ABSTRACT: A selective electrochemical biosensor was developed for detecting carbendazim (CBZ) based on well-dispersed colloidal boron nitride (BN) nanocrystals and gold nanoparticles (Au NPs). BN was synthesized by "solvent cutting" to modify a glassy carbon electrode (GCE), and Au NPs were then electrodeposited. A single-stranded oligonucleotide with methylene blue (MB) was modified to the electrode surface through gold–sulfur bonds. A double-stranded DNA was formed in the presence of an aptamer. The aptamer chain can specifically bind to the target CBZ. When the aptamer binds to CBZ, the electroactive substance MB labeled at one end of the complementary chain can effectively contact the electrode surface. Detection of CBZ is realized by simultaneously monitoring the MB signal enhancement. The CBZ concentration was determined in a wide linearity range from 0.1 ng mL⁻¹ to 100 µg mL⁻¹, with a low detection limit of 0.019 ng mL⁻¹. This biosensor exhibited excellent selectivity and acceptable repeatability and was applied in cucumber, kiwifruit, and water samples with good recoveries, demonstrating that the strategy has remarkable potential and offers a good platform for CBZ detection.

1. INTRODUCTION

Carbendazim (CBZ), a benzimidazole fungicide,¹ has been widely used in various agricultural production systems to control plant diseases from pathogens due to wide spectrum activity and low production costs.² However, the structure of CBZ contains a benzimidazole ring, which is difficult to degrade in the environment due to its long half-life. Additionally, it is absorbed into organisms by roots, seeds, or leaves of plants through the food chain.³ The excessive use of CBZ leads to pesticide residues and poses a threat to humans and other organisms.⁴,⁵ Therefore, it is highly desirable to explore a reliable method for detecting CBZ. Many analytical techniques have been carried out for investigating benzimidazole fungicides, including high-performance liquid chromatography,⁶ mass spectrometry,⁷ liquid chromatography coupled with mass spectrometry,⁸ UV–visible spectrometry,⁹ surface-enhanced Raman scattering,¹⁰ and fluorescence spectroscopy.¹¹ Although these analytical methods have been used to detect CBZ, they still have several drawbacks, such as high cost, intricate pretreatment process, and long test time.¹² In contrast, electrochemical techniques are superior because of their low cost, high efficiency, and ease of use.¹³ The electrochemical sensor monitors the electrical signal changes by changing the component concentration in the electrochemical system. All kinds of electrochemical sensors and biosensors have irreplaceable advantages. They can perform recognition with a specific receptor on the basis of common sensors. Hence, the CBZ detection of biosensing platforms using biological recognition molecules as a sensitive element is needed. The biosensing detection process is the specific binding of a target to its biological recognition molecules (enzymes, antibodies, aptamers, antigens, and microorganisms) to give rise to signal changes.¹⁴ In addition, an aptamer (Apt), as a kind of receptor, can improve the selectivity of the biosensor. Apt, a single-stranded oligonucleotide (DNA or RNA), can screen from the oligonucleotide fragment library by the “exponential enrichment” systematic evolution of ligands.¹⁵–¹⁷ With strong stability, high affinity, strong specificity, and easy modification, Apt can specifically bind to the target.¹⁸ Recently, Wu et al. constructed a new dual-mode aptasensor for microcystin-LR ultrasensitive detection using MoS₂-PtPd materials with a limit of detection (LOD) of 0.045 ng mL⁻¹.¹⁹ Likewise, Su et al. proposed a fluorescent aptasensor using Au NPs and rhodamine B as an indicator with a LOD of 2.33 nM.²⁰ To further enhance the sensing performance, previous investigations concentrated on the properties...
of electrode materials. Electrode nanomaterials have a great influence on sensor stability and play a role in aptasensor construction for pesticide determination.

As a new material with similar properties to graphene, boron nitride (BN) has gradually attracted attention due to its superior properties, such as easy functionalization, high surface area, and chemical stability. BN has been extensively employed for electrochemical sensor fields by deposition on the surface of a glassy carbon electrode (GCE). Kokulnathan’s group used h-BN and bismuth oxide to fabricate a flutamide electrochemical sensor. In addition, Adeel et al. constructed an Apt/AuNPs/BN/FTO sensor for myoglobin with a detection limit of 34.6 ng mL^{-1}. However, the application of h-BN is hindered because of the limit of dispersivity and polydispersed size. It is a challenge to look for a kind of size-controlled and well-dispersed BN nanocrystal as the fundamental material in the aptasensor field.

