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# Development of a multicolor upconversion fluorescence immunoassay for the simultaneous detection of thiamethoxam and dextran by magnetic separation†

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The contents of both pesticide residues and dextran are important parameters for evaluating the quality of sugarcane. In this study, a multicolor upconversion fluorescence immunoassay for the simultaneous detection of thiamethoxam and dextran was established on the basis of magnetic separation. Antigens of thiamethoxam and dextran were coupled to magnetic nanoparticles as the separation elements. Monoclonal antibodies of thiamethoxam (6C7D12) and dextran (3C6F7) were conjugated with the upconversion nanoparticles of NaYF<sub>4</sub>:Yb,Er with an emission wavelength at 544 nm and NaYF<sub>4</sub>:Yb,Tm with an emission wavelength at 477 nm to prepare the signaling elements, respectively. Due to the difference in the emission wavelength, the signaling elements bound on the separation elements could be detected simultaneously after separation by an external magnetic field. After optimization, the half-maximal inhibitory concentration (IC<sub>50</sub>) values of the immunoassay for thiamethoxam and dextran were 0.46 and 49.33 ng mL<sup>-1</sup>, respectively. The assay showed no cross-reactivity with the analogs of thiamethoxam and dextran except for clothianidin (8.7%). The average recoveries of thiamethoxam and dextran in sugarcane juice were 82.9–93.3% and 87.5–97.2%, respectively. The results indicated that the immunoassay could meet the requirements for the simultaneous quantitative detection of thiamethoxam and dextran.

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## 1 Introduction

Thiamethoxam is a kind of high-efficiency and broad-spectrum insecticide that has been widely used to control aphids, sugarcane moth borers and whiteflies in sugarcane planting. Due to its high toxicity to honeybees, thiamethoxam has been temporarily prohibited by the European Commission.<sup>1</sup> In addition, thiamethoxam has been observed to pose a risk to other nontarget organisms, including humans. Jameel *et al.* found that thiamethoxam could cause different hazardous effects by interaction with DNA as well as alteration in oxidative stress markers and biological parameters.<sup>2</sup> Importantly, the toxic effect is continued in the next generation and might be at the genetic level. Caron-Beaudoin *et al.* proved that thiamethoxam was an endocrine disruptor due to its effects on aromatase activity and hormone production.<sup>3</sup> Dextran, a high molecular weight polysaccharide, is commonly found in sugarcane,

sugarcane juice and sugar,<sup>4</sup> and it is produced by dextranase that is secreted by infected microorganisms (*Leuconostoc mesenteroides*).<sup>5</sup> Dextran can cause some negative effects on the sugar industry, such as sugar loss, viscosity increases, influencing crystallization, and false increases in rotation.<sup>6,7</sup> Therefore, it is necessary to detect thiamethoxam and dextran in sugarcane.

At present, the detection of thiamethoxam mainly depends on instrument-based methods, such as high-performance liquid chromatography (HPLC), HPLC-tandem mass spectrometry and so on.<sup>8,9</sup> The methods are sensitive and accurate, but they have some shortcomings, such as expensive testing instruments, complicated sample pretreatment, time-consuming and laborious detection, and difficulty meeting the requirements of the rapid detection of a large number of samples. For dextran, the alcohol haze method<sup>10</sup> and Roberts' copper method<sup>11</sup> have been widely used in the sugar industry, but their sensitivity and specificity are unsatisfactory.<sup>12</sup> The enzyme-HPLC method<sup>13</sup> and Optical Activity Ltd. Polarimetric Method (DASA)<sup>14</sup> have great sensitivity and specificity, but the test cost is high, and the test cannot be used routinely in the sugar industry.

