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Novel Chemiluminescence Immunoassay for the Determination of Zearalenone in Food Samples Using Gold Nanoparticles Labeled with Streptavidin−Horseradish Peroxidase

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ABSTRACT: A novel highly sensitive chemiluminescence immunoassay (CLIA) was developed to detect zearalenone in food samples by using both biotinylated zearalenone conjugates and gold (Au) nanoparticles labeled with streptavidin−horseradish peroxidase for signal amplification. Biotinylated zearalenone−ovalbumin conjugates and Au nanoparticles labeled with streptavidin−horseradish peroxidase were synthesized separately. The concentrations of immunoreagents and the reaction times of these immunoreagents were optimized to improve the performances of analytical methods. For the CLIA based on biotinylated zearalenone conjugates and Au nanoparticles labeled with streptavidin−horseradish peroxidase, the limit of detection was 0.008 ng/mL and the IC_{50} was 0.11 ng/mL. The linear working range was 0.02−0.51 ng/mL. The cross-reactivities with the zearalenone analogues (α-zearalanol, zearalanone, α-zearalenol, β-zearalanol, and β-zearalenol) were 32, 17, 12, 0.3, and 0.1%, respectively. The recovery rates in spiked food samples were 97−117%, and the intraday and interday relative standard deviations were both <10%. Parallel analysis of natural food samples showed a good correlation between this novel CLIA and liquid chromatography−tandem mass spectrometry. This method provides a rapid, accurate, and highly sensitive method to determine levels of zearalenone in food samples.

KEYWORDS: *zearalenone, chemiluminescence, immunoassay, biotin−streptavidin, gold nanoparticles*

INTRODUCTION

Zearalenone (ZEN) is an estrogenic mycotoxin[1] that is produced by *Fusarium* species, such as *Fusarium graminearum* and *Fusarium roseum*.[2,3] The purification of ZEN from moldy corn was first reported by Stob and his team in 1962. As an environmental factor that disrupts endocrine function, ZEN can induce the accumulation of high levels of estrogen in animals and, thus, cause diseases and harmful effects associated with the reproductive system,[4] including abortion and stillbirth. Further research is needed to establish whether ZEN has the potential to cause cancer in humans. Nonetheless, contamination by ZEN could not only have severe effects on the agricultural economy but also compromise food safety issues and threaten human health. Babies and children are more vulnerable to the effects of ZEN than adults.[5] There is thus an urgent need for approaches that are highly sensitive, accurate, and rapid analytical methods to detect ZEN.

At present, the most commonly used immunoassays to detect and quantify ZEN use enzyme-linked immunosorbent assays (ELISAs),[6] colloidal gold test strips,[7] and electrochemical immunosensors.[8] Of these, ELISAs and colloidal gold test strips are used extensively for the rapid and simultaneous detection of different analytes on a large scale. However, owing to the limited sensitivities of approaches that involve ELISA and colloidal gold test strips, these methods are not suitable for the detection of trace levels of contamination, which are needed to detect potentially harmful levels of ZEN in food consumed by children or babies. The greater focus on the potential of electrochemical immunosensors can be ascribed to their high sensitivity and amenability to being modified by materials that enable signal amplification. Nonetheless, the complicated detection process used by these sensors, which requires that samples are detected individually, renders them slow and expensive.

Like ELISA, chemiluminescence immunoassay (CLIA) procedures involve the action of an enzyme on a substrate. Nonetheless, the luminescent signal generated by CLIA enables better sensitivity and a wider working range than the colorimetric readout of ELISA.[9] On the basis of the advantages (such as rapidity, lower cost, sensitivity) of the chemiluminescence immunoassay, pathogen bacteria[10] (*Escherichia coli* O157:H7, *Yersinia enterocolitica*, *Salmonella typhimurium*, and *Listeria monocytogenes*) and illicit drugs (clenbuterol[11]) were detected by chemiluminescence immunoassay in food. Earlier papers that described practical applications of CLIA involved the detection of mycotoxins, such as fumonisin B1,[12] ochratoxin A,[13] and aflatoxin B1.[14] However, the sensitivities in these studies were insufficient to detect trace amounts of these toxins.

Over the past few years, many signal amplification procedures have improved the sensitivity of immunoassays. These include the biotin−streptavidin system,[15] Au nanoparticles (AuNPs),[16] magnetic beads,[17] and novel chemiluminescent enhancers.[18] Approaches for signal amplification involved either the use of biotinylated conjugates to increase the number of associated horseradish peroxidase (HRP) molecules modified with streptavidin or the association of

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multiple enzymes, antibodies, or chemiluminescent enhancers on one carrier (for example, either a AuNP or magnetic bead). However, none of these signal amplification procedures were yet demonstrated to be able to detect mycotoxins in food samples.