Here, we presented a novel electrochemical Apt biosensor based on well-dispersed colloidal BN for detecting CBZ. Colloidal BN was composed of boric acid and melamine nanocrystals combined with glycol solvent. Although BN is a reliable insulator, BN is restricted by a large band gap. Anchoring gold nanoparticles (Au NPs) can enhance electron–hole separation and narrow band gap and functionalize BN easily. The AuNPs/BN/GCE as a transducer was used to immobilize the capture probe (CP) with labels of methylene blue (MB) and a sulfhydryl group at its 5′ and 3′ terminal carboxylated Apt to form double strands. DNA biosensors rely on electrochemical transduction of hybridization between probes and complementary sequences. The detection of hybridization is accomplished using the reduction signal of MB. MB, an organic photosensitive dye of the phenothiazine family, is a redox indicator with electrochemical activity. It can be inserted into the nucleic acid double strand and plays a role in being adsorbed on the probe by a large number of specific interactions with guanine bases on DNA. MB acts as a cation, and the guanine on the DNA strand has electrochemical activity and shows electronegativity. The strong affinity between the two promotes MB to enter the GCE, resulting in a substantial increase of the current signal. The response performance between Apt and CBZ was investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods. Moreover, the aptasensor was further used for determining the CBZ content in three different actual samples.

2. RESULTS AND DISCUSSION

2.1. Structural and Morphological Analysis of the BN/AuNP Composites. Formation of the nanomaterials was characterized by X-ray diffraction (XRD), Raman spectroscopy, and scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Figure 1A illustrates the XRD spectra of colloidal BN. The three diffraction peaks characteristic of BN at 26.46, 42.38, and 55.31° correspond to the (002), (100), and (004) planes (JCPDS card no. 34-0421), verifying the crystalline nature of the BN nanomaterial. The crystalline structure of BN was studied by Raman spectroscopy (Figure 1B). The characteristic band of BN at 1368 cm^{-1} corresponds to the E_{2g} phonon mode. These results are consistent with that of BN reported in other literature.

SEM was used to corroborate the surface morphologies of the nanocomposites, and TEM was applied to investigate the structure and morphology. SEM and TEM images of BN and Au NP composites are shown in Figure 2. BN colloidal nanocrystals represent a flake-like structure with many random mesopores in the layer (Figure 2A). In Figure 2B, Au NPs were well dispersed on the surface of BN. In addition, there are distinct lattice fringes of pristine BN (Figure 2C, looking messy and inconsistent), revealing that boron nitride has high crystallinity. Several spherical nanoparticles were on the surface of BN. The BN/AuNP nanostructure exhibits a single layer structure with a high surface composite (Figure 2D).

The dispersibility experiments of BN (2 mg) in water (2 mL) and ethanol (2 mL) from 0 to 48 h are shown in Figure S1. BN is evenly dispersed without precipitation after being dissolved in water and ethanol for 48 h. The dispersibility of BN is satisfactory due to the imperfect nitridation of the BO species. In addition, the X-ray powder diffraction peak 101 of the sample is not cracked, and radial grains containing boron nitride are less than 100 nm.

2.2. Electrochemical Characterization. The stepwise procedure of the aptasensor in each step was characterized by CV and electrochemical impedance spectroscopy (EIS) (0.1 mol L^{-1} KCl and 5.0 mmol L^{-1} [Fe(CN)_{6}]^{3-/-4} as the redox probe solution). The frequency of CV curves changes from ~0.3 to 0.4 V at a scan rate of 50 mV s^{-1}. As shown in Figure 3A, a pair of reversible redox peaks appeared on the bare GCE at ~0.154 and 0.323 V because of the high electron transfer efficiency. The response on the AuNPs/BN/GCE was better than that achieved on the bare GCE. However, the signal decreased after adding CP on the AuNPs/BN/GCE because the successful immobilization of probe DNA impeded the electron exchange. After treating 6-mercapto-1-hexanol (MCH), the current is further reduced. It can be explained that the insulating MCH layer formed on the electrode surface prevents electron transfer. Moreover, the peak current decreased owing to the presence of Apt, revealing that the insulating Apt layer acts as a barrier for electronic communication and mass transfer.
transfer. These phenomena indicate that the material has been loaded on the electrode.