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Immunoassays have the advantages of high sensitivity and specificity, simple and rapid operation, low cost and a large analysis capacity,<sup>15,16</sup> and they have been used in the rapid screening and monitoring of a large number of targets, including thiamethoxam and dextran. Kim *et al.* prepared an anti-thiamethoxam polyclonal antibody (pAb) and developed an enzyme-linked immunosorbent assay (ELISA), with the half-maximal inhibitory concentration (IC<sub>50</sub>) of 9.0 ng mL<sup>-1</sup>.<sup>17</sup> Kim *et al.* prepared a monoclonal antibody (mAb, IC<sub>50</sub> of 0.2 ng mL<sup>-1</sup> in ELISA) for thiamethoxam and developed an automated flow fluorescent immunoassay.<sup>18</sup> Ye *et al.* reported a mAb-based ELISA (IC<sub>50</sub> of 0.87 ng mL<sup>-1</sup>) and immunochromatographic strip for the detection of thiamethoxam.<sup>19</sup> For dextran, Liang *et al.* reported an anti-dextran mAb and immunonephelometry for the quantitative detection of dextran.<sup>20</sup> Wang *et al.* developed a mAb-based sandwich ELISA (limit of detection, LOD of 3.9 ng mL<sup>-1</sup>)<sup>21</sup> and competitive ELISA (IC<sub>50</sub> of 204.2 ng mL<sup>-1</sup>)<sup>22</sup> for dextran. However, there is currently no method for the simultaneous detection of thiamethoxam and dextran. In addition, immunoassays need to be developed toward more simple, rapid, sensitive and accurate methods.

In recent years, lanthanide-doped upconversion nanoparticles (UCNPs) have attracted increasing attention from researchers because they are excited by near-infrared (NIR) light (typically 980 nm) and emit different wavelengths of visible and ultraviolet light.<sup>23,24</sup> UCNPs have the advantages of large Stokes shifts, great chemical and physical stability, high resistance to photobleaching and low potential biological toxicity.<sup>25–29</sup> Compared with that of conventional fluorescence materials, the excitation light of UCNPs (NIR light) generally cannot be absorbed by the sample matrix, which can improve the signal-to-noise ratio and detection sensitivity of immunoassays.<sup>30,31</sup> In addition, the UCNPs doped with different lanthanides possess varied luminescence spectra, which are very suitable for the simultaneous detection of multiple analytes by immunoassays under the same excitation light source.

In this paper, anti-thiamethoxam and anti-dextran mAbs were prepared by cell fusion, and a multicolor upconversion fluorescence immunoassay (UCFIA) for the simultaneous detection of thiamethoxam and dextran was developed by using UCNPs as signaling elements and magnetic nanoparticles (MNPs) as the separation elements. The sensitivity, specificity and accuracy of the immunoassay were evaluated by the detection of standards, analogs and spiked samples.

## 2 Materials and methods

### 2.1 Materials

The standards of thiamethoxam, imidacloprid, acetamiprid, thiacloprid, nitenpyram and clothianidin were purchased from Dr Ehrenstorfer (Germany). Imidacloprid (97.82%) was provided by Nantong Jiangshan Agrochemical and Chemicals Co., Ltd. (Jiangsu, China). Dextran 2000 and its analogs were supplied by Pharmacia Company (Shanghai, China). Bovine serum albumin (BSA), ovalbumin (OVA) and tetramethylbenzidine (TMB) were purchased from Sigma Chemical Co., Ltd. (Missouri, USA). The antigens of dextran (dextran 40–BSA and dextran 40–OVA) were

obtained from Medical College, Xiamen University. Gelatin was purchased from Saiguo Biotechnology Co., Ltd. (Guangdong, China). Goat anti-mouse immunoglobulin–horse radish peroxidase (GAM–HRP) conjugate was purchased from Boster Biological Technology Co., Ltd. (California, USA). Polyoxyethylene sorbitan monolaurate (Tween-20) was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). H<sub>2</sub>O<sub>2</sub> was purchased from Guanghua Technology Co., Ltd. (Guangdong, China). The amine-functionalized UCNPs of NaYF<sub>4</sub>:Yb,Er and NaYF<sub>4</sub>:Yb,Tm,<sup>31,32</sup> and amine-functionalized Fe<sub>3</sub>O<sub>4</sub> MNPs<sup>33</sup> were prepared as previously described and stored in the laboratory. A 25% glutaraldehyde solution was supplied by Sino-pharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals used in this study were of analytical grade. BALB/c mice were obtained from the Center of Comparative Medicine of Yangzhou University (Jiangsu, China). All animals used in this study and animal experiments were approved by Department of Science and Technology of Jiangsu Province (license number: SYXK (SU) 2017-0007).