This study established three kinds of CLIA and optimized all of the analytical parameters of each method in a step-by-step manner. The sensitivities of the three types of CLIA increased after their adaptation to enhance signal amplification. The method that used the biotinylated zearalenone−ovalbumin conjugates and AuNPs labeled with streptavidin−horseradish peroxidase (HRP) was shown to be able to detect ZEN in spiked and naturally contaminated samples. This analytical method provides a rapid and accurate approach to detect trace amounts of contaminants in food samples.

MATERIALS AND METHODS

Materials. Biotin, ZEN, ZEN analogues (α-zearalanol, β-zearalanol, α-zearalenol, β-zearalenol, and zearalanone), tetrachloroauric(III) acid, *O*-carboxymethyloxime (CMO), and *p*-iodophenol were purchased from Sigma Chemical (St. Louis, MO, USA). Ovalbumin (OVA), nonfat dried milk, and luminol were obtained from Sangon Biotech (Shanghai, China). Tween-20 was obtained from Generay Biotech (Shanghai, China). Monoclonal antibodies against ZEN (2C9) were prepared in our laboratory[19] and had the same features as those characterized previously.[20] Sulfo-NHS-LC-biotin was purchased from Molecular Probes (Eugene, OR, USA). Streptavidin−HRP was obtained from Anaspec (Fremont, CA, USA). Both the ZEN-free and naturally contaminated food samples were provided by the Shanghai Entry-Exit Inspection and Quarantine Bureau.

Equipment. White 96-well microtiter plates were purchased from Greiner Bio (Würzburg, Germany). The chemiluminescence microplate reader (Infinite F200) was supplied by Tecan (Männedorf, Switzerland). The horizon shaker (MX2) was from Finepcr (Seoul, Korea). The ultraperformance liquid chromatography (UPLC) system and UPLC BEH C18 column were supplied by Waters (Milford, MA, USA). The QTrap MS/MS system was obtained from Applied Biosystems (Foster City, CA, USA).

Methods. *Synthesis of ZEN Conjugates.* The ZEN−HRP conjugate was synthesized as described previously.[21,22] The ZEN−6′-carboxymethyloxime (ZEN−oxime) conjugate was first prepared using ZEN and CMO as described previously.[22] The conjugation of ZEN−oxime and HRP was performed as previously reported in our laboratory.[20] The ZEN−OVA conjugate was prepared using an approach similar to that used to prepare ZEN−HRP.

Biotinylation of ZEN−OVA. The ZEN−OVA conjugate was modified with biotin essentially as described previously,[23] but with slight modifications. First, ZEN−OVA was dissolved in reaction buffer (0.1 M Na_3PO_4, 0.15 M NaCl, pH 7.2), and sulfo-NHS-LC-biotin was added to this solution slowly. The solution was stirred and allowed to react for 2 h. Second, the biotinylated derivative was purified by gel filtration on a Sephadex G-25 column. The biotinylated derivative (ZEN−OVA−biotin) was detected by spectrophotometric measurement at 280 nm.

Preparation of Streptavidin−HRP−Au Nanoparticles. A modified version of the citrate reduction method was used to prepare AuNPs with diameters of 15 nm.[24] The sizes and shapes of the AuNPs were characterized by transmission electron microscopy and by spectroscopy in the UV−visible region of the spectrum. The pH values of solutions of AuNPs were adjusted to pH 8.0 by the addition of 0.2 M potassium carbonate buffer as described previously.[25] First, 1 mL of streptavidin−HRP (0.08 mg/mL) was dissolved in 2 mM borate buffer (BB, pH 7.4). The mixture (1 mL) was then slowly and continuously added to 10 mL of the suspension of AuNPs over the course of 1 min and then stirred for 30 min at room temperature. Thereafter, 1.0 mL of 10% bovine serum albumin (BSA) (m/v) was dissolved in 2 mM BB (pH 7.4) and added to the mixture. The mixture was stirred for 30 min at room temperature. To increase the homogeneity of the preparation of AuNPs labeled with streptavidin−HRP (streptavidin−HRP−AuNPs), the solution was centrifuged at 1000*g* for 20 min and the pellet discarded. The supernatant was collected and centrifuged at 7000*g* for 30 min. The streptavidin−HRP-labeled AuNPs were washed three times with each step involving the addition of 10 mL of 2 mM BB (pH 7.4) and centrifuging at 7000*g* for 30 min. Finally, the streptavidin−HRP−AuNPs were stored in 4 °C in 10 mM phosphate buffer saline (PBS).

Development of Direct Competitive CLIA. Three different types of direct competitive CLIA (DC-CLIA) were developed using the biotinylated ZEN−OVA- and streptavidi-HRP-labeled AuNPs system to evaluate the effects of signal amplification and to further improve the performance of CLIA. A schematic illustration that compares these DC-CLIA variants is shown in Figure 1.