EIS is used to perform the surface features of different stages during the fabrication of the aptasensor. The Nyquist plots of impedance spectroscopy show a straight line and a semicircle domain, corresponding to the processes of limited diffusion and limited electron transfer. The \( R_{ct} \) value is estimated from the semicircle diameter of the EIS Nyquist diagram. Surfaces of different electrodes are given in Figure 3B, and the frequency changed from 100 kHz to \(-0.1 \) Hz. Curve a is the impedance diagram of the bare electrode. After BN and gold modification (curve b), the gradual decrease of \( R_{ct} \) indicates that the modification of colloidal BN and Au NPs can significantly improve the electron transfer performance. However, the modification of CP, MCH, and Apt resulted in the further increased \( R_{ct} \) (curves c–e), indicating that the immobilization of these materials hinders the electronic exchange between the redox probe and the electrode.

To confirm the feasibility and sensitivity of this electrochemical aptasensor for CBZ, the aptasensor was performed in phosphate buffers (PBs) with and without CBZ. The current response of DPV is verified in Figure 4. In the absence of CBZ, there was a low value of 13.64 \( \mu \)A. After adding CBZ (100 \( \mu \)g mL\(^{-1}\)), the peak current of the DPV spectrum increased accordingly. The current changes indicated that CBZ and Apt combined with each other.

2.3. Optimization of Detection Conditions. The performance of this electrochemical aptasensor can be affected by several vital factors, such as pH, the concentration of Apt, and the binding time of CBZ with Apt. Accordingly, it is necessary to optimize parameters to achieve excellent performance.

Figure 5A shows the effects of the PB pH value the oxidation peak current. The peak current response of DPV increased with the increase of the pH value from 5.0 to 7.0 and then decreased with increasing pH, illustrating the optimal DPV response at 7.0. A PB pH value of 7.0 was used throughout the subsequent experiments.

The electrochemical behavior of Apt ranging from 0.5 to 3 \( \mu \)M was investigated (Figure 5B). The current of absorbed
MB increased while the concentration of Apt on the surface of the modified electrode was from 0.5 to 1.5 μM owing to the increase in the binding amounts of Apt and CBZ. Higher Apt concentrations caused reduction of the MB signals because intermolecular hybridization of excessive Apt led to a decrease of Apt immobilization on the GCE. Therefore, an Apt concentration of 1.5 μM was chosen in later studies.

The binding time of CBZ with Apt plays a great role in the performance of the electrochemical aptasensor (Figure 5C). The aptasensor was immersed in the CBZ solution for 40, 45, 50, 55, 60, 65, and 70 min. The peak current greatly increased as the binding time increased before 45 min and slightly decreased after 45 min, revealing the saturation of binding sites for Apt at 45 min. Therefore, 45 min was chosen in further experiments to determine CBZ.

**2.4. Electrochemical Detection of CBZ.** CBZ in the range of 0.1 ng mL⁻¹ to 100 μg mL⁻¹ was tested with DPV under the optimized experimental conditions to assess the sensitivity and dynamic ranges of the electrochemical aptasensor. The Apt/MCH/CP/AuNPs/BN/GCE was immersed in different concentrations of CBZ for 45 min. From Figure 6A, the oxidation current signal of the aptasensor increased as the CBZ concentration continuously increased. Figure 6B shows a good linear relationship, and the linear regression equation is \( I_p (\mu A) = 1.575 \log C (\mu g mL^{-1}) + 24.586 \) (\( R = 0.9912 \)). The LOD was 0.019 ng mL⁻¹, calculated according to the equation LOD = \( 3S_a/b \). Different analytical results for CBZ detection are shown in Table 1. Compared with other methods, the AuNPs/BN composites provide a large specific surface area to increase the loading of CBZ, and the aptasensor has a broad linear range and a low detection limit for CBZ detection. Therefore, the aptasensor can realize sensitive detection of CBZ.