### 2.2 Preparation of thiamethoxam antigens

The hapten of thiamethoxam was synthesized according to previous articles (Fig. S1†).<sup>17,19</sup> The product was collected and characterized by electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR) spectroscopy: ESI-MS, *m/z*, 362.1 [M + H]<sup>+</sup>; <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$ : 7.79 (s, 1H), 5.16 (s, 2H), 5.11 (s, 2H), 4.88 (s, 2H), 3.48 (t, *J* = 6.9 Hz, 2H), 2.98 (s, 3H), 2.84 (t, *J* = 6.9 Hz, 2H). The hapten was conjugated with BSA and OVA by the active ester method to prepare the immunogen and coating antigens, and the procedure was performed as described previously.<sup>34</sup> The conjugates were identified by UV-vis spectroscopy, and the hapten densities were estimated by the following formula: ( $\epsilon_{\text{conjugate}} - \epsilon_{\text{carrier protein}}$ )/ $\epsilon_{\text{hapten}}$ . The molar ratios (hapten/protein) of immunogen and coating antigen were 20 : 1 and 10 : 1, respectively.

### 2.3 Preparation of mAb

The BSA conjugates (thiamethoxam hapten–BSA and dextran 40–BSA) were used to immunize BALB/c female mice by an intraperitoneal injection. The immunization strategy and the cell fusion procedures were performed as described in a previous article.<sup>35</sup> After fusion, the cells were distributed into 96-well culture plates, and the supernatants from the plates were analyzed by indirect competitive ELISA (ic-ELISA) when hybridomas had grown to nearly 30% of the well area. The wells showing inhibitions in the ic-ELISAs were subcloned by the limiting dilution method. Ascites were obtained by the intraperitoneal injection of BALB/c female mice and purified by a protein A column (GE Healthcare, USA). After dialysis for 3 days, the mAbs were stored at –20 °C.

### 2.4 ic-ELISA protocol

All incubations were carried out at 37 °C, and the plates were washed five times with 10 mM phosphate-buffered saline containing 0.05% Tween 20 (PBST, pH = 7.4) after incubation. The 96-well microtiter plates (Corning) were coated with coating



antigen (thiamethoxam hapten-OVA or dextran 40-OVA) in carbonate-bicarbonate buffer (50 mM, pH = 9.6) for 2 h. The plates were then blocked by the incubation of 1% gelatin in PBS (200  $\mu$ L per well) for 1.5 h. Twenty-five microliters of analyte (thiamethoxam or dextran 2000) and antibody in PBS were added to the blocked plate, followed by incubation for 1 h. The diluted (1/20 000 in PBS) GAM-HRP (50  $\mu$ L per well) was added to the plate for the incubation of 1 h. The bound GAM-HRP was detected by the addition of 50  $\mu$ L per well TMB solution (100  $\mu$ L of 1% hydrogen peroxide and 400  $\mu$ L of 0.6% TMB in dimethyl sulfoxide added to 25 mL of citrate acetate buffer, pH = 5.5). After incubation for 15 min, the reaction was stopped by adding 25  $\mu$ L of 2 M  $\text{H}_2\text{SO}_4$ , and the absorbance at 450 nm was measured by a SpectraMax M5 microplate reader (Molecular Devices, USA).

## 2.5 Preparation of antibody-conjugated UCNPs and antigen-conjugated MNPs

The antibody-conjugated UCNPs were prepared by the classical glutaraldehyde cross-linking method.<sup>36</sup> Ten milligrams of amine-functionalized  $\text{NaYF}_4\text{:Yb,Er}$  (or  $\text{NaYF}_4\text{:Yb,Tm}$ ) UCNPs were dispersed into 5 mL of PBS by ultrasonication for 30 min. Then, 100 mg of sodium borohydride and 1.25 mL of 25% glutaraldehyde were added and shaken for 1 h at room temperature (RT). The UCNPs were separated by centrifugation for 5 min at  $6000 \times g$ . After washing 3 times with PBS (20 mL), the precipitate was resuspended in 5 mL of PBS, and 0.6 mg of anti-thiamethoxam antibody (or anti-dextran antibody) was added and subjected to a shaking reaction for 6 h at RT. The unbound antibody was removed by centrifugation for 5 min at  $6000 \times g$ , and 5 mL of PBS containing 1% BSA was added to block the nonspecific and unreacted sites of the UCNPs by shaking reaction for 6 h. After washing 3 times with PBS, the antibody-conjugated UCNPs were dispersed in 5 mL of PBS and stored at 4 °C. The antigens (thiamethoxam hapten-OVA and dextran 40-OVA) were conjugated with MNPs by a similar method except that the MNPs were separated by an external magnetic field.