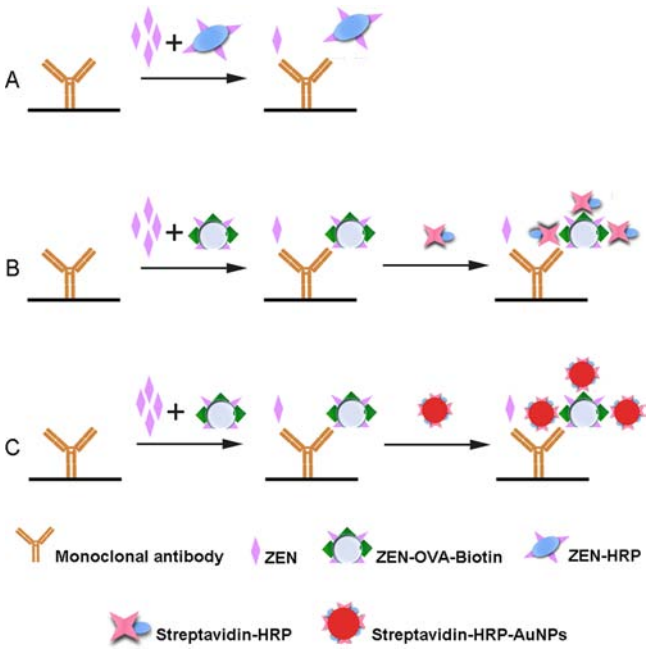

Figure 1. Schematic illustration of three DC-CLIAs.

As shown in Figure 1A, a 96-well microplate was coated with monoclonal antibody (2C9) following overnight incubation at 4 °C, with 2C9 antibodies diluted in 0.05 M carbonate buffer (pH 9.6). After three washings with 10 mM PBS containing 0.05% Tween-20 (PBST), 300 μL of 5% (m/v) skimmed milk (in 10 mM PBS) was added for 2 h at 37 °C to block the wells. Then the microplate wells were washed three times with 10 mM PBST. Diluted sample extracts or ZEN standard solutions were mixed with the same volume of ZEN−HRP. The mixtures (100 μL) were then added in the blocked wells and incubated for 1 h at 37 °C. After three washings with 10 mM PBST, 150 μL of substrate (1 mM luminol, 2 mM *p*-iodophenol, and 0.1 mM H_2O_2, in 0.1 M Tris-HCl buffer, pH 8.6) was added in the microplate, and the luminescence of the solution was immediately determined using a chemiluminescence microplate reader.

As shown in Figure 1B, the second variant involved replacing ZEN−HRP with ZEN−OVA−biotin and streptavidin−HRP. The steps used to coat and block the wells were identical to those described for the scheme shown in Figure 1A. For the steps that followed, diluted sample extracts or standard ZEN solutions were mixed with the same volume of ZEN−OVA−biotin, and then the mixtures (100 μL) were added in the blocked wells and incubated for 1 h at 37 °C. Next, streptavidin−HRP was added to react with the biotin in the wells. Finally, 150 μL of substrate solution (the same as that mentioned above) was added to each well, and the luminescence of the solutions was determined immediately.

As shown in Figure 1C, the third variant involved replacing streptavidin−HRP with streptavidin−HRP−AuNPs to increase the

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sensitivity of the approach. The first two procedures were similar to those described above (Figure 1B), with the only differences between the protocols lying in differences in the concentrations of coating antibody and ZEN−OVA−biotin used. Then streptavidin−HRP−AuNPs solution was added to react with the ZEN−OVA−biotin in the wells. Next, 150 μL of substrate solution was added before the luminescence of the reaction solutions was determined. The calibration curves of three types of DC-CLIA were prepared by Origin 8.0 software.

Optimization of DC-CLIA. To improve the performances of the three types of DC-CLIA, the concentrations of coating antibody, ZEN−HRP, ZEN−OVA−biotin, streptavidin−HRP, and streptavidin−HRP−AuNPs were optimized for each DC-CLIA by determining values of both the 50% inhibitory concentration (IC_{50}) and maximum relative light units (RLU_{max})/IC_{50}. The immunoreaction times (30, 60, 90, or 120 min) using ZEN−HRP, ZEN−OVA−biotin, streptavidin−HRP, and streptavidin−HRP−AuNPs were also optimized for each DC-CLIA by determining IC_{50} and RLU_{max}/IC_{50} values. Whereas a lower IC_{50} value denotes greater sensitivity of an assay, a higher RLU_{max}/IC_{50} denotes both stronger signal intensity and greater sensitivity. The concentrations of methanol in ZEN standard solutions (0, 10, 20, 30, and 40%) were evaluated by inhibition curves to reduce the negative effect of methanol on sensitivity.