**2.5. Stability, Reproducibility, and Interferences of the Atpasensor.** The capability of the system was monitored by DPV under the conditions (pH = 7, concentration of 1.5 μM, and binding time of 45 min). To investigate the stability of the aptasensor, the Apt/MCH/CP/AuNPs/BN/GCE was stored in the lab at room temperature. The aptasensor retained 99.8% of the initial response after 10 days of storage in the refrigerator, indicating that its stability was excellent. The reproducibility of the aptasensor was demonstrated by four Apt/MCH/CP/AuNPs/BN/GCEs for detecting 10 ng mL⁻¹ CBZ. The relative standard deviation (RSD) was calculated to be 3.37%. The interference is another essential analytical parameter to evaluate the performance of the aptasensor. Tenfold concentration (100 ng mL⁻¹) pesticides (trichlorfon, chlorpyrifos, carbaryl, triazolone, and amitrole) were tested to assess the specificity of the aptasensor. The responses were recorded on the Apt/MCH/CP/AuNPs/BN/GCE along with the DPV response to

---

**Figure 4.** DPV signals of carbendazim (CBZ): aptasensor in PB (black) and aptasensor treated with 100 μg mL⁻¹ CBZ in PB (red).

**Figure 5.** Optimization of assay conditions: (A) pH value of the electrolyte, (B) Apt concentration, and (C) binding time of CBZ with Apt.

**Figure 6.** (A) DPV signals of CBZ at pH 7 on the Apt/MCH/CP/AuNPs/BN/GCE with different concentrations (\((a-h): 0, 1 \times 10^{-10}, 1 \times 10^{-9}, 1 \times 10^{-8}, 1 \times 10^{-7}, 1 \times 10^{-6}, 5 \times 10^{-5}, \) and \(1 \times 10^{-4} \) g mL⁻¹). (B) The linear relationship for the increasing concentration of CBZ.
aptasensor had outstanding specificity for the detection of CBZ. The well-validated recoveries for CBZ were determined by monitoring the electrochemical signal of MB (Table 2). The principle was applied for the determination of CBZ in cucumber, kiwifruit, and tap water samples with satisfactory recoveries. The aptasensor is a viable and promising tool with great application potential in the sensor field and may lay a foundation for the monitoring of CBZ. In addition, the scope of application of well-dispersed BN colloidal nanocrystals is greatly broadened, and BN is promising for use in many other applications.

### 4. EXPERIMENTAL SECTION

#### 4.1. Materials.
Gold chloride tetrahydrate (HAuCl₄·4H₂O), CBZ, boric acid, melamine, and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were obtained from Aladdin Industrial Corporation (Shanghai, China, https://www.aladdin-e.com). MCH was procured from Sigma-Aldrich (https://www.sigmaaldrich.com). Tris(hydroxymethyl)aminomethane was obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China, https://www.macklin.com). Methanol, absolute ethanol, glycol, potassium ferricyanide (K₃Fe(CN)₆), and potassium ferrocyanide (K₄Fe(CN)₆) were obtained from Sigma-Aldrich (Shanghai, China, http://www.sangon.com). A variety of PBs were obtained by mixing Na₂HPO₄ (0.1 M) and NaH₂PO₄ (0.1 M) stock solutions. Deionized water was used throughout the experiments. All synthetic DNA sequences were as presented in ref 1 and provided by Sangon Inc. (Shanghai, China, http://www.sangon.com). The base sequences of synthetic oligonucleotides are shown as follows

#### 3. CONCLUSIONS

A novel electrochemical aptasensor based on the competition principle was applied for the determination of CBZ in cucumber, kiwifruit, and tap water samples with satisfactory recoveries. The aptasensor is highly promising for real sample analysis.

### Table 1. Comparison of the Aptasensor with Other Methods for CBZ Detection

| method                  | linear range (g mL⁻¹) | LOD (g mL⁻¹) | reference |
|-------------------------|-----------------------|--------------|-----------|
| biosensor               | 1 × 10⁻¹⁰ to 1 × 10⁻⁸ | 8.2 × 10⁻¹²  | 1         |
| spectroscopy            | 0–300 × 10⁻⁶         | 5 × 10⁻⁶     | 2         |
| HPLC                    | 0.2–4 × 10⁻⁴         | 0.2 × 10⁻⁶   | 6         |
| UV–vis spectrophotometry| 5 × 10⁻⁹ to 6 × 10⁻⁷ | 2.1 × 10⁻⁶   | 9         |
| Raman scattering        | 0–5.2 × 10⁻⁴         | 3.4 × 10⁻⁶   | 10        |
| electrochemical sensor  | 1.9 × 10⁻⁴ to 4.5 × 10⁻⁷ | 6.71 × 10⁻¹⁰ | 12        |
| aptasensor              | 1 × 10⁻¹⁰ to 1 × 10⁻⁴ | 1.9 × 10⁻¹¹  | this work |

Figure 7. Selectivity evaluation of the aptasensor detection of chlorpyrifos, triazolone, amitrole, carbaryl, and CBZ. 10 ng mL⁻¹ CBZ (Figure 7). These results indicated that the aptasensor had outstanding specificity for the detection of CBZ.

