



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Zr-doped CoOOH nano-enzymes with enhanced oxidase-like activity for the colorimetric determination malathion in Foods

Shixin Li¹, Xiaomin Pang¹, Xue Wang, Zhixiang Xu, Yufeng Sun*

Key Laboratory of Food Processing Technology and Quality Control in Shandong Province, College of Food Science and Engineering,
Shandong Agricultural University, Tai'an 271018, China

ABSTRACT: Herein, a colorimetric platform is presented based on zirconium-doped cobalt oxyhydroxide (Zr-CoOOH) nano-enzymes for the rapid detection of malathion (MAL). The CoOOH nano-enzymes showed excellent oxidase-like activity. With the use of 3,3',5,5'-tetramethylbenzidine (TMB) as the chromogenic substrate, the Zr-CoOOH nano-enzymes can oxidize the colorless TMB to the blue product (oxTMB), and the UV-visible spectral absorption band of oxTMB is 652 nm. The oxidase-like activity of Zr-CoOOH nano-enzymes was significantly enhanced, which was attributed to the high affinity between Zr-CoOOH nano-enzymes and TMB. The metal in the Zr-CoOOH nano-enzymes can be combined with the phosphate thiolate groups in the MAL molecule, thus inducing an enhanced catalytic activity of MAL for the reaction. The Zr-CoOOH+TMB+MAL system can achieve the quantitative detection of MAL. Under the optimized conditions, the constructed sensor exhibited a linear relation with MAL over the range of concentration from 0.1-10 μM , with a detection limit of 0.023 μM . The sensing platform showed excellent anti-interference and batch stability. This strategy expands the application of nano-enzymes to the area of rapid detection.

Keywords: Nano-enzymes; Zr doped Cobalt oxyhydroxide; Colorimetric detection; Malathion

1. Introduction

Malathion (MAL), one of the organophosphorus pesticides, has widely used in agricultural production as a high-effective insecticide. The extensive use of MAL leads to residual in soil, water and atmosphere. Because of the residues, human health will be seriously threatened through the food chain. In addition, MAL has been categorized as a Group 2A carcinogen according to the World Health Organization's International Agency for Research on Cancer in 2007. Therefore, it is imperative to develop a rapid and sensitive analytical method for detection of MAL.

The corresponding analytical methods for detection MAL, such as liquid chromatography-mass spectrometry (ALothman, et al., 2019), capillary electrophoresis (García-Ruiz, et al., 2005), and the surface-enhanced Raman scattering (Zhang, et al., 2022b), are authoritative but with the disadvantages of time-consuming, professional operation and expensive equipment. In order to address those challenges, a series of

*Corresponding author
sunyufeng1208@163.com (Y.F. Sun).

Received 27 August 2025
Received in revised form 29 November 2025
Accepted 6 January 2026

sensing platforms including electrochemiluminescence (Li, et al., 2024a; Li, et al., 2025a) colorimetric (Zhang, et al., 2025a), fluorescent sensor (Li, et al., 2025c; Han, et al., 2025) and electrochemical sensor (Zhang, et al., 2025b) have been employed in food safety. Among these methods, colorimetric sensors are outstanding due to their fast analysis speed, easy operation, and high efficiency.

Traditional colorimetric sensors often require bio-enzymes to assist in the construction of sensing systems. For example, an ultrasensitive colorimetric immunosensing method was developed by combining the enzyme-nanoprobe induced biomineralization of cupric subcarbonate with a sensitive copper chromogenic reaction (Li, et al., 2018). Lin et al researchers used bio-enzyme encapsulated liposomes to achieve signal amplification for colorimetric detection ochratoxin A (Lin, et al., 2018). However, bio-enzymes are easily deactivated, difficult to store, and high cost, which are challenges in constructing colorimetric sensing systems.

Nano-enzymes are functional nanomaterials with enzyme-like activities and have drawn extensive concerns during to the advantages of adjustable catalytic activity, robust stability, and cost efficiency (Liu, et al., 2024; Tian, et al., 2024). According to catalytic mechanisms, nano-enzymes can be divided into peroxidase, catalase, oxidases, superoxide dismutase and hydrolases and have shown great potential in medicine, food safety, industrial applications and other fields (Lu, et al., 2024). In view of the above characteristics, nano-enzymes have emerged as an excellent candidate for constructing colorimetric sensing systems.

Cobalt hydroxide (CoOOH) is an emerging transition metal hydroxyl oxide. Due to its surface properties, wide absorption band, good redox properties and biocompatibility, CoOOH has received extensive attention in the field of optical analysis (Zhang, et al., 2022a). A series of fluorescence sensors have been developed using the quenching property of CoOOH (Wu, et al., 2019; Shen, et al., 2021). And, the peroxidase-like activity of CoOOH has been employed to construct sensing platform (Jin, et al., 2019; Li, et al., 2019). Although nano-enzymes have been widely used in various fields, improving their catalytic activity remains a challenge. Cheng researcher significantly enhanced the oxidase-like activity of Fe-Co dual-atom nano-enzymes doped with S element by regulating the local electronic structure of the active site (Cheng, et al., 2024). Zn single-atom nano-enzymes with oxidase-like activity was improved by forming Zn—N—B bond in situ during high temperature pyrolysis (Li, et al., 2025b). Hence, heteroatom doping is undoubtedly an effective method for regulating the catalytic activity of nano-enzymes.