Specificity Study. To evaluate the specificity of the antibody in gold nanoparticles labeled with streptavidin−horseradish peroxidase CLIA method, cross-reactivity of the anti-ZEN monoclonal antibody with five ZEN analogues and several other mycotoxins (deoxynivalenol, fumonisin B1, and aflatoxin B1) were determined. ZEN analogues include α-zearalanol, β-zearalanol, α-zearalenol, β-zearalenol, and zearalanone. The cross-relativities were determined using the individual analogue, respectively. Using biotin−streptavidin−HRP−AuNPs DC-CLIA, the standard curves of ZEN with different concentrations were established first, and then different concentrations (1000, 100, 10, 1, 0.1, 0.01, 0.001, and 0 ng/mL) of each analyte instead of ZEN were mixed with the same volume of the ZEN−OVA−biotin concentration (0.05 μg/mL), which had been optimized in the earlier procedure (Table 1). The standard curves were prepared by

Table 1. Optimum Concentrations or Dilution Ratios of Immunoreagents for Three DC-CLIAs

	optimum final concentration (μg/mL)				optimum dilution ratio
analytical method	coating antibody	ZEN−HRP	ZEN−OVA−biotin	streptavidin−HRP	streptavidin−HRP−AuNPs
DC-CLIA	0.9	1	–[a]	–	–
biotin−streptavidin−HRP DC-CLIA	0.5	–	0.1	0.1	–
biotin−streptavidin−HRP−AuNPs DC-CLIA	0.5	–	0.05	–	1/20

[a] –, not used.

Origin 8.0 software. The *X*-axis of the standard curve was indicated as log concentration. The *Y*-axis (B/B_0) represents the percentage of relative light units (RLU) of standard solutions divided by that of the RLU at 0 ng/mL. Then the IC_{50} (50% inhibition) for each analyte was calculated respectively. Cross-reactivity of the anti-ZEN monoclonal antibody with each compound was evaluated according to the following formula:

$$\text{cross-reactivity (\%)} = \frac{IC_{50}\text{ for ZEN}}{IC_{50}\text{ for the structure-related compounds}} \times 100\%$$

Sample Preparation and Recovery Studies. The ZEN-free samples (corn, wheat, noodles, and biscuit) that were kindly provided by the Shanghai Entry-Exit Inspection and Quarantine Bureau did not contain ZEN. Before extraction, the samples were ground and dried by overnight incubation in a 60 °C incubator. Recovery rates of spiked food samples (corn, wheat, noodles, and biscuit) at 1, 2.5, 5, 10, 15, 20, and 25 μg/kg were determined as described previously.[24−27] Standard ZEN solution was added to 10 g food samples, homogenized, and then allowed to stand at room temperature overnight. After the addition of 40 mL of methanol/water (70:30, v/v) to each sample, the samples were shaken vigorously for 30 min at room temperature on a horizontal shaker. After being allowed to stand for 10 min, the supernatant was centrifuged at 4000*g* for 20 min and diluted five times with 10 mM PBS before analysis. To evaluate the reliability of this method, each sample was measured three times in one day and repeated three times on different days.

Analysis by LC-MS/MS. Forty-six natural samples (including 15 corn, 15 wheat, 10 noodles, and 6 biscuit samples) were analyzed using biotin−streptavidin−HRP−AuNPs DC-CLIA and LC-MS/MS. Procedures used for LC-MS/MS were essentially those described previously,[28] with some minor modifications. Each sample was determined three times in triplicate; hence, nine measurements were taken over all. First, the samples were ground and dried overnight in a 60 °C incubator. The extraction solvent mixture (40 mL, acetonitrile/water/acetic acid, 79:20:1, v/v/v) was added to the 10 g food samples and shaken vigorously on a horizontal shaker for 60 min at room temperature. Second, the samples were allowed to stand for 10 min before the supernatants were centrifuged at 2500 *g* for 10 min. The supernatants were then mixed with the same volume of extraction solvent mixture (acetonitrile:water:acetic acid, 20:79:1, v/v/v) and passed through a 0.22 μm filter before being injected into the LC-MS/MS instrument. Quantitative LC-MS/MS results were analyzed using Analyst software (AB SCIEX, Framingham, MA, USA).