#### 2.6. Analytical Application of the Aaptasensor
To estimate the feasibility of this method, the aptasensor was used to detect CBZ in kiwifruit, cucumber, and tap water samples. A certain amount of CBZ (0, 1, 5, and 10 μg mL⁻¹) was added to three different samples (2 mL) to form spiked samples. The samples were tested by DPV and were also determined by gas chromatography–mass spectrometry (GC-MS). The well-validated recoveries for CBZ changed from 92.8 to 104.6% and the RSD was in the range between 2.4 and 5.7%, indicating that the electrochemical aptasensor is highly promising for real sample analysis.

#### Table 2. Detection of CBZ in Water, Cucumber, and Kiwifruit Samples

| sample          | found (μg mL⁻¹) | RSD (%) | recovery (%) | found (μg mL⁻¹) | RSD (%) | recovery (%) |
|-----------------|----------------|---------|--------------|----------------|---------|--------------|
| tap water       | 1.00           | 0.95 ± 0.02 | 2.7          | 95.0           | 1.003 ± 0.02 | 5.2          | 100.3        |
|                 | 5.00           | 5.02 ± 0.12 | 3.1          | 100.4          | 4.94 ± 0.06  | 3.9          | 98.8         |
|                 | 10.0           | 10.45 ± 0.10 | 3.2         | 104.5          | 10.3 ± 0.12  | 4.3          | 103.0        |
| cucumber        | 1.00           | 1.03 ± 0.21 | 3.8          | 103.0          | 1.01 ± 0.04  | 5.1          | 101.0        |
|                 | 5.00           | 5.10 ± 0.09 | 2.4          | 102.0          | 5.23 ± 0.15  | 3.6          | 104.6        |
|                 | 10.0           | 9.72 ± 0.01 | 4.5          | 97.2           | 10.05 ± 0.20 | 3.4          | 100.5        |
| kiwifruit       | 1.00           | 0.99 ± 0.17 | 5.7          | 99.0           | 0.93 ± 0.03  | 5.4          | 93.0         |
|                 | 5.00           | 4.77 ± 0.20 | 3.3          | 95.4           | 4.64 ± 0.09  | 4.2          | 92.8         |
|                 | 10.0           | 9.89 ± 0.08 | 2.9          | 98.9           | 9.55 ± 0.24  | 4.8          | 95.5         |
CBZ-aptamer: 5′-GGGCACACAAACCGATGGTCCAGC-CACCAGATGCAACGCGCCCAACCAGG-3′
MB-CP probe: 5′-MB-CGCGGGTGCCGGGTTGGGCTGTGAGCATTGGGGCTGACCCATCGTTGT- GTTGCCCG-(CH2)6-SH-3′

4.2. Preparation of BN. Initially, boric acid and melamine (n1/n2 = 1:6) were mixed and ground. The precursor mixture was heated to 900 °C (10 °C min⁻¹) and maintained for 2 h under 5% H2/N2 flow in a horizontal tube furnace. Afterward, 100 mL of ethylene glycol was used to prun the resulting solids (2 g) for 2 h. Finally, the dialysis process of the BN dispersion was performed on the membrane for 1 week and then filtered (0.45 μm). The desirable BN colloidal nanomaterial was used in the following process.

4.3. Synthesis and Characterization of AuNPs/BN/GC. Before loading, the GCE was polished with alumina paste (0.05 μm) and rinsed thoroughly with deionized water, followed by ultrasonic cleaning for 30 s with nitric acid, anhydrous ethanol, and deionized water. BN (1 mg) was dispersed into 1 mL of deionized water via 40 min of ultrasonic mixing to obtain a uniform suspension, and 10 μL of the mixture was dropped on the cleaned GCE. Au NPs were then deposited on the surface of the BN/GCE by constant potential deposition.29 In brief, the BN/GCE was immersed into a solution of 0.01 M Na2SO4 containing HAuCl4 (2.5 mm) and then electrodeposited under a potential of −0.2 V for 300 s. Finally, the AuNPs/BN/GCE was dried in air for further use.

