21.87 ng/mL. ZEN antigen was diluted to 1800, 900, 450, 225, 112.5, 56.25 and 28.13 ng/mL. DON antigen was diluted to 2100, 1050, 525, 262.5, 131.25, 65.63 and 32.82 ng/mL. After antigens were fixed on sensor chip, the corresponding antibodies at different gradient concentrations were introduced into sensor. The degree of antigen-antibody binding was detected by the SPR biosensor.

When baseline was stable, PBS buffer solution was injected into SPR biosensor. 1 mL of 5 μg/mL antibody solution was injected at a flow rate of 1 μL/s. The displayed signal value was recorded when antigens combined with their corresponding antibodies. The binding index was calculated by the difference between displayed signal value and baseline value. The binding index indicated the degree of antigen-antibody binding. The optimal concentration of mycotoxin antigens was determined based on the highest binding index.

2.6. Establishment of SPR standard competition curve

1 mL of four mycotoxin antigen at gradient concentration was prepared, respectively. 5 μg corresponding mycotoxin antibody was added to bind antigen. Binding signal was obtained by SPR detection.

The binding signal was represented by B_0 when competitive antigen concentration was 0 ng/mL. Similarly, the binding signal was represented by B when the competitive antigen was at other concentrations. Standard competition curve was plotted via setting the logarithm of the competing antigen concentration as abscissa and the value of B/B_0 as the ordinate. Through the regression curve equation and the

correlation coefficient, the LOD and the detection range (IC_{20}-IC_{80}) were calculated.

2.7. SPR-specific assay

The specificity of SPR was tested against other mycotoxins as follows: aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G1 (AFG2), α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), 15-acetyl-DON (15-AcDON), nivalenol (NIV), ochratoxin B (OTB), ochratoxin C (OTC), fumonisin B1 (FB1) and fumonisin B2 (FB2). The corresponding IC_{50} was determined and calculated. The cross-reactivity (CR) was calculated as percentage of the IC_{50} of each target mycotoxin divided by the IC_{50} of other mycotoxins.

2.8. Method validation

100 g blank corn and wheat samples were ground to uniform powder. Then 3 g grounded sample was weighed into 50 mL centrifuge tubes. Spiking solution of mycotoxins was added to the tubes to provide corresponding concentrations (Table 3). After 30 min, 10 mL water solution containing 20 % methanol was added to each tube. Then the tubes were vortexed for 1 min and shaken for 5 min at 1000 rpm. The tubes were centrifuged at 4 ^{o}C, 12000 rpm, for 10 min. 200 μL supernatant was mixed with 800 μL PBS buffer and then taken to SPR analysis. The analytical cycle was repeated 3 times. According to the test results, the recovery and standard deviation (RSD) for each concentrations of mycotoxins were calculated.

2.9. HPLC-MS/MS analysis

Sample treatment and spiking levels were the same as *2.8*. The HPLC-MS/MS system consisted of a Shimadzu (Kyoto, Japan) HPLC system equipped with a

QTRAP 5500 triple quadrupole mass spectrometer of ABI Sciex (Foster City, USA). An Ultra Aqueous C18 column (100×2.1 mm, 3.0 μm particle size) was employed for separation of the target compounds. Mobile phases were water containing 1 % formic acid and 0.1 % ammonium formate (phase A), and methanol containing 5 % water, 1 % formic acid and 0.1 % ammonium formate (phase B). The flow rate of mobile phase was kept at 0.4 mL/min with the following linear gradient elution. The proportion of phase B maintained at 0% for 1 min, then linearly increased from 50% to 100% in 1 min and kept constant for 6 min. The column was equilibrated with 0% phase B for 3 min before the next injection. The overall time between two consecutive injections was 11 min. The column temperature was 35 °C and the injection volume was 10 μL. AFB1, OTA and ZEN were ionized at negative ESI mode, while DON were ionized at positive ESI mode. All of them were detected using multiple reaction monitoring mode. Capillary and inlet voltage were 5000 V and 10V, respectively. Source gas temperature and sheath gas temperature were 350 °C and 400 °C, respectively. Desolvation gas and collision induced dissociation gas were nitrogen and argon, respectively. MS/MS parameters are as follows: AFB1 $[M+H]^+$ m/z 313→285 and m/z 313→128, OTA $[M+H]^+$ m/z 404→239 and m/z 404→102, DON $[M+H]^+$ m/z 297→231 and m/z 297→249, ZEN $[M-H]^-$ m/z 317→175 and m/z 317→131. The concentration of mycotoxins was calculated by standard addition method.