Herein, we found that CoOOH nanosheets synthesized by the hydrothermal method possess a novel enzyme-like catalytic activity, the activity of an enzyme-like oxide enzyme. In the absence of H₂O₂, CoOOH nano-enzyme can catalyze colorless TMB to blue. The catalytic activity of CoOOH nano-enzyme was further enhanced through Zr doping (Zr-CoOOH). A platform for colorimetric sensing was constructed by leveraging the synergistic effect of MAL on the oxidase-like activity of Zr-CoOOH nano-enzyme. This method provides a new strategy for the development and improvement of the catalytic activity of nano-enzymes.

2. Experimental section

2.1. Materials and reagents

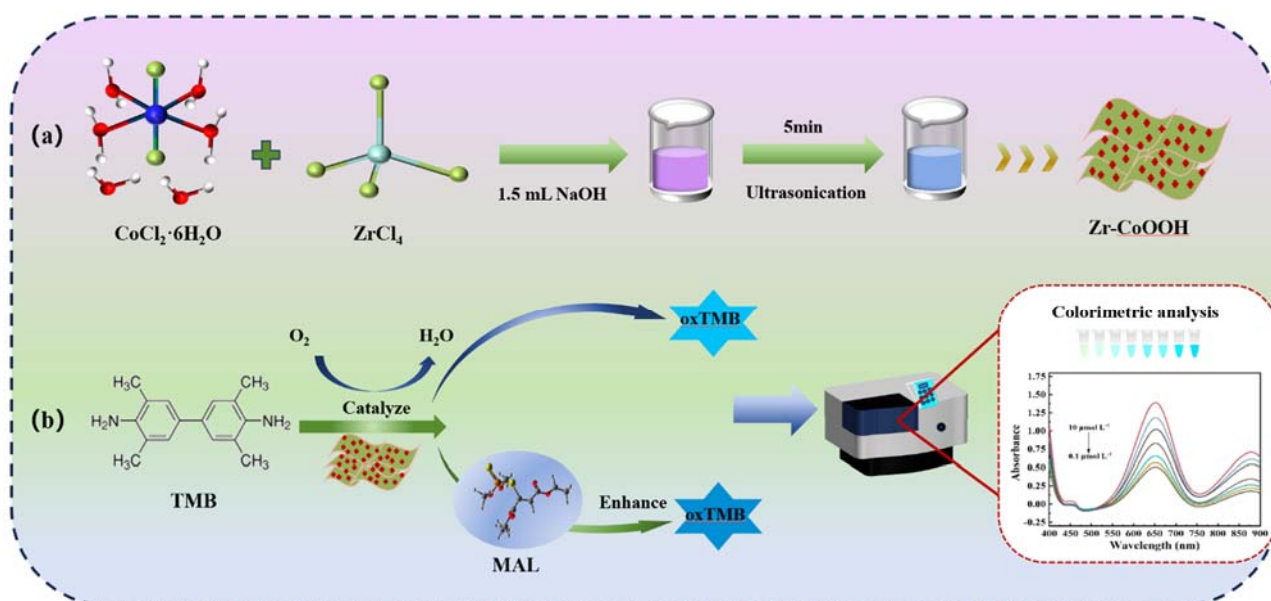
Apple and cantaloupe samples were purchased at the Tai'an market (Shandong, China). 3,3',5,5'-tetramethylbenzidine (TMB), glutathione (GSH), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, ZrCl_4 , $\text{C}_4\text{H}_{10}\text{O}_6\text{Zn}$ and Na_2SO_4 were supplied in Shanghai Aladdin Biochemical Technology Co., Ltd. 2,2-Diphenyl-1-picrylhydrazyl (DPPH, 96 %), 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS, 98 %) obtained from Macklin Co., Ltd. Malathion (MAL), methyl parathion (MP), trichlorfon (TCF), chlorpyrifos (Chl) and glyphosate (GLY) were bought by the Agricultural Chemical Control Institute of the Ministry of Agriculture in Beijing. Glucose (Glu), ascorbic acid (AA), acetonitrile, CH_3COOH , KCl, NaCl and NaOH were acquired through Tianjin Kaitong Chemical Reagent Co., Ltd. All experiments utilized double distilled water (DDW) derived by Aike ultra-pure water facility in Chengdu, China. A mixture of 0.2 M Na_2HPO_4 solvent and 0.2 M NaH_2PO_4 at varying ratios to produce phosphate buffered saline (PBS) of various pH levels. A sample solution of 10.0 mM of MAL was made up by melting 330.36 mg of MAL in 100.0 mL of ethanol. The working solution was obtained by diluting the stock solution with PBS at different concentrations.