4.4. Aptasensor Fabrication. MB-CP was first mixed with fresh 1 mM TCEP and incubated for 1 h to reduce the disulfide bond. Then, 5 μL of the MB-HP probe was fixed on the surface of the AuNPs/BN/GCE and allowed to dry. The electrode dried naturally at room temperature. Subsequently, the CP/AuNPs/BN/GCE was cleaned by 10 mM Tris-HCl buffer (pH 7) and dried in air. Afterward, to block nonspecific sites, 6 μL of MCH (1 mM) was loaded on the electrode surface and kept for 1 h, followed by rinsing with Tris-HCl buffer. The electrode was covered with 5 μL of Apt (5 μmol/L) and stored at 4 °C overnight. Extra nonspecific absorbed Apt was removed with Tris-HCl buffer.

4.5. Real Sample Preparation. CBZ in cucumber, kiwifruit, and water samples was detected to evaluate the applicability of the aptasensor. The cucumber and kiwifruit samples were purchased from a local market, and the water samples were obtained from tap water. The cucumber and kiwifruit samples were mashed into juice, separately. Then, the juice samples were filtered using funnels and centrifuged for 10 min at 4000 rpm. Furthermore, a filter (0.22 μm) was used to filter the supernatant solutions. Then, the pH was adjusted to 7 before detection and these spiked samples with different concentrations of CBZ were measured by DPV. To evaluate the detection results of the aptasensor, the results were compared with GC-MS measurements.

# ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04326.

Apparatus; electrochemical; detection BN dispersibility; and time distribution graphs of BN in deionized water (left) and ethanol (right) at 0, 1, 2, 3, 4, 5, 6, 16, 18, 22, 25, 31, 41, 42, and 48 h (Figure S1) (PDF)

# AUTHOR INFORMATION

Corresponding Authors
Yuan Qin – Shaanxi Key Laboratory of Earth Surface System and Environmental Carrying Capacity, College of Urban and Environmental Science, Northwest University, Xi’an 710127, China; Key Laboratory of Watershed Geographic Sciences, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China; Email: qinyuanas123@stumail.nwu.edu.cn
Huayu Huang – Shaanxi Key Laboratory of Earth Surface System and Environmental Carrying Capacity, College of Urban and Environmental Science, Northwest University, Xi’an 710127, China; Key Laboratory of Watershed Geographic Sciences, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China

Authors
Ruijie Wang – Shaanxi Key Laboratory of Earth Surface System and Environmental Carrying Capacity, College of Urban and Environmental Science, Northwest University, Xi’an 710127, China
Yangzi Li – Shaanxi Key Laboratory of Earth Surface System and Environmental Carrying Capacity, College of Urban and Environmental Science, Northwest University, Xi’an 710127, China
Zhenfeng Lin – Shaanxi Key Laboratory of Earth Surface System and Environmental Carrying Capacity, College of Urban and Environmental Science, Northwest University, Xi’an 710127, China
Rong Nie – School of Chemistry and Chemical Engineering, Lanzhou City University, Lanzhou 730070, China
Yifei Shi – Shaanxi Environment Investigation and Assessment Center, Xi’an 710054, China

Complete contact information is available at: https://pubs.acs.org/doi/10.1021/acsomega.1c04326

Notes
The authors declare no competing financial interest.

# ACKNOWLEDGMENTS

This work was supported by the Program for Key Research and Development Plan in Shaanxi Province (No. 2019SF-243), the Innovation Capability Support Program of Shaanxi Province (No. 2020TD-022), and Open Program of Key Laboratory of Watershed Geographic Sciences, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences (WSGS2020008).