## 2.6 Procedures of multicolor UCFIA

Due to the interaction of antigens and antibodies, the signaling elements and separation elements formed immunocomplexes. In the presence of thiamethoxam and dextran, these compounds competed with the separation elements to bind signaling elements, thus dissociating the immunocomplexes. Under the action of an external magnetic field, the amount of separated signaling elements is reduced, which leads to a decrease in the fluorescence intensity. In addition, the emission wavelengths of  $\text{NaYF}_4\text{:Yb,Er}$  (544 nm) and  $\text{NaYF}_4\text{:Yb,Tm}$  (477 nm) are different, and thus, thiamethoxam and dextran could be detected simultaneously. Briefly, 400  $\mu$ L of thiamethoxam and dextran 2000 standard or sample solution were mixed with 80  $\mu$ L of thiamethoxam hapten-OVA-MNPs and dextran 40-OVA-MNPs (1 : 1, v/v) in a tube by vortexing for 1 min. Then, 400  $\mu$ L of anti-thiamethoxam mAb- $\text{NaYF}_4\text{:Yb,Er}$  and anti-dextran mAb- $\text{NaYF}_4\text{:Yb,Tm}$  (1 : 1, v/v) in PBS was

added for incubation for 1 h with shaking. After separation with a magnetic separator, the MNPs were washed 3 times with PBS. Subsequently, 500  $\mu$ L of PBS was added, and the bound signaling elements were detected by a fluorescence spectrophotometer with a 980 nm laser transmitter (Changchun Laser Optoelectronics Technology Co., Ltd., China) at wavelengths of 544 nm and 477 nm.

## 2.7 Optimization of the multicolor UCFIA

The experimental parameters of the immunoassay, including the dosage of MNPs conjugated with antigens, incubation time, concentration of NaCl, pH value and methanol content, were optimized. The immunoassay was used to detect the standards of thiamethoxam and dextran 2000 under volumes of antigen-conjugated MNPs from 40  $\mu$ L to 160  $\mu$ L, incubation times from 10 to 70 min, concentrations of NaCl from 0.05 mol  $\text{L}^{-1}$  to 0.4 mol  $\text{L}^{-1}$ , pH values from 5 to 9, and methanol concentrations from 0% to 20%. The standard curves of thiamethoxam and dextran were established by plotting the changes in the fluorescence intensities at 544 nm and 477 nm, respectively, *versus* the logarithm of the analyte concentration. The changes in the fluorescence intensity were calculated by the following equation:  $\Delta I = I_0 - I$  ( $I_0$  and  $I$  represent the fluorescence intensity without and with analyte, respectively). The parameters that resulted in the immunoassay with the highest  $F_{\text{max}}/\text{IC}_{50}$  were desirable.

## 2.8 Cross-reactivity (CR)

The specificity of the immunoassay was determined by the CR. The analogs of thiamethoxam and dextran were detected by the immunoassay. The CRs were calculated by the following formula:  $\text{CR} = (\text{IC}_{50} \text{ of analyte} / \text{IC}_{50} \text{ of analog}) \times 100\%$ .

## 2.9 Analysis of spiked samples

Thiamethoxam-free sugarcane (confirmed by ultraperformance liquid chromatography coupled to tandem mass spectrometry<sup>37</sup>) was collected from a market in Nanjing, China; the sugarcane was juiced, and the solid residue was removed by filtration. Ten grams of juice was spiked with thiamethoxam and dextran simultaneously at concentrations of 20 ng  $\text{g}^{-1}$  thiamethoxam and 20  $\mu\text{g g}^{-1}$  dextran 2000, 100 ng  $\text{g}^{-1}$  thiamethoxam and 40  $\mu\text{g g}^{-1}$  dextran 2000, and 400 ng  $\text{g}^{-1}$  thiamethoxam and 80  $\mu\text{g g}^{-1}$  dextran 2000. After appropriate dilution, the concentrations of thiamethoxam and dextran 2000 in the spiked samples were simultaneously detected by the immunoassay, and the recoveries were calculated. Each spiked sample was analyzed in triplicate.