3. Results and Discussion

3.1. Cross-reactivity profile

According to Table 1, the remaining mycotoxins did not cross-react except some mycotoxins had low cross-reaction rate with their metabolites. As we know, antibodies can specifically recognize and bind to antigens. However, antibodies could be less recognizable for substances with similar chemical structure. As shown in cross-reactivity assay, the low cross-reaction rate of antibodies revealed desirable specificity of the detection method (Moon, et al., 2018). Some commercial test kits also demonstrated low cross-reactivity profile towards masked toxins (Chen, Hu, Sun, Li, & Qin, 2016; Tangni, Motte, Callebaut, & Pussemier, 2010). Therefore, it was shown that the low cross-reaction rate of antibodies guaranteed a high specificity in our SPR method.

3.2. Optimization of antigen concentration

A sensor chip image of the four mycotoxin antigens were shown in Fig. S2. Antigens were spotted on the chip surface with different concentrations. The adsorption sites of mycotoxin antigens can be clearly observed. Moreover, the antigen concentration is positively correlated with the brightness of the spot. The results indicated the optimal antigen concentrations of AFB1, OTA, ZEN and DON were 100 ng/mL, 87.5 ng/mL, 112.5 ng/mL and 131.25 ng/mL, respectively. Antigen adsorption on the sensor chip surface is a complex process. The insufficient antigen concentration may cause the relatively scarce amount of antigen on the chip surface, which may weaken the antigen-antibody binding and result in poor stability of the

combination. On the contrast, the excessive antigen concentration will cause steric hindrance, decreasing antigen-antibody hybridization efficiency. Therefore, the determination of a suitable concentration of antigen for stabilizing the binding of antigen-antibody and achieving high binding efficiency is indispensable (Dorokhin, Haasnoot, Franssen, Zuilhof, & Nielen, 2011).

3.3. SPR standard competition curve

Typical SPR spectra and the standard curve of ABF1 was shown in Fig. 2 and Fig. 3A, respectively. The detection range was 0.99-21.92 ng/mL (Table 2). The IC50 and LOD was 4.67 ng/mL and 0.59 ng/mL, respectively. This LOD was better than LOD (2.51 ng/mL) of palm-sized SPR detection (Moon, Byun, Kim, Lim, Jeong, et al., 2018), which was comparable to LOD (0.1 ng/mL) of HPLC-MS/MS (Sadhasivam, et al., 2017). In terms of detection range, the current method was similar or better than AFB1 determination based on competitive dispersion of gold nanorods (X. Xu, Liu, Li, & Ying, 2013) and fluorescent sensor systems based on nanostructured polymeric membranes (Sergeyeva, et al., 2017). The standard curve of OTA was shown in Fig. 3B. The detection range was 1.98-28.22 ng/mL (Table 2). The IC50 and LOD was 7.48 ng/mL and 1.27 ng/mL, respectively. This LOD or detection range was better than those of nanogold-based lateral flow immunoassay detection (Moon, Kim, & Lee, 2013) and label-free quartz crystal microbalance immunosensor detection (Pirinçci, et al., 2018). This linear relationship was better than that of broad-specificity photoelectrochemical immunoassay detection (R^2=0.986) (Qileng, et al., 2018). The standard curve of ZEN was shown in Fig. 3C. The detection range was 10.37-103.31

ng/mL (Table 2). The IC50 and LOD was 32.73 ng/mL and 7.07 ng/mL, respectively. This LOD was similar with rapid immunechromatographic strip detection in maize (F. Zhang, et al., 2018), where its LOD was 1.5 ng/mL. The standard curve of DON was shown in Fig. 3D. The detection range was 5.31-99.37 ng/mL (Table 2). The IC50 and LOD was 22.96 ng/mL and 3.26 ng/mL, respectively. This LOD was better than ELISA method (Y. Xu, Yang, Huang, Li, He, et al., 2018), where its LOD was 9.83 ng/mL.

As mentioned above, the established SPR method was proved having high sensitivity. The LOD of ZEN, AFB1, DON and OTA were lower than the minimum residue limit of China national standard and commission regulation of EU.