2.2. Instruments and apparatus

The ultraviolet-visible (UV-Vis) absorption spectra was noted by using a UV-2600i spectrophotometer (Japan). Transmission electron microscopy (TEM) images were made by Hitachi H-600 Microscopy Incorporated (Japan). Fourier transform infrared (FT-IR) spectra within an area of $4000\text{--}500\text{ cm}^{-1}$ were measured in a NICOLET iS10 infrared Spectrophotometer (America). The flowing rate of the carrier gas (N_2) was 10 ml min^{-1} .

2.3. Synthesis of Zr-CoOOH nano-enzymes

The synthesis method of Zr-CoOOH nano-enzymes was accorded to the same laboratory (Pang et al., 2024). Synthesis schematic of Zr-CoOOH nano-enzymes was shown in Scheme 1. (a).



Scheme 1. (a) Synthesis schematic of Zr-CoOOH nano-enzymes. (b) Schematic representation of colorimetric detection of MAL based on OXD-like activity of Zr-CoOOH nano-enzymes.

2.4. Method determination of activity of Zr-CoOOH nano-enzymes

Briefly, 0.1 mg of Zr-CoOOH was dispersed into 1.0 mL of DDW by sonication for further use. Subsequently, 400 μL of Zr-CoOOH dispersion and 200 μL of TMB solution (1.0 mM) were mixed together and then added into 1400 μL of acetate buffer (pH 3.0~5.5). The absorbance of the resulting mixture was determined at 652 nm after 10 min of incubation.

2.5 The evaluation of Zr-CoOOH enzyme activity

The steady-state kinetic analysis of Zr-CoOOH was examined. Kinetic experiments were performed via the addition of different concentrations of TMB in Zr-CoOOH acid buffer. Then, the evolution of absorbance at 652 nm within 10 min was determined by UV spectrophotometer. Kinetic parameter of K_m of the enzyme could be calculated out of the Michaelis- Menten kinetic equation (1) and Lineweaver-Burk equation (2), in which v is the initial rate of the enzymatic reaction, V_{max} is the maximum rate of hydrolysis, K_m is the Michaelis constant, and $[S]$ is the concentration of MAL (Huang et al., 2022; Wu et al., 2024).

$$v = \frac{V_{max} [S]}{K_m + [S]} \quad (1)$$

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}[S]} \quad (2)$$

2.6. The measurement of antioxidant ability

According to Hua's method, the antioxidant properties of the Zr-CoOOH nanozymes were determined by standard ABTS and DPPH radical scavenging assays (Hua et al., 2025). For the DPPH radical scavenging activity, a 62.5 μM ethanolic DPPH solution was mixed with various concentrations of the Zr-CoOOH nanozymes solutions (1, 10, 20, 30, 40, and 50 $\mu\text{g mL}^{-1}$) in a 1:1 ratio and incubated. The absorbance was then measured at 517 nm. For the ABTS assay, the ABTS stock solution was prepared by mixing 7 mM ABTS solution with 2.45 mM potassium persulfate solution and allowing the mixture to react in the dark for 17 h. Subsequently, the working solution obtained by diluting the original ABTS solution, was incubated in the dark with various concentrations (1, 10, 20, 30, 40, 50, and 60 $\mu\text{g mL}^{-1}$) of the Zr-CoOOH nanozymes. Finally, the absorbance was recorded at 734 nm. The scavenging activity for DPPH or ABTS radicals was determined following Equation (3):

$$\% \text{ Inhibition} = \frac{(\text{Absorbance (Control)} - \text{Absorbance (Test)})}{\text{Absorbance (Control)}} \times 100 \quad (3)$$

2.7. Sample preparation

Apple and cantaloupe fruit samples were used to determine the recovery tests by a colorimetric method based on Zr-CoOOH nano-enzymes to investigate the accuracy. The edible parts of apple and cantaloupe samples were crushed using a blender, respectively, and then preserved at a temperature between -20°C and

-16 °C for the next experiment. An initial step involved adding 5.0 g of the samples into a 50 mL centrifuge tube. Subsequently, 1 mL of MAL standard solution with specific concentrations (0.5 μM , 1.0 μM and 5.0 μM) was added to the previous mixture, and the resulting mixture was incubated for 4 h at room temperature. Then, the obtained mixtures underwent vortexed for 3 min and sonicated for 5 min. Next, the supernatant was carefully transferred into a 50 mL centrifuge tube which contained 1.0 g NaCl. And the resulting mixture was oscillated vigorously for 3 min. Following 30 min of incubation, the upper (acetonitrile) layer (3.0 mL) was carefully pipetted and transferred to a 50 mL centrifuge tube, and the sample in the tube was evaporated to dryness by means of nitrogen blowing at 80 °C under N_2 protection. The extract was redissolved in 200.0 μL of acetone solution and diluted with PBS (pH 6.0) until the total volume reached 6.0 mL, then the diluted solution was purified by 0.22 μm membrane filtration. Ultimately, the filtrate was measured using a colorimetric method based on Zr-CoOOH nano-enzymes.