# 3 Results and discussion

## 3.1 Determination of the mAb subtype and sensitivity in ic-ELISA

After subcloning, one hybridoma cell line that stably secreted anti-thiamethoxam mAb was named 6C7D12. The subtype of the mAb was determined as IgG3 by an Isotyping Kit for Mouse Monoclonal Antibody (Sino Biological Inc., China). The  $\text{IC}_{50}$ ,



LOD ( $IC_{10}$ ) and linear range ( $IC_{10}$  to  $IC_{90}$ ) of the ic-ELISA developed by the mAb were  $0.36 \text{ ng mL}^{-1}$ ,  $0.02 \text{ ng mL}^{-1}$  and  $0.02\text{--}5.88 \text{ ng mL}^{-1}$ , respectively. The sensitivity of the presented mAb for thiamethoxam was better than that of the pAb prepared by Kim *et al.* ( $IC_{50}$  of  $9.0 \text{ ng mL}^{-1}$ )<sup>17</sup> and the mAb prepared by Ye *et al.* ( $IC_{50}$  of  $0.87 \text{ ng mL}^{-1}$ )<sup>19</sup> and slightly lower than that of the mAb prepared by Kim *et al.* ( $IC_{50}$  of  $0.2 \text{ ng mL}^{-1}$ ).<sup>18</sup>

For dextran, three hybridoma cell lines that stably secreted anti-dextran mAb were obtained and named 3C6F7, 3E5D2 and 4B9A5. The subtype of 3C6F7 and 3E5D2 mAbs was IgG1, and that of 4B9A5 mAb was IgG2a. Among them, 3C6F7 had the highest titer and the best sensitivity, and the  $IC_{50}$ , LOD and linear range in the ic-ELISA were  $120.1 \text{ ng mL}^{-1}$ ,  $12.8 \text{ ng mL}^{-1}$  and  $12.8\text{--}1217.3 \text{ ng mL}^{-1}$ , respectively. The sensitivity of the presented mAb was better than that of the mAb prepared by Wang *et al.* ( $IC_{50}$  of  $204.2 \text{ ng mL}^{-1}$  in ic-ELISA).<sup>21</sup>

### 3.2 Specificity of the mAbs in ic-ELISA

The analogs of thiamethoxam, including imidaclothiz, imidacloprid, acetamiprid, thiacloprid, nitenpyram and clothianidin, were used to evaluate the specificity of the ic-ELISA developed by the anti-thiamethoxam mAb (6C7D12). The ic-ELISA showed CRs of 11.3% and 1.1% for clothianidin and imidacloprid, respectively. The other analogs of thiamethoxam exhibited no

CR (less than 0.1%) by the ic-ELISA determination. For dextran, the ic-ELISA developed by the anti-dextran mAb (3C6F7) showed no CR (less than 0.1%) with sucrose, glucose, starch and  $\beta$ -glucan. These results indicated that the anti-thiamethoxam and anti-dextran mAbs showed high specificity in the ic-ELISAs.

### 3.3 Optimization of the multicolor UCFIA

As shown in Fig. 1, under the excitation wavelength of 980 nm, the signaling element of thiamethoxam ( $\text{NaYF}_4\text{:Yb,Er}$  UCNPs conjugated with anti-thiamethoxam mAb) had four obvious emission peaks at 409, 526, 544 and 657 nm, among which the fluorescence intensity at 544 nm was the highest. For the signaling element of dextran ( $\text{NaYF}_4\text{:Yb,Tm}$  UCNPs conjugated with anti-dextran mAb), there were three obvious emission peaks at 362, 452 and 477 nm, among which 477 nm had the highest fluorescence intensity. When the two signaling elements were mixed equally, the emission spectra did not overlap at 544 nm and 477 nm. Therefore, the fluorescence intensities at 544 nm and 477 nm were selected for the simultaneous detection of thiamethoxam and dextran in the multicolor UCFIA.

To construct a sensitive method, the multicolor UCFIA was optimized. The optimal volume of MNPs conjugated with thiamethoxam and dextran antigens was  $40 \mu\text{L}$  because the