3.4. Initial in-house validation

As shown in Table 3, average recoveries and RSD of AFB1 in corn samples ranged from 101.33% to 106.67% and from 4.96% to 9.38%, respectively. Average recoveries and RSD of AFB1 in wheat samples ranged from 92.67% to 104.89 % and from 4.45% to 9.80 %, respectively. According to recent report, RSD of a novel electrochemical piezoelectric label free immunosensor used for AFB1 detection in ground nut was 4.78% (Chauhan, et al., 2015). RSDs of a novel electrochemical immunosensor using single-walled carbon nanotubes/chitosan for AFB1 detection in corn ranged from 4.4% to 8.4% (Xian, et al., 2016). Comparatively, RSD of this SPR detection method is similar to other immunosensor methods.

Average recoveries and RSD of OTA in corn samples ranged from 102.27% to 111.17% and ranged from 3.63% to 3.71%, respectively. Average recoveries and RSD

of OTA in wheat samples ranged from 95.00 % to 98.50 % and from 2.52 % to 8.81 %, respectively. Recent study showed that recovery of broad-specificity photoelectrochemical immunoassay detection ranged from 79.6 to 201 % (Qileng, Wei, Lu, Liu, Cai, et al., 2018). Recovery and RSD of a magnetically controlled fluorescence aptasensor detection in corn ranged 95-103 % and 5.6-7.2 % (Qian, et al., 2018). Recovery and RSD of one-step detection by dot immunoassay using a nanobody-alkaline phosphatase fusion protein in cereal ranged 93.7-101% and 7.5-9.8% (Tang, Wang, Lv, Hu, & Liu, 2018). Recovery and RSD of highly-stable anti-fouling black phosphorene nanosensor detection in beer and grape juice ranged 98.8-103.3 % and 2.71-4.01 % (Xiang, et al., 2018). By comparison, our SPR method showed a preferable accuracy than other sensor or immunoassay detection methods.

Average recoveries and RSD of ZEN in corn samples ranged from 90.58% to 103.67% and from 4.04% to 8.09%, respectively. Average recoveries and RSD of ZEN in wheat samples ranged from 89.25% to 100.22% and from 3.89% to 9.74%. The past studies had showed that recovery of fluoroimmunoassay detection using CdTe/CdS/ZnS quantum dots in maize ranged 82.6-105.2% (F. Zhang, Liu, Sheng, Zhang, Liu, et al., 2018). Recovery of a novel bioassay based on aptamer-functionalized magnetic nanoparticle detection ranged 90.4-114.75% in wheat and 80.76-119.66% in maize (Niazi, et al., 2018). Compared with fluoroimmunoassay and bioassay detection methods, our SPR method achieved superior recovery and RSD.

Average recoveries and RSD of DON in corn samples ranged from 88.33% to 104.13% and from 3.63% to 8.99%. Recoveries and RSD of DON in wheat samples

ranged from 92.44% to 103.60% and from 5.02% to 9.73%. This recovery is better than recoveries of 87.39-106.88% and 101.43-113.72% performed by immunological detection method in corn and wheat (Y. Xu, Yang, Huang, Li, He, et al., 2018).

The calculated average recoveries were between 85% and 115%, and the RSDs were all less than 10 %, meeting the requirement of food safetyand commission regulation EC No. 1881 (Commission Regulation (EC), 2006). The result indicated that established method is accurate and highly sensitive compared with recently developed SPR immunoassays (Lee, Park, Byun, Kim, & Kim, 2017; Rehmat, Mohammed, & Anal, 2018; Sun, Wu, & Zhao, 2017).

3.5. HPLC-MS/MS validation

As shown in Table 3, values of concentration detected by both SPR method and HPLC-MS/MS method were near to the corresponding spiked concentrations. The variance between SPR method and HPLC-MS/MS method were within 10 % in most cases, which suggested that accuracy of established SPR method is comparable to HPLC-MS/ MS (Dorokhin, Haasnoot, Franssen, Zuilhof, & Nielen, 2011).