3. Results and discussion

3.1. Morphological and structure characteristics of CoOOH and Zr-CoOOH nano-enzymes

The morphology of CoOOH and Zr-CoOOH nano-enzymes were characterized using TEM. With the irregular thin-type structural CoOOH, as shown in Fig. 1a and Fig. 1b. After Zr element doping, Zr-CoOOH possessing a multi (six)-edge typed super-thin layer structure (Fig. 1c and Fig. 1d). This kind of structure offered a greater specific surface area as well as an increased number of bonding locations for analytes than CoOOH (Zou et al., 2022; Fan et al., 2024).

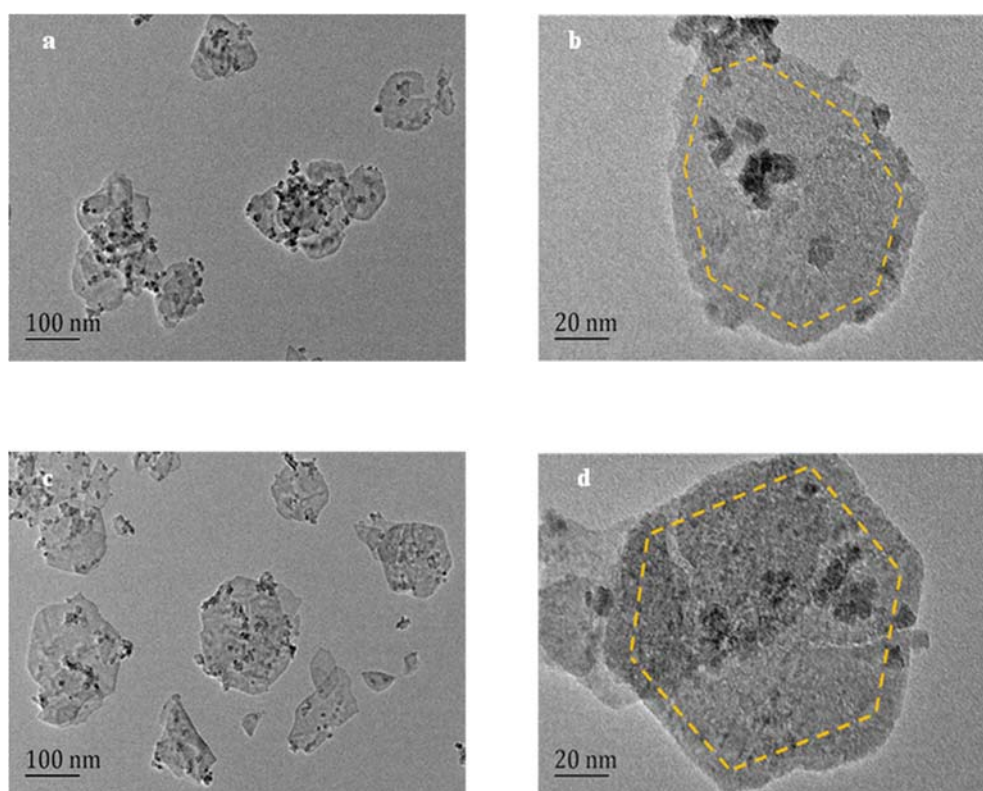


Fig. 1. TEM images of (a) 100 nm CoOOH and (b) 20 nm CoOOH, (c) 100 nm Zr-CoOOH nano-enzymes and (d) 20 nm Zr-CoOOH nano-enzymes.

The chemical compositions of ZrOOH and CoOOH were studied using FT-IR spectroscopy. The FT-IR spectra of CoOOH, ZrOOH and Zr-CoOOH nano-enzymes were shown in Fig. 2a. In the spectrum of CoOOH, the peaks appeared at 3629 cm^{-1} , 1350 cm^{-1} and 666 cm^{-1} . The reasons were corresponded to the stretching vibration of the interlayer hydroxyl group on -OH, the vibration within the Co-O bond of cobalt oxides, and the role of Co-O^{2-} , respectively. In the case of ZrOOH, peaks at 3409 cm^{-1} and 1550 cm^{-1} each correlated to -OH and C=O on the ZrOOH surface. The FT-IR spectrum of Zr-CoOOH nano-enzymes showed characteristic peaks of CoOOH and ZrOOH, and the emergence of these characteristic peaks indicated the successful synthesis of Zr-CoOOH (Shinde, 2022).