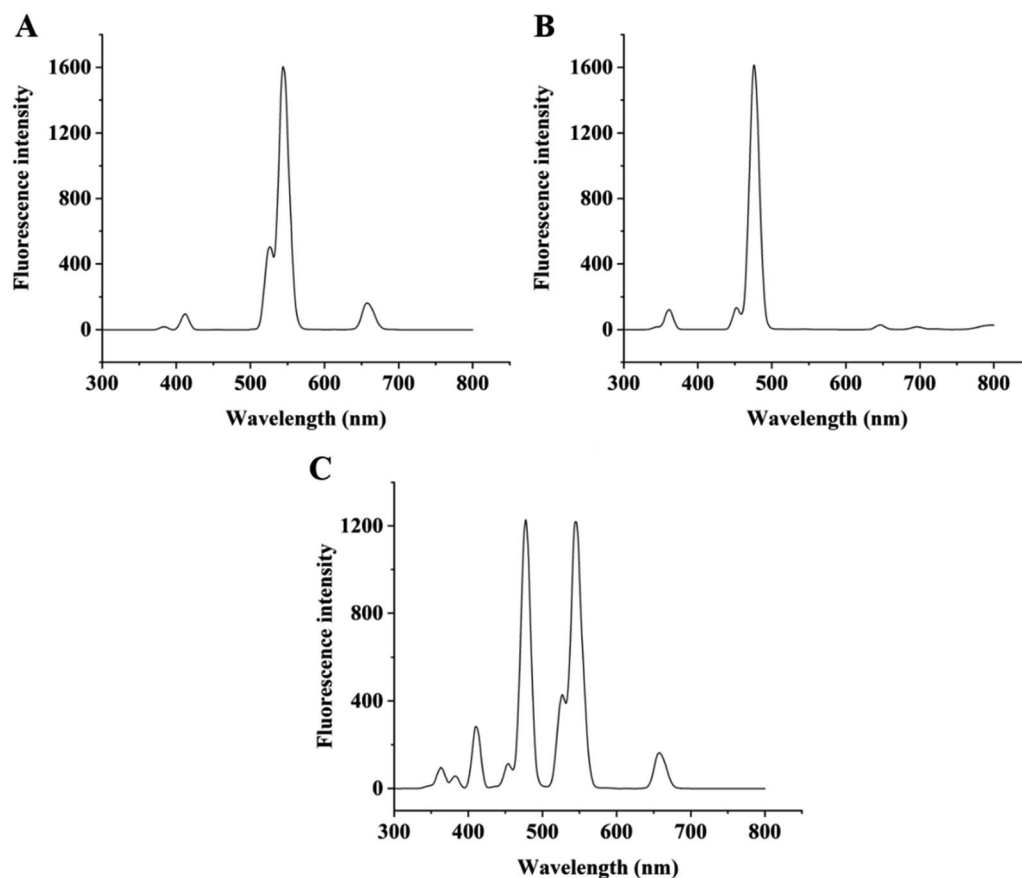


Fig. 1 The emission spectra of signaling elements. (A)  $\text{NaYF}_4\text{:Yb,Er}$  UCNPs conjugated with anti-thiamethoxam mAb; (B)  $\text{NaYF}_4\text{:Yb,Tm}$  UCNPs conjugated with anti-dextran mAb; (C) the mixture of thiamethoxam and dextran signaling elements.



multicolor UCFIA showed the highest fluorescence intensities at 544 nm (thiamethoxam) and 477 nm (dextran) (Fig. S2†). When the incubation time was 60 min, the value of the fluorescence intensity reached a plateau (Fig. S3†). As shown in Fig. S4,† the optimal concentrations of NaCl for the detection of thiamethoxam and dextran were  $0.2 \text{ mol L}^{-1}$  and  $0.14 \text{ mol L}^{-1}$ , respectively. Since thiamethoxam and dextran were detected simultaneously, the optimal conditions should be selected according to the results of both compounds. Therefore,  $0.2 \text{ mol L}^{-1}$  NaCl was selected. The multicolor UCFIA showed a higher  $F_{\text{max}}/IC_{50}$  in the detection of both thiamethoxam and dextran when the pH value was 7.4 (Fig. S5†). Although the optimal methanol content for thiamethoxam and dextran was

2.5% and 0%, respectively, organic solvent was required as a cosolvent in the preparation of the standard solution and sample extraction. Thus, 2.5% methanol was selected as the optimal methanol content. In summary, the optimal conditions of the immunoassay were  $80 \mu\text{L}$  of thiamethoxam hapten-OVA-MNPs and dextran 40-OVA-MNPs (1 : 1, v/v), incubation for 60 min,  $0.2 \text{ mol L}^{-1}$  NaCl, pH = 7.4 and 2.5% methanol.