4. Conclusions

A simultaneous detection method of multi-residue using SPR technique was established to quantify AFB1, OTA, ZEN and DON in corn and wheat with high sensitivity, accuracy and specificity. The LODs ranged from 0.59 to 3.26 ng/mL and the RSDs were less than 10%, which can meet the requirement of food safety. The results obtained from the current SPR method were in good agreement with those from HPLC-MS/MS method. but SPR method has the advantages of fast and

convenient, real-time monitoring and minimum sample pretreatment. In the future, with the further development of technology research, the method may also be applied to other fields such as biology and medical areas along with potential market development prospects.

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Legends

Fig. 1 A**:** Schematic illustration of SPR sensor chip modification process. B: Schematic illustration of optical setup and sensor chip in SPR working system. Signal was represented by change in reflectivity (%) (ΔR%)

Fig. 2 Typical SPR spectra of mycotoxin standard solution

Fig. 3 Standard curve line of AFB1 (A), OTA (B), ZEN (C) and DON (D)

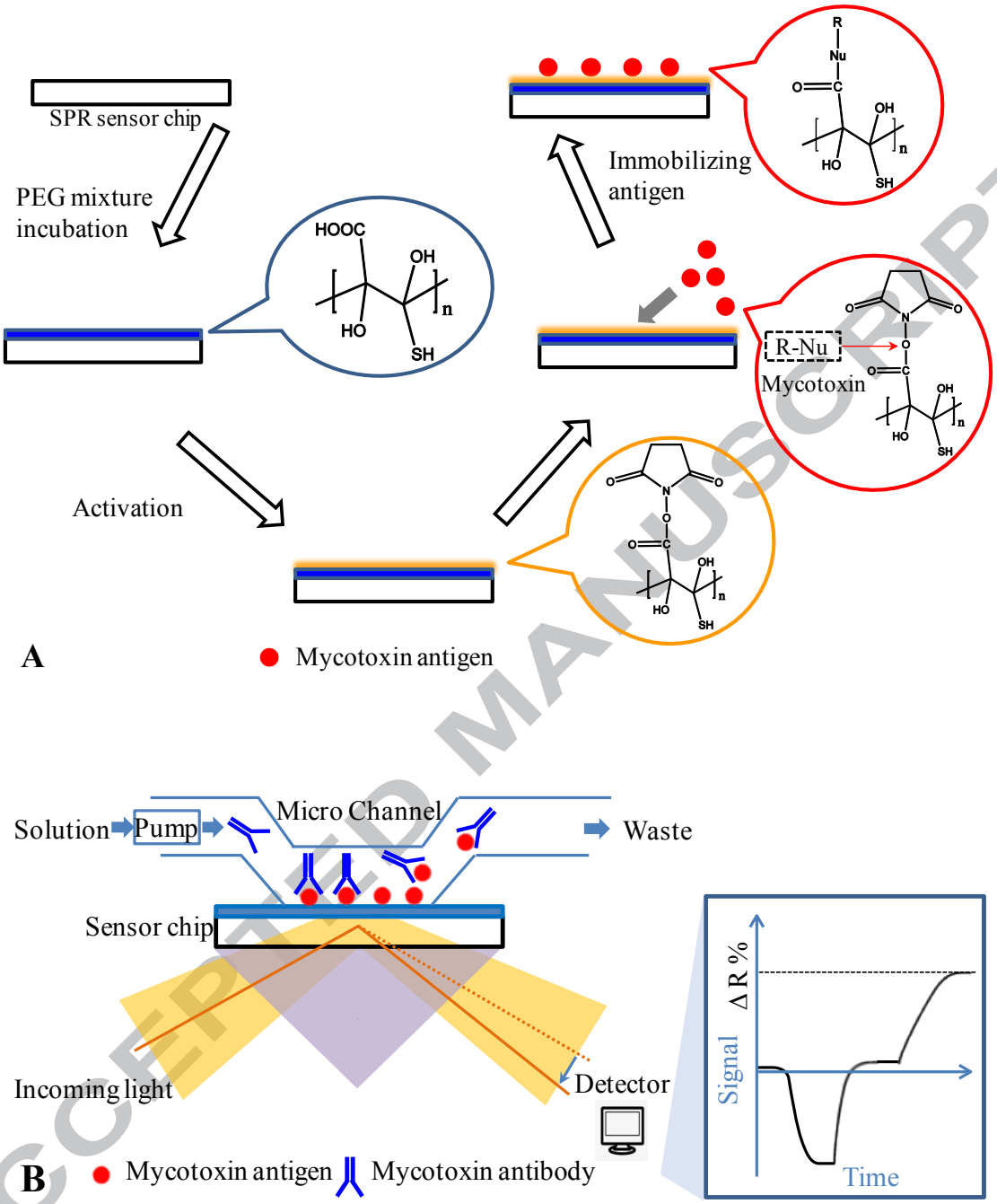

Fig. 1

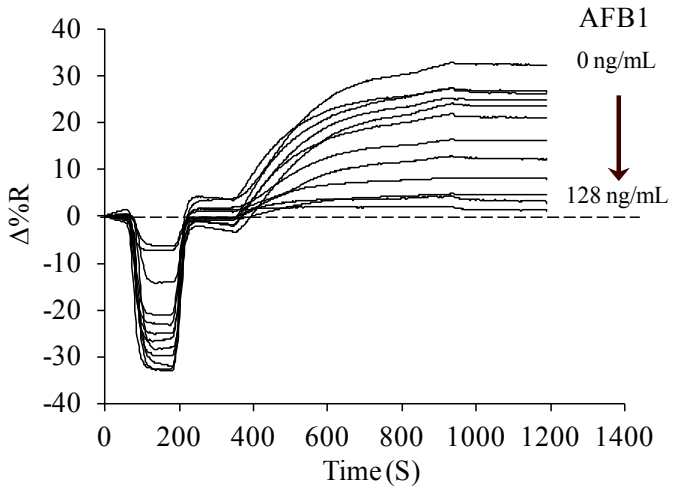

Fig. 2

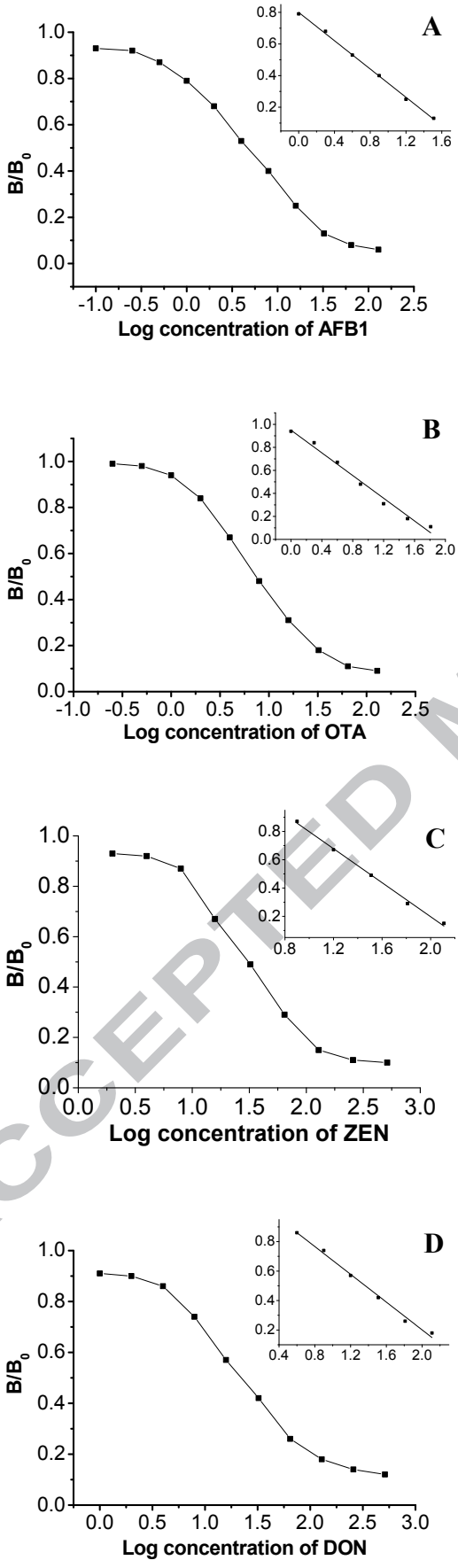

Fig. 3

Tables

Table 1

The cross-reactivity result of AFB1, OTA, ZEN and DON by SPR test

Mytoxins	Cross-reaction rate (%)			
	AFB1	ZEN	DON	OTA
AFB1	100	0	0	0
ZEN	0	100	0	0
DON	0	0	100	0
OTA	0	0	0	100
AFB2	19.1	0	0	0
AFG1	17.2	0	0	0
AFG2	4.4	0	0	0
a-ZEL	0	15.3	0	0
P-ZEL	0	11.5	0	0
15-AcDON	0	0	16.2	0
NIV	0	0	0	0
OTB	0	0	0	6.2
OTC	0	0	0	1.8
FBI	0	0	0	0
FB2	0	0	0	0