RESULTS AND DISCUSSION

Optimization of Three Variants of DC-CLIA. Both IC_{50} and RLU_{max}/IC_{50} values were used to evaluate the performance of the various CLIA approaches. The IC_{50} value, which indicates the 50% inhibitory concentration, is usually used in optical immunoassay analysis. Nonetheless, for CLIA-based approaches, the differential effects of reagents on RLU relative to optical density necessitate the use of other tools to evaluate performance. The RLU_{max}/IC_{50} ratio provides a useful parameter to assess the influence of different factors on CLIA performance, with a higher RLU_{max}/IC_{50} ratio (higher RLU_{max} and lower IC_{50}) indicating higher signal intensity and sensitivity.[29] Reduced concentrations of ZEN−OVA−biotin and streptavidin−HRP−AuNPs decreased IC_{50} (Figure 2A,B) and RLU_{max} values. However, the highest RLU_{max}/IC_{50} values for each assay (Figure 2A,B) indicated the optimum conditions. The optimum concentrations were selected by considering both the IC_{50} value and RLU_{max}/IC_{50}. The optimum final concentration of ZEN−OVA−biotin was 0.1 μg/mL (1/2,000), when RLU_{max}/IC_{50} was the highest and IC_{50} was the lowest when compared with values determined at other concentrations (Figure 2A). Using the same criteria, the optimum dilution ratio of streptavidin−HRP−AuNPs was 1/20 (Figure 2B). The results of efforts to determine the optimum concentrations or dilution ratios of the reagents used in the three types of DC-CLIA are shown in Table 1.

The effects of immunoreaction time for each step of the three types of CLIAs were studied to improve the sensitivities of the immunoassays. Consideration of both IC_{50} and RLU_{max}/IC_{50} values indicated that overnight incubation with coating antibody at 4 °C was optimum, whereas 60 min incubation periods were optimal for streptavidin−HRP−AuNPs (Figure