### 3.4 Sensitivity of the multicolor UCFIA

The calibration curves of thiamethoxam and dextran under the optimal conditions are shown in Fig. 2. The linear regression equations of thiamethoxam and dextran were  $y = 255.29x +$

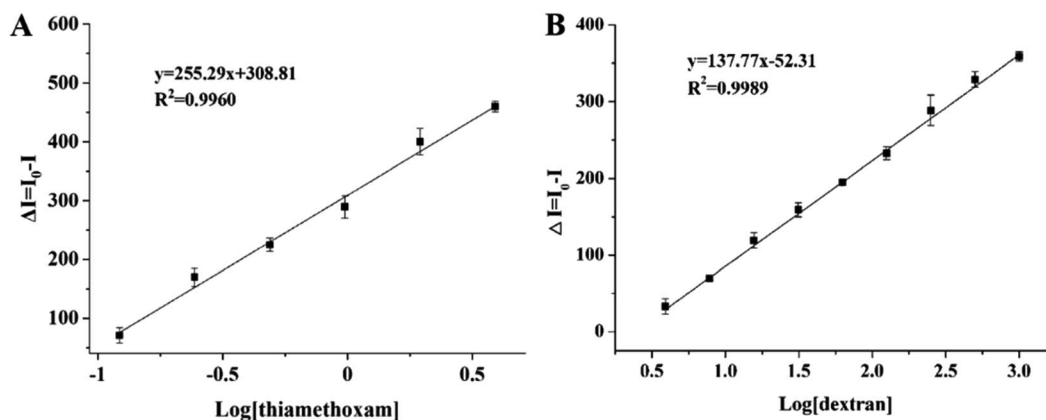


Fig. 2 The calibration curve of the multicolor UCFIA for (A) thiamethoxam and (B) dextran 2000.

Table 1 Cross-reactivity of thiamethoxam toward some of its analogs

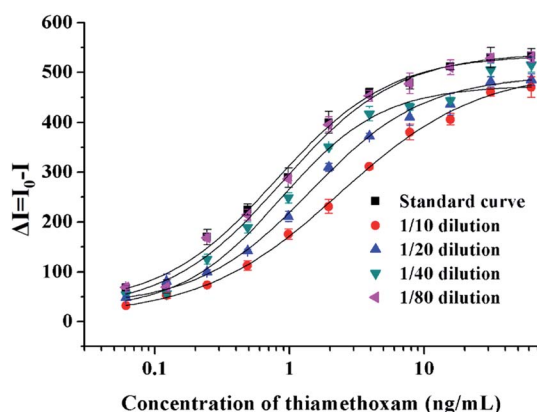
Analyte	Structure	$IC_{50}$ (ng $\text{mL}^{-1}$ )	Cross-reactivity (%)
Thiamethoxam		0.46	100
Imidaclothiz		>1000	<0.05
Imidacloprid		30.4	1.5
Acetamiprid		>1000	<0.05
Thiacloprid		>1000	<0.05
Nitenpyram		>1000	<0.05
Clothianidin		5.3	8.7





**Table 2** Cross-reactivity of dextran 2000 toward some of its analogs

Compound	IC <sub>50</sub> (ng mL <sup>-1</sup> )	Cross-reactivity (%)
Dextran 2000	49.33	100
Sucrose	>10 000	<0.5
Glucose	>10 000	<0.5
Starch	>10 000	<0.5
β-Glucan	>10 000	<0.5

**Fig. 3** The matrix interference of sugarcane on the multicolor UCFA for the detection of thiamethoxam.

308.81 ( $R^2 = 0.9960$ ) and  $y = 137.77x - 52.31$  ( $R^2 = 0.9989$ ), respectively. The IC<sub>50</sub>, limit of detection (LOD, IC<sub>10</sub>) and linear range (IC<sub>10</sub> to IC<sub>90</sub>) of the multicolor UCFA for thiamethoxam were 0.46 ng mL<sup>-1</sup>, 0.09 ng mL<sup>-1</sup> and 0.09–2.34 ng mL<sup>-1</sup>, respectively. For dextran 2000, the IC<sub>50</sub>, LOD and linear range were 49.33 ng mL<sup>-1</sup>, 4.39 ng mL<sup>-1</sup> and 4.39–544.43 ng mL<sup>-1</sup>, respectively. Compared with that of the ic-ELISAs for thiamethoxam and dextran in this study (see the section

“Determination of mAb subtype and sensitivity in ic-ELISA”), the sensitivity of the multicolor UCFA was similar with that of the ic-ELISA. However, the multicolor UCFA had significant advantages in the detection time and operation procedure. More importantly, the multicolor UCFA could detect thiamethoxam and dextran simultaneously.