# REFERENCES

(1) Eissa, S.; Zourob, M. Selection and characterization of DNA aptamers for electrochemical biosensing of carbendazim. Anal. Chem. 2017, 89, 3138–3145.
(2) Qin, B.; Li, Z.; Hu, F.; Hu, C.; Chen, T.; Zhang, H.; Zhao, Y. Highly sensitive detection of carbendazim by using terahertz time-domain
spectroscopy combined with metamer. IEEE Trans. THz Sci. Technol. 2018, 8, 149–154.
(3) Wang, S.; Su, L.; Wang, L.; Zhang, D.; Ma, Y.; et al. Colorimetric determination of carbendazim based on the specific recognition of aptamer and the poly-diallyldimethylammonium chloride aggregation of gold nanoparticles. Spectrochim. Acta, Part A 2020, 228, No. 117809.
(4) McCarroll, N. E.; Protzel, A.; Ioannou, Y.; Stack, H. F.; Jackson, M. A.; Waters, M. D.; Dearfield, K. L. A survey of EPA/OPP and open literature on selected pesticide chemicals: III. Mutagencity and carcinogeticity of benomyl and carbendazim. Mutat. Res. 2002, 512, 1–35.
(5) Goldman, J. M.; Rehnborg, G. L.; Cooper, R. L.; Gray, L. E.; Hein, J. F.; et al. Effects of the benomyl metabolite, carbendazim, on the hypothalamic-pituitary reproductive axis in the male rat. Toxicology 1989, 57, 173–182.
(6) Lopez, L. F.; Lopez, A. G.; Riba, M. V. HPLC method for simultaneous determination of fungicides: carbendazim, metalaxyl, folpet, and propiconazole in must and wine. J. Agric. Food Chem. 1989, 37, 684–687.
(7) Gilbert-López, B.; García-Reyes, J. F.; Ortega-Barralés, P.; Molina-Díaz, A.; Fernández-Alba, A. R. Analyses of pesticide residues in fruit-based baby food by liquid chromatography/electrospray ionization time-of-flight mass spectrometry. Rapid Commun. Mass Spectrom. 2007, 21, 2059–2071.
(8) Hogendoorn, E.; Zoonen, P. V. Recent and future developments of liquid chromatography in pesticide trace analysis. J. Chromatogr. A 2000, 892, 435–453.
(9) Larki, A.; Pourreza, N.; Rastegarzadeh, S. Determination of fungicide carbendazim in water and soil samples using dispersive liquid-liquid microextraction and microvolume UV−vis spectrophotometry. Talanta 2015, 134, 24–29.
(10) Furini, L. N.; Sanchez-Cortes, S.; López-Tocón, I.; Otero, J. C.; Aroca, R. F.; Constantino, C. Detection and quantitative analysis of carbendazim herbicide on Ag nanoparticles via surface-enhanced Raman scattering. J Raman Spectrosc. 2015, 46, 1095–1101.
(11) Garrido Frenich, A.; Zamora, D. P.; Martínez Vidal, J. L.; Martínez Galera, M. Standardization of SPE signals in multicomponent analysis of three benzimidazolic pesticides by spectrofluorimetry. Anal. Chim. Acta 2003, 477, 211–222.
(12) Maximino, E. M.; Lima, F. D.; Cardoso, C.; Arruda, G. J. Modification of carbon paste electrodes with recrystallized zeolite for simultaneous quantification of thiram and carbendazim in food samples and an agricultural formulation. Electrochim. Acta 2018, 259, 66–76.
(13) Zhang, Z.; Guo, C.; Zhang, S.; He, L.; Wang, M.; Peng, D.; Tian, J.; Fang, S. Carbon-based nanocomposites with aptamer-templated silver nanoclusters for the highly sensitive and selective detection of platelet-derived growth factor. Biosens. Bioelectrochem. 2017, 89, 735–742.
(14) Thévenot, D. B.; et al. Electrochemical biosensors: recommended definitions and classification. Biosens. Bioelectrochem. 2001, 16, 121–131.
(15) Couture, S.; Ellington, A. D.; Gerber, A. S.; Cherry, J. M.; Doudna, J. A.; Green, R.; Hanna, M.; Pace, U.; Rajagopal, J.; Szostak, J. W. Mutational analysis of conserved nucleotides in a self-splicing group I intron. J. Mol. Biol. 1990, 215, 345–358.
(16) Tuerk, C.; Gold, L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 1990, 249, 505–510.
(17) Robertson, D. L.; Joyce, G. F. Selection in vitro of an RNA enzyme that specifically cleaves single-stranded DNA. Nature 1990, 344, 467–468.
(18) Xu, G.; Hou, J.; Zhao, Y.; Bao, J.; Yang, M.; Fa, H.; Yang, Y.; Li, L.; Huo, D.; Hou, C. Dual-signal aptamer sensor based on polydopamine-gold nanoparticles and exonuclease I for ultrasensitive malathion detection. Sens. Actuators, B 2019, 287, 428–436.