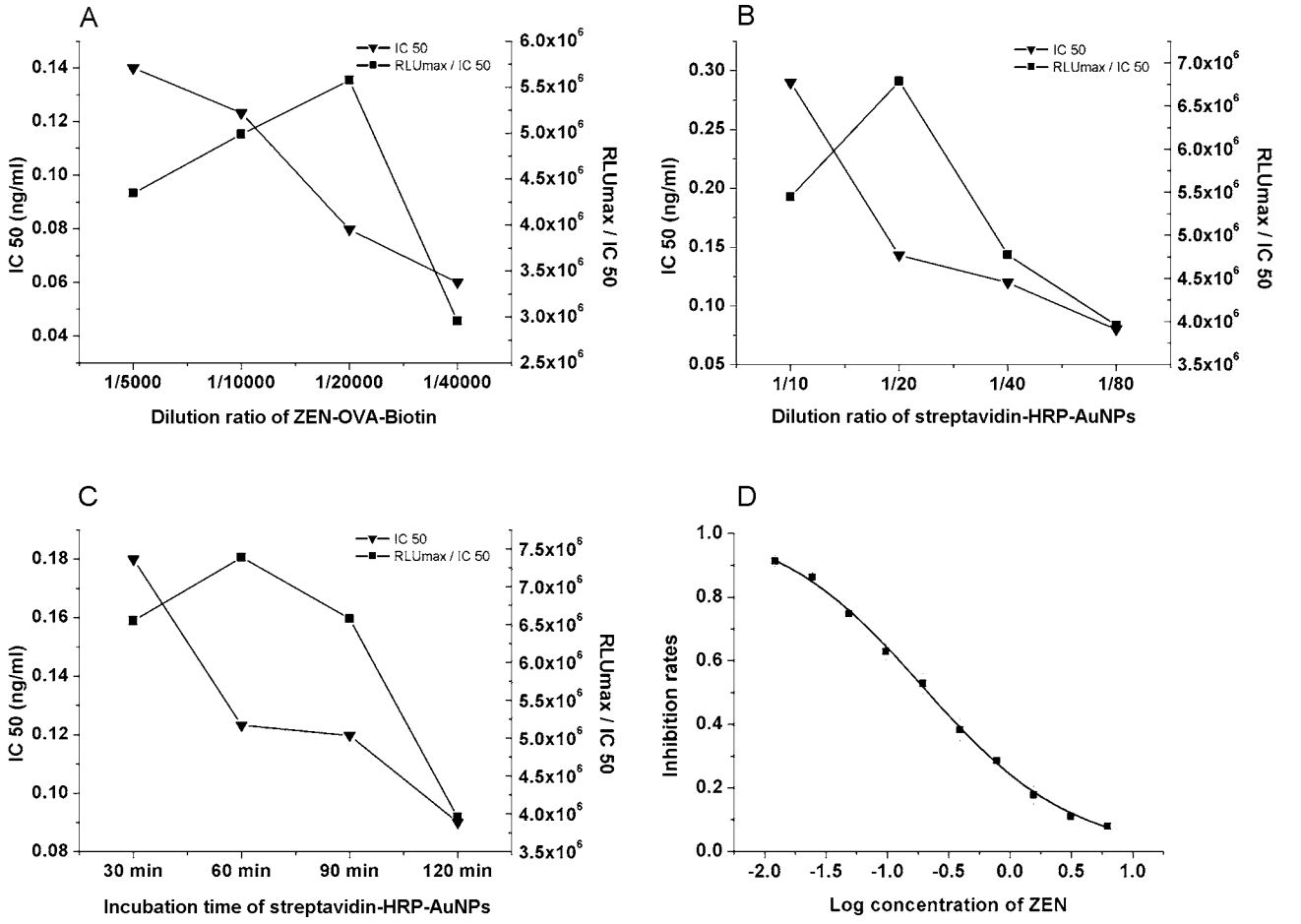

Figure 2. Optimization of biotin−streptavidin (SA)−HRP−AuNPs DC-CLIA by IC_{50} and RLU_{max}/IC_{50}, and calibration curve of this method ($Y = -0.301X + 0.166$, $R^2 = 0.9931$).

Table 2. Detection Characteristics of Three DC-CLIAs

analytical method	limit of detection (ng/mL)	dynamic working range (ng/mL)	IC_{50} (ng/mL)	calibration curve
DC-CLIA	0.05	0.17−2.97	0.54	$Y = -0.346X + 0.404$ ($R^2 = 0.9998$)
biotin−streptavidin−HRP DC-CLIA	0.01	0.05−1.22	0.23	$Y = -0.366X + 0.258$ ($R^2 = 0.9947$)
biotin−streptavidin−HRP−AuNPs DC-CLIA	0.008	0.02−0.51	0.11	$Y = -0.301X + 0.166$ (R^2 =0.9931)

2C) and other reagents. Different concentrations of methanol (from 0 to 40%) in ZEN solutions were assessed to reduce the effects of organic solvents on sensitivities. Comparison of the inhibition curves indicated that a methanol content in the competition buffer of <20% was optimal. A methanol content >20% both reduced the sensitivity of the assay and narrowed the working range. The inhibition curve of DC-CLIA using the ZEN−OVA−biotin and streptavidin−HRP−AuNPs system shown in Figure 2D was prepared using data collected under these optimum conditions, which were also used to test rates of recovery from spiked samples.

Comparisons of Three Types of DC-CLIA. These optimized conditions were used to show that the limits of detection (10% inhibition of the maximal chemiluminescence intensity), dynamic working ranges (20−80% inhibition of the maximal chemiluminescence intensity), IC_{50} (50% inhibition of the maximal chemiluminescence intensity) values (Table 2), sensitivities, and working ranges were improved when using the biotin−streptavidin−HRP system and improved even further when using the biotin−streptavidin−HRP−AuNP system (Figure 1B,C). The detection limit (0.008 ng/mL) and linear working range (0.02−0.51 ng/mL) determined using biotin−streptavidin−HRP−AuNPs DC-CLIA were superior to those determined for DC-CLIA (0.05 and 0.17−2.97 ng/mL, respectively) and biotin−streptavidin−HRP DC-CLIA (0.01 and 0.05−1.22 ng/mL, respectively). The three standard curves are presented in Figure 3.

Compared with indirect competitive approaches, direct competitive (DC) analytical methods are more rapid and less labor intensive. Furthermore, direct competitive analytical methods are more suitable for standardization and commercialization, because the antibody can be supplied already coated on the surfaces of microplate wells.[30]

The modification procedures used for most direct competitive immunoassays for small-molecule analytes usually involve the conjugation of analytes with HRP. However, the effect of this single step for signal amplification was limited, and often unsuitable for the detection of trace amounts of analyte.

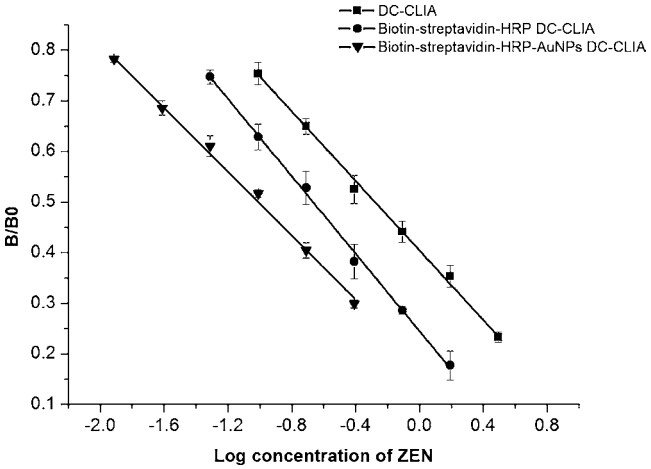

Figure 3. Calibration curves of three types of DC-CLIA.