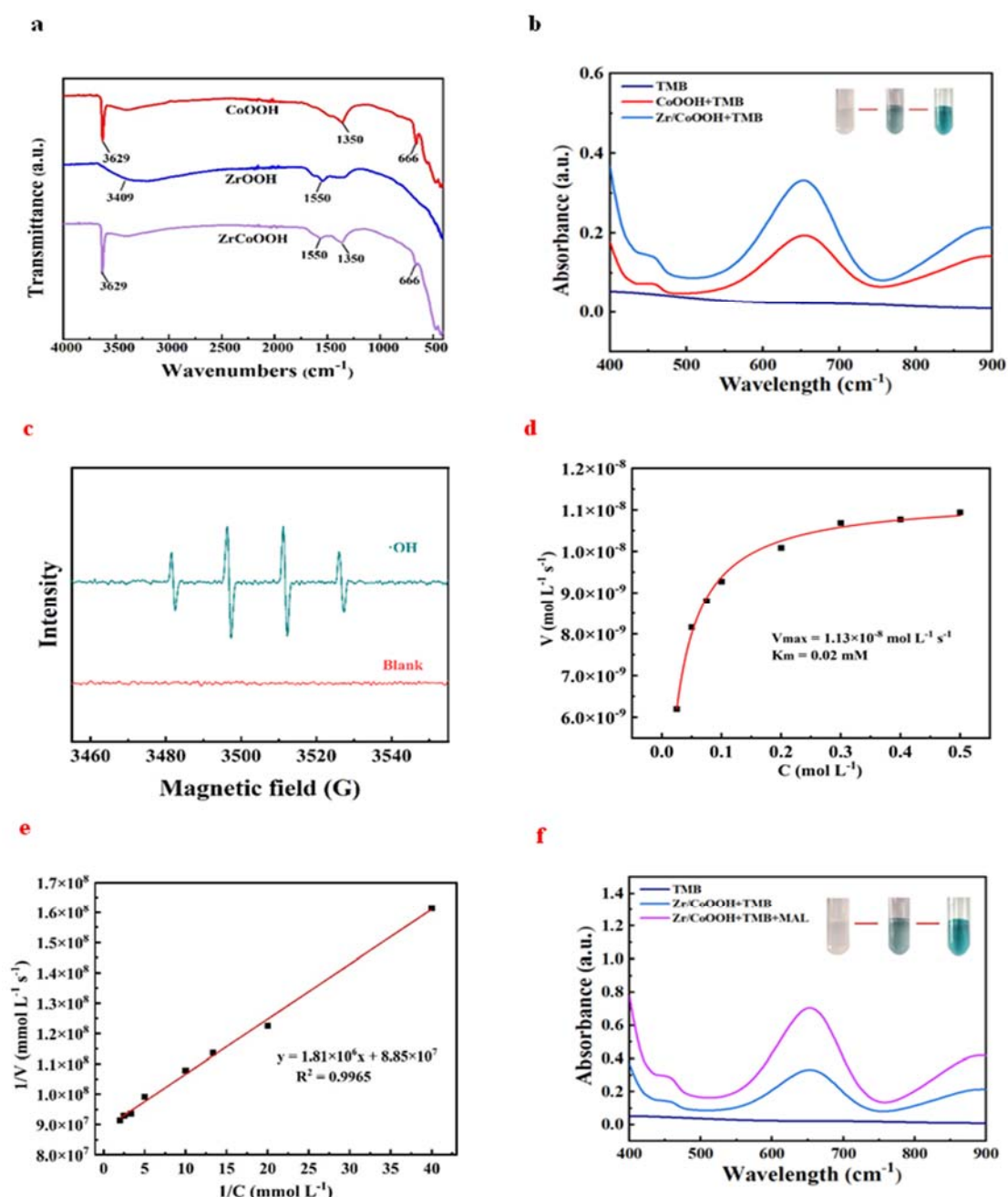


Fig. 2. (a) FT-IR spectra of the CoOOH, ZrOOH, Zr-CoOOH nano-enzymes. (b) UV-Vis absorption spectra of the system under different conditions. (c) EPR spectra of $\cdot\text{OH}$ radicals. (d) Steady-state kinetic analysis of Zr-CoOOH as an oxidase-like enzyme with different concentrations of TMB. (e) Lineweaver-Burk plots of the double reciprocal of the Michaelis-Menten equation. (f) The UV-visible absorption spectra of TMB, Zr-CoOOH+TMB and Zr-CoOOH+TMB+MAL.

The chemical compositions and near-surface valence states of CoOOH and Zr-CoOOH were investigated by X-ray photoelectron spectroscopy (XPS). The survey spectra of both materials are presented in Fig. S1a. For CoOOH, characteristic peaks corresponding to cobalt (Co 2p, approximately 780.2 eV), oxygen (O 1s, ~530.9 eV), and carbon (C 1s, ~284.8 eV) were detected, confirming its successful preparation. In the case of Zr-CoOOH, an extra signal for zirconium (Zr 3d, ~182.1 eV) was evident, verifying the effective incorporation of Zr into the structure. In the Zr 3d spectrum of Zr-CoOOH (Fig. S1b), two characteristic peaks were observed at binding energies of 181.8 and 184.0 eV. The measured peak area ratio of approximately 3:2 is consistent with the theoretical ratio for the spin-orbit doublet of Zr 3d_{5/2} and Zr 3d_{3/2}, thereby confirming the presence of Zr⁴⁺ oxidation states (Xu et al., 2023). These findings provide further evidence for the successful synthesis of Zr-CoOOH (Pang et al., 2024).