AFB1: aflatoxin B1, OTA: ochratoxin A, ZEN: zearalenone, DON: deoxynivalenol

Table 2

Standard curve equation, LOD and detection range of AFB1, OTA, ZEN and DON

Mycotoxins	Standard curve equation	LOD	Detection range
AFB1	y=-0.4468x+0.7990 (R^2=0.9975)	0.59 ng/mL	0.99-21.92 ng/mL
OTA	y=-0.5020x+0.9546 (R^2=0.9911)	1.27 ng/mL	1.98-28.22 ng/mL
ZEN	y=-0.6009x+1.4103 (R^2=0.9979)	7.07 ng/mL	10.37-103.31 ng/mL
DON	y=-0.4715x+1.1417 (R^2=0.9937)	3.26 ng/mL	5.31-99.37 ng/mL

AFB1: aflatoxin B1, OTA: ochratoxin A, ZEN: zearalenone, DON: deoxynivalenol

Table 3

LC-MS/MS validation of AFB1, OTA, ZEN and DON

Matrices	Addition level (ng/mL)	Value of concentration detected by SPR (ng/mL)	Recovery (%)	RSD (%)	Value of concentration detected by LC-MS/MS (ng/mL)	Variance (%)
AFB1						
Corn	5	5.10±0.28	102.00±5.66	9.38	4.81	5.80%
	10	10.67±0.29	106.67±2.87	4.96	9.68	9.87%
	15	15.20±0.75	101.33±4.99	8.59	14.55	4.33%
Wheat	5	4.70±0.33	94.00±6.53	9.8	4.86	-3.20%
	10	9.27±0.26	92.67±2.62	4.45	9.02	2.47%
	15	15.73±0.86	104.89±5.72	8.53	14.98	5.02%
OTA						
Corn	20	22.23±0.45	111.17±2.25	3.67	20.58	8.27%
	50	51.13±1.10	102.27±2.19	3.71	48.97	4.33%
	80	87.23±1.55	109.04±1.94	3.36	76.85	12.98%
Wheat	20	19.00±0.29	95.00±1.47	2.52	21.23	-11.15%
	50	48.83±3.03	97.67±6.06	8.81	48.74	0.19%
	80	78.80±2.38	98.50±2.97	5.11	78.99	-0.24%
ZEN						
Corn	30	29.13±1.41	97.11±4.69	8.09	28.11	3.41%
	50	51.83±1.88	103.67±3.76	5.7	46.52	10.63%
	80	72.47±1.90	90.58±2.37	4.04	75.88	-4.27%
Wheat	30	30.07±1.71	100.22±5.71	9.74	29.03	3.46%
	50	47.97±2.57	95.93±5.14	8.54	49.56	-3.19%
	80	71.40±1.80	89.25±2.25	3.89	78.23	-8.54%
DON						
Corn	8	7.07±0.17	88.33±2.12	3.63	7.68	-7.67%
	15	15.37±0.83	102.44±5.56	8.99	13.56	12.04%
	25	26.03±1.06	104.13±4.25	6.32	25.68	1.41%
Wheat	8	8.27±0.45	103.33±5.62	9.73	7.89	4.71%
	15	13.87±0.49	92.44±3.28	5.02	15.64	-11.82%
	25	25.90±1.08	103.60±4.32	6.93	24.86	4.16%

AFB1: aflatoxin B1, OTA: ochratoxin A, ZEN: zearalenone, DON: deoxynivalenol

Declaration of Interest Statement

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

Highlights

- A SPR method for simultaneous analysis of AFB1, OTA, ZEN and DON in corn and wheat is presented.
- A specific and sensitive SPR sensor chip was fabricated based on self-assembled monolayer.
- Mycotoxins were detected with indirect competitive immunoassay format.
- Application of a SPR technique significantly increases the sensitivity of the method.