In this study, signal amplification detection methods based on the principle of DC-CLIA were developed using ZEN−OVA−biotin−streptavidin−HRP. The mechanism of the signal amplification was based on the specificbinding of biotin and streptavidin, and Au nanoparticles can be labeled with streptavidin−horseradish peroxidase. Then through the specific binding, the detected signal was amplified step by step. Compared with the detection limit of DC-CLIA (0.05 ng/mL), the sensitivity increased 5 times when using the ZEN−OVA−biotin and streptavidin−HRP systems (0.01 ng/mL). After biotinylation, one molecule of ZEN−OVA−biotin could not only react with coating antibody but also react with multiple molecules of streptavidin−HRP, thereby amplifying the enzymatic signals. This modified chemiluminescence analysis approach, which takes advantage of the affinity of biotin for streptavidin, had been reported after early studies investigated its use for detecting hormones[15] and fentanyl analytes.[31] However, it has not been used and reported for the detection of mycotoxins. For the rapid detection of analytes in food by chemiluminescence immunoassay, the researchers also focused on the signal amplification approaches. HRP-functionalized mesoporous silica nanoparticle was prepared to decrease the sensitivity for staphylococcal enterotoxin B. The detection limit (4 pg/mL) was significantly better than with ELISA and laser-induced fluorescence immunoassay,[32] but according to the more complex procedures for preparation, AuNPs have wider applications than other nanoparticles in rapid detection immunoassay.

In this paper, the signal amplification technique based on the ZEN−OVA−biotin and streptavidin−HRP−AuNPs system was used to acquire better performance as multiple streptavidin−HRP molecules in one AuNP molecule. The association of several streptavidin−HRP molecules with each AuNP could amplify enzymatic signals and increase the sensitivities of analytical methods. Double-codified AuNPs (DC-AuNPs) that were employed in the early studies included both an anti-α-fetoprotein IgG HRP conjugate[18] and an anti-human IgG peroxidase conjugate,[25] but these have not been reported for streptavidin−HRP−AuNPs.

Specificity Study. Using the DC-CLIA based on ZEN−OVA−biotin and streptavidin−HRP−AuNPs, the cross-reactivities with the ZEN analogues (α-zearalanol, zearalanone, α-zearalenol, β-zearalanol, and β-zearalenol) were 32, 17, 12, 0.3, and 0.1%, respectivily. As shown above, the biotin−streptavidin−HRP−AuNPs DC-CLIA shows cross-reactivity for zearalanone (17%), α-zearalenol (12%), and an especially a high cross-reactivity for α-zearalanol (32%). Comparison with the previous studies about the cross-reactivity of anti-ZEN antibodies in ELISA and a commercial ELISA kit,[6,33] there were also high cross-reactivities for ZEN analogues. The reasons were the similar structure between ZEN and its analogues, and the antibodies were prepared by ZEN−(CMO)−protein conjugates, which were immunogens. Moreover, α-zearalanol plays a most important role in uterine cytoplasmic receptor of rat (in decreasing order, α-zearalanol > α-zearalenol > β-zearalanol > ZEN > β-zearalenol),[34] which indicates more severe toxicities of α-zearalanol than of ZEN in mammals.

No cross-reactivity was observed with other mycotoxins including deoxynivalenol, fumonisin B1, and aflatoxin B1 (<0.01%). These results indicated that this method has good specificity for ZEN.

Recovery Studies. Recovery rates of spiked food samples that were analyzed by biotin−streptavidin−HRP−AuNPs CLIA are shown in Table 3. The relative standard deviations (RSDs) were 4.1−8.2% for intraday comparisons and 5.3−9.4% for interday comparisons. Results for the recovery studies of biotin−streptavidin−HRP−AuNPs DC-CLIA were accurate and showed good reproducibility. As the first report of the

Table 3. Recovery Rates of Spiked Food Samples

	concentration of ZEN (μg/kg)				
sample	spiked	detected	intraday RSD[a] (%) (n = 3)	interday RSD (%) (n = 9)	recovery rate (%)
corn	25	25.9	4.9	5.8	104
	20	21.6	5.7	5.3	105
	15	15.9	5.1	7.4	106
	10	10.7	7.1	7.7	107
	5	4.85	7.4	7.9	97
	2.5	2.63	6.9	7.8	105
	1	1.05	7.5	8.4	105
wheat	25	28.2	6.3	8.2	113
	20	22.9	5.7	7.3	115
	15	17.6	7.4	6.8	117
	10	10.9	7.0	7.2	109
	5	5.63	6.2	8.4	113
	2.5	2.65	7.7	7.1	106
	1	1.04	7.3	8.0	104
noodles	25	27.8	6.7	7.1	111
	20	20.7	5.3	7.5	104
	15	15.6	6.4	6.7	104
	10	10.4	7.5	9.4	104
	5	5.44	6.3	6.9	109
	2.5	2.76	8.2	7.8	110
	1	1.07	7.3	8.5	107
biscuit	25	26.2	6.8	7.9	105
	20	20.3	4.1	7.5	102
	15	14.5	5.9	7.8	97
	10	10.4	5.6	8.2	104
	5	5.31	6.1	7.4	106
	2.5	2.60	8.1	7.9	104
	1	1.05	6.9	7.5	105

[a]RSD, relative standard deviation.

use of streptavidin−HRP−AuNPs, the results reported here indicate the effectiveness of this approach to signal amplification and the reliability of this method for the analysis of ZEN levels in food samples.