3.2. Catalytic properties of Zr-CoOOH nano-enzymes

TMB was chosen as a chromogenic substrate to investigate the properties of the synthesized Zr-CoOOH nano-enzymes. As shown in Fig. 2b, no UV absorption peak occurred within the wavelength scope between 400-900 nm when TMB was present alone. The colorless substrate TMB was oxidized by hydroxyl radicals ($\cdot\text{OH}$) in CoOOH to its blue oxide oxTMB, and this oxidation mechanism indicated that CoOOH nano-enzymes had oxidase-like activity (Zhang et al., 2022a). Thus when CoOOH was present, a rapid and notable color reaction occurred, turning the solution blue, which resulted in a clear UV absorption peak detected at approximately 652 nm. In contrast, in a TMB system of the same concentration the UV absorption peak for the Zr-CoOOH nano-enzymes was obviously higher than that for the CoOOH nano-enzymes, which was probably due to the reduction in nano-enzymes size achieved through Zr doping, thereby enhancing their catalytic activity (Tahir et al., 2025).

3.3. Catalytic mechanism of Zr-CoOOH nano-enzymes

The color change in the reaction system where TMB produced oxTMB was dependent on the concentration of O₂. The dissolved oxygen played a crucial role as an electron acceptor during the process of TMB oxidation. Initially, due to the oxidase activity, Zr-CoOOH nano-enzymes catalyzed the disintegration from absorbed O₂ to generate O₂^{•−}. Owing to the unsteady nature of O₂^{•−}, it was immediately converted to HO₂[•] and ¹O₂ by a disproportionation reaction under acidic conditions. The formation of the superoxide HO₂[•] effectively facilitated the generation of highly reactive $\cdot\text{OH}$ within an acidic buffer, leading to the oxidation of TMB to oxTMB. The specific reaction formula has been established based on previous studies (Zhang et al., 2022a). Furthermore, the electron paramagnetic resonance (EPR) spectrum revealed signals corresponding to $\cdot\text{OH}$ radicals and presents a typical signal of 1:2:2:1, confirming the existence of $\cdot\text{OH}$ (Fig. 2c). The study demonstrated that the efficacy of Zr-CoOOH nano-enzymes in catalyzing the transformation of dissolved oxygen to $\cdot\text{OH}$ radicals. Subsequently, these ROS promoted the oxidation of colorless TMB to its blue oxidized form (oxTMB). The specific reactions are as follows:





3.4. Kinetic evaluation of Zr-CoOOH nano-enzymes

The catalytic activity of Zr-CoOOH nano-enzymes was investigated utilizing the theory and method of enzyme kinetics. The Michaelis-Menten constant (K_m) obtained through the Lineweaver-Burk equation has found extensive application in characterizing the affinity and maximum initial rate between an enzyme and its substrate (Mekonnen et al., 2024). The magnitude of the K_m value bears an inverse relationship to the affinity of Zr-CoOOH nano-enzymes for the substrate (Wu et al., 2024). As shown in Fig. 2d and Fig. 2e, with TMB serving as the substrate, the K_m value and $V_{\max}$ of Zr-CoOOH nano-enzymes are found to be 0.02 mM and $1.13 \times 10^{-8} \text{ M s}^{-1}$, respectively. The kinetic parameters (K_m and V_m) of the Zr-CoOOH nano-enzymes were compared with those of other oxidase-like nanozymes. The results (Table S2) indicate that while the V_m value of the Zr-CoOOH nano-enzymes is slightly lower than those of other enzymes, its K_m value is lower than the reported values for similar substances, implying a superior substrate affinity (Li, et al., 2024b; Fan, et al., 2024; Han, et al., 2025; Zhang, et al., 2025a;). Fig. 2d was generated by fitting the TMB concentration to the reaction rate v via the Michaelis-Menten equation. The inverse of the TMB concentration was fitted to the inverse of v by creating a Lineweaver-Burk plot from the double inverse of the Michaelis-Menten equation, which resulted in Fig. 2e. The equation of linear return was $y = 1.81 \times 10^6 x + 8.85 \times 10^7$ ($R^2 = 0.9965$). The low K_m value by Zr-CoOOH nano-enzymes demonstrated the high affinity. The result is attributed to the ultra-thin morphology, small size, and high specific surface area possessed by Zr-CoOOH nanosheets, allowing the substrate to be easily adsorbed at the active site on their surface for catalytic oxidation. These findings suggest that the high catalytic efficiency of Zr-CoOOH nano-enzymes is largely dependent on their affinity with the substrate.