### 3.5 Specificity of the multicolor UCFA

As shown in Tables 1 and 2, the multicolor UCFA had no CR with the analogs of thiamethoxam and dextran, except for clothianidin (8.7% CR) and imidacloprid (1.5% CR). These CRs are mainly derived from the anti-thiamethoxam mAb, which was also present in the ic-ELSA for thiamethoxam (see the section “Specificity of the mAbs in ic-ELISA”). The results indicated that the multicolor UCFA had good specificity and was similar to the ic-ELISAs developed with the same mAbs.

### 3.6 Analysis of spiked samples by the multicolor UCFA

Before detection of the spiked samples, the matrix interference was evaluated by dilution with PBS. One gram of sugarcane juice that was not spiked with analytes was diluted with PBS 10 times and used to prepare standard curves of thiamethoxam. As shown in Fig. 3, the standard curve prepared using the matrix with an 80-fold dilution was similar to those in the matrix-free buffer, which meant that the matrix effect was eliminated. Since dextran is usually present in sugarcane, the original concentration of dextran in sugarcane juice was detected by a serial dilution. The detection results are summarized in Table 3. The original concentration of dextran in sugarcane was out of the linear range under the 50- and 100-fold dilutions. The results of the 200-, 400- and 800-fold dilutions were 99 740, 102 240 and 97 200 ng g<sup>-1</sup>, respectively. The average original concentration of dextran in sugarcane was 99 727 ng g<sup>-1</sup>, with a relative standard deviation (RSD) of 2.53%, which indicated that the 200-fold dilution could remove the matrix interference.

**Table 3** The original concentration of dextran in the sugarcane juice

Dilution factor	50	100	200	400	800
Detection results (ng g <sup>-1</sup> )	>554	>554	498.7	255.6	121.5
Original concentration (ng g <sup>-1</sup> )	>27 200	>55 400	99 740	102 240	97 200

**Table 4** Average spiked recoveries of thiamethoxam and dextran 2000 in the spiked sugarcane juice

Analyte	Original concentration	Spiked concentration	Detection value	Recovery (%)	RSD (%)
Thiamethoxam (μg kg <sup>-1</sup> )	0	20	16.6 ± 0.5	82.9	3.1
		100	93.3 ± 6.1	93.3	6.5
		400	354.8 ± 17.6	88.7	5.0
Dextran 2000 (μg g <sup>-1</sup> )	99.7	20	109.3 ± 5.0	91.3	4.6
		40	122.2 ± 2.5	87.5	2.1
		80	174.7 ± 6.3	97.2	3.6



To detect thiamethoxam and dextran simultaneously, 200-fold dilution was used in the detection of spiked samples.

Table 4 shows the detection results of the spiked samples. The average recoveries of thiamethoxam and dextran 2000 in sugarcane juice were 82.9–93.3% with an RSD of 3.1–6.5% and 87.5–97.2% with an RSD of 2.1–4.6%, respectively. These results indicated that the accuracy of the multicolor UCFIA could meet the requirements for the simultaneous detection of thiamethoxam and dextran 2000 in sugarcane juice.

## 4 Conclusion

In this study, mAbs of thiamethoxam and dextran were prepared, and they showed good sensitivity and specificity through ELISA evaluations. Based on the different emission wavelengths of the UCNPs NaYF<sub>4</sub>:Yb,Er and NaYF<sub>4</sub>:Yb,Tm, the mAbs of thiamethoxam and dextran were conjugated with these UCNPs to develop a multicolor UCFIA by using MNPs as a separation element. The IC<sub>50</sub> and the linear range were 0.46 ng mL<sup>-1</sup> and 0.09–2.34 ng mL<sup>-1</sup> for thiamethoxam, respectively, and 49.33 ng mL<sup>-1</sup> and 4.39–554.43 ng mL<sup>-1</sup> for dextran. The multicolor UCFIA showed high sensitivity and significant advantages in the detection time and operation procedure. More importantly, the multicolor UCFIA realized the simultaneous detection of thiamethoxam and dextran. The multicolor UCFIA had no CR with the analogs of thiamethoxam and dextran, except for clothianidin (8.7%) and imidacloprid (1.5%). The recoveries of spiked samples showed that the assay was accurate and could be used to detect thiamethoxam and dextran. Therefore, this study presented an alternative tool for the simultaneous determination of thiamethoxam and dextran.

## Conflicts of interest

There are no conflicts to declare.

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