Natural Food Samples Analysis. Natural samples were measured using both biotin−streptavidin−HRP−AuNPs DC-CLIA and LC-MS/MS. The results are shown in Table 4.

Table 4. Comparison of the Capacities of a Biotin−Streptavidin−HRP−AuNPs DC-CLIA with LC-MS/MS for the Detection of ZEN in Food Samples

sample	biotin−streptavidin−HRP−AuNPs DC-CLIA (μg/kg), mean ± SD[a]	LC-MS/MS (μg/kg), mean ± SD
corn	23.2 ± 1.3	21.6 ± 1.4
corn	21.9 ± 1.4	22.7 ± 1.6
corn	4.45 ± 0.2	–[b]
wheat	22.3 ± 1.5	24.1 ± 1.3
wheat	19.7 ± 1.4	21.2 ± 1.0
biscuit	3.57 ± 0.1	–

[a]SD, standard deviation (n = 3). [b]–, not detected.

Given that the detection limit of LC-MS/MS was 10 μg/kg, levels of ZEN in food samples below this level could not be detected by LC-MS/MS, but could be detected using CLIA. Four samples were subjected to quantitative analysis by both methods. The correlation between the two methods was analyzed by Pearson correlation using SPSS software (11.5 version). The result showed significant correlation (P < 0.01), which indicated a good correlation between the two methods.

Comparison of other highly sensitive methods for the detection of ZEN (Table 5), such as electrochemical immunosensors,[8,35] electrochemical immunoassays,[36] and surface plasmon resonance,[37] indicated that the detection limit of our method was similar to that of electrochemical immunosensors and immunoassays, but the procedures of the latter methods were relatively more complex. Methods based on surface plasmon resonance method are also complicated and require both additional cleanup steps and expensive instruments. Surface plasmon resonance may not be suitable for detection of analytes in industrial or agricultural settings, although it offers high sensitivity and a wide dynamic working range.

Compared with the best broadly comparable alternatives, the novel CLIA using biotin−streptavidin−HRP−AuNPs described in this study is rapid, accurate, easy to use, and highly sensitive. This method also provides a platform for the detection of small-molecule analytes, including mycotoxins, drugs, and pesticide residues.

In conclusion, a novel chemiluminescence immunoassay to determine ZEN levels in food samples was prepared using a system that involves ZEN−OVA−biotin and streptavidin−HRP−AuNPs. Use of the biotin−streptavidin system and AuNPs for signal amplification improved the sensitivity of detection and the breadth of dynamic working ranges. For the CLIA using biotin−streptavidin−HRP−AuNPs systems, the limit of detection was 0.008 ng/mL and the IC_{50} was 0.11 ng/mL. The linear working range was 0.02−0.51 ng/mL. The recovery rates in spiked food samples were 97−117%, and the RSD values were all <10%. Analysis of natural samples using this novel CLIA and LC-MS/MS revealed a good correlation between the two methods. The method is highly sensitive, rapid, and accurate and has a wide working range. The use of chemiluminescence microplate readers in agricultural and industrial settings could enable detection of trace amounts of analytes using this platform.

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Author Contributions

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Notes

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Table 5. Comparison of Highly Sensitive Methods for the Detection of ZEN

analytical method	matrices	limit of detection	IC_{50}	dynamic working range	ref
chemiluminescence immunoassay	corn, wheat, noodles, biscuit	0.008 ng/mL	0.11 ng/mL	0.02−0.51 ng/mL	this study
electrochemical sensor (screen-printed electrode)	maize, baby food	0.007 ng/mL	0.088 ng/mL	–[a]	Hervás et al.[8]
electrochemical sensor (glass carbon electrode)	maize, baby food	0.011 ng/mL	0.079 ng/mL	–	Hervás et al.[35]
electrochemical immunoassay	corn, wheat, noodles, biscuit	0.002 ng/mL	0.054 ng/mL	0.004−9.5 ng/mL	Wang et al.[36]
surface plasmon resonance	corn	0.3 μg/kg	–	0.3−3000 ng/mL	Choi et al.[37]

[a]–, not mentioned.

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