3.5. The response mechanism of Zr-CoOOH nano-enzymes to MAL

As shown in Fig. 2f, UV-Vis spectroscopy was applied to investigate the mechanism for determination MAL by the nano-enzymes and TMB system. The illustration showed the color changing of the solution in different systems. Compared with Zr-CoOOH+TMB system, the color of Zr-CoOOH+TMB+MAL system was significantly deepened, and the signal of the UV absorption peak at 652 nm was remarkably enhanced. These changes indicated that MAL strengthened the catalytic activity of TMB. The augmented oxidase activity of Zr-CoOOH nano-enzymes may be owing to the binding of metals on their surface to the phosphorothioate groups within MAL molecules, which leads to MAL-specific adsorption onto Zr-CoOOH nano-enzymes. In addition, the lone pair electrons of the aldehyde group in MAL can be used as mediator molecules to facilitate electrons transfer from Zr-CoOOH nano-enzymes to dissolved oxygen, generating highly reactive $\text{O}_2^{\cdot-}$ and ${}^1\text{O}_2$, which then oxidize more TMB molecules into the blue product oxTMB, making the color of the reactive solution change from blue to dark blue (Li et al., 2022). This laid the foundation for the subsequent MAL

detection experiments. Schematic representation of colorimetric detection of MAL on the basis of oxidase-like activity of Zr-CoOOH nano-enzymes is exhibited in Scheme 1. (b).

3.6. Optimization of catalytic conditions for Zr-CoOOH nano-enzymes

In order to maximize the catalytic performance of the enzyme, the reaction parameters for the Zr-CoOOH nano-enzymes were optimized. Fig. 3a showed the effects of different volumes of Zr-CoOOH nano-enzymes and TMB on the catalytic activity. The absorbance was maximum when 0.4 mL of Zr-CoOOH nano-enzymes and 0.2 mL of TMB were added. Consequently, the amount of Zr-CoOOH nano-enzymes and TMB were determined to be 0.4 mL and 0.2 mL as the optimal additions, and the subsequent conditions were analyzed based on these data.

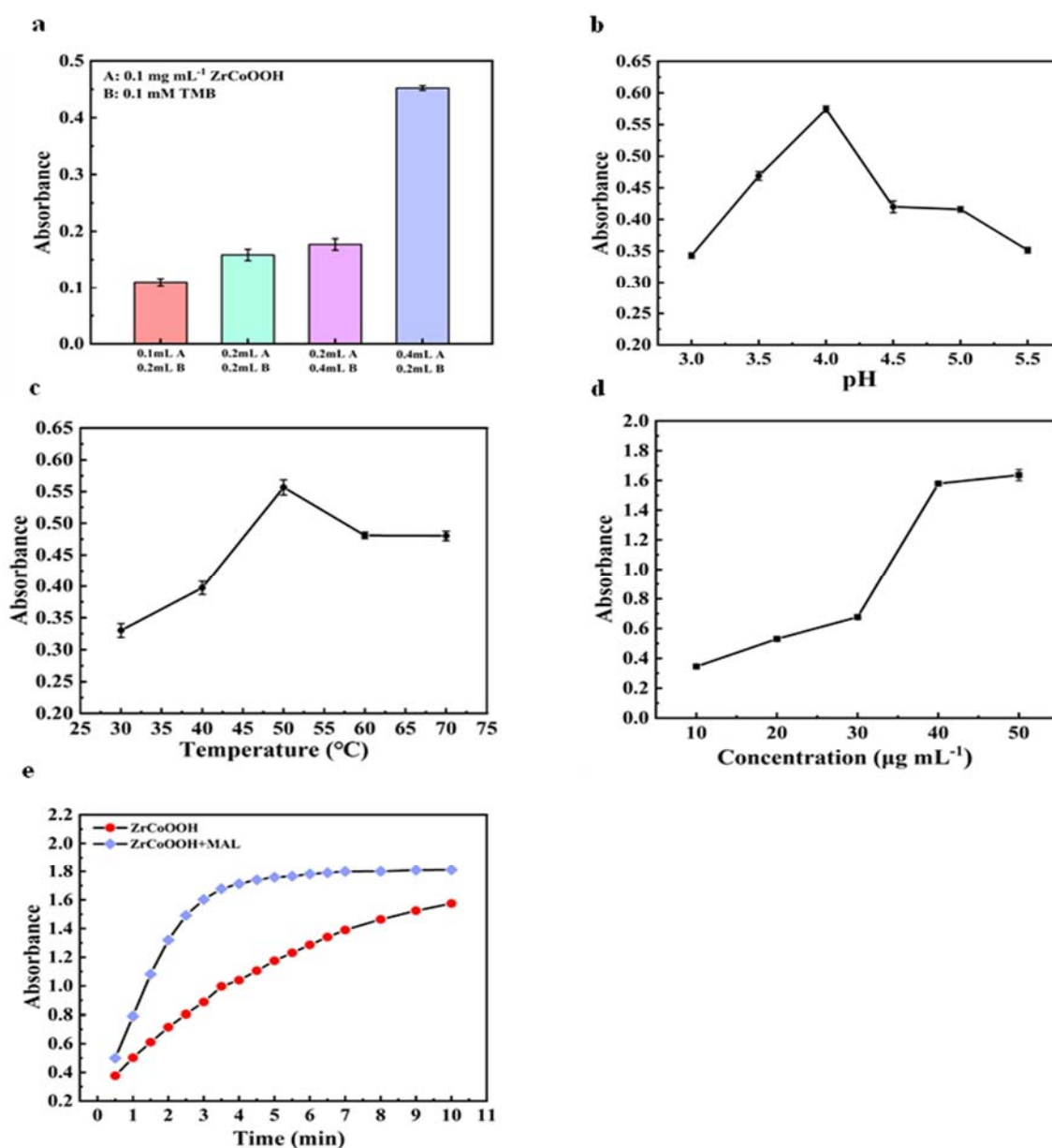


Fig. 3. (a) Absorbance spectra of the system under different additive amounts of Zr-CoOOH nano-enzymes and TMB at acetate buffer system. (b) The influence of pH on the oxidase-like activity of Zr-CoOOH nano-enzymes. (c) The influence of temperature on the oxidase-like activity of Zr-CoOOH nano-enzymes. (d) The influence of concentration on the oxidase-like activity of Zr-CoOOH nano-enzymes. (e) The effect of time on the oxidase-like activity of Zr-CoOOH nano-enzymes and Zr-CoOOH+TMB+MAL.

The pH level significantly influences the catalytic performance of nano-enzymes. As depicted in Fig. 3b, the absorbance first increases and then decreases within the pH range of 3.0 to 5.5, peaking at pH 4.0. Thus pH 4.0 was chosen as the optimal pH to continue the following experiments.

The study on the impact of temperature on the catalytic activity of the nano-enzymes involved varying the temperature from 30 °C to 70 °C, and the maximum value of absorbance was reached at 50 °C. So 50 °C was determined to be the optimal temperature (Fig. 3c), and 50 °C was selected for subsequent experiments.

Furthermore, the concentration of the Zr-CoOOH nano-enzymes was subjected to further optimization (Fig. 3d). It was evident that, as the concentration of the Zr-CoOOH nano-enzymes increased, the absorbance initially rose and then plateaued after reaching 40 $\mu\text{g mL}^{-1}$, indicating that a concentration of 40 $\mu\text{g mL}^{-1}$ is the optimal level for the Zr-CoOOH nano-enzymes.

As the catalytic activity of the nano-enzymes is tightly associated with the response time, we studied the absorbance at 652 nm for the Zr-CoOOH+TMB system and the Zr-CoOOH+TMB+MAL system. In Fig. 3e, the absorbance of both systems increased with the length of catalytic reaction time, and the absorbance of the Zr-CoOOH+TMB+MAL system stabilized at about 5 min. Considering the necessity of rapid detection of pesticide residues, 5 min was chosen as the optimal reaction time for further experiments.

3.7. Antioxidant activity of the Zr-CoOOH nano-enzymes

The antioxidant potential of Zr-CoOOH nano-enzymes was evaluated using DPPH and ABTS assays (Hua et al., 2025). The DPPH scavenging activity increased steadily over the concentration range of 1 to 50 $\mu\text{g mL}^{-1}$, reaching a maximum of approximately 80% at 40 $\mu\text{g mL}^{-1}$ (Fig. 4a). As shown in Fig. 4b, the ABTS radical scavenging ability increased with increasing concentrations of Zr-CoOOH nano-enzymes (1-60 $\mu\text{g mL}^{-1}$), with a scavenging rate exceeding 70% at 40 $\mu\text{g mL}^{-1}$. ABTS can be oxidized to green-colored ABTS $\cdot^+$ in the presence of potassium persulfate. When Zr-CoOOH nano-enzymes are present, the formation of ABTS $\cdot^+$ is inhibited, resulting in a reduction of the solution color. These results preliminarily indicate that Zr-CoOOH nano-enzymes possess multi-radical scavenging capabilities.

3.8. Analytical performance of the Zr-CoOOH nano-enzymes

Based on the direct enhancement of Zr-CoOOH-like oxidase activity caused by MAL, a rapid and simple colorimetric sensing platform was constructed specifically for detection MAL. Under optimal conditions, the signal responses of various MAL concentrations were recorded. The blue color intensity of the Zr-CoOOH+TMB+MAL system progressively increased with rising MAL concentration until saturation occurred beyond 10 μM , and the signal growth plateaued, indicated complete interaction between MAL and Zr-CoOOH nano-enzymes (as shown in Fig. 4c). The linear equation for the Zr-CoOOH+TMB+MAL method was ultimately fitted as $A = 0.0672C + 0.7406$ (C represents the MAL concentration, $R^2 = 0.9939$). The limited of detection (LOD) was determined to be 0.023 μM ($S/N = 3$) over a linear range between 0.1-10 μM , as illustrated in Fig. 4d. The comparison of the property between Zr-CoOOH nano-enzymes and the prior described MAL colorimetric detection analytical methods is summarized in Table S1. By contrast with most

of the reported analytical approaches to detecting MAL, the Zr-CoOOH+TMB+MAL method exhibited a linearity range from 0.1-10 μM , superior to Ir NPs (0.1-5 μM) (Liang, et al., 2022), PDA-Pd/NCs (0-8 μM) (Kang, et al., 2023), FeCo-NC DAzyme (0.05-4.0 μM) (Li, et al., 2024b), and the polyaniline-ZnO-NiO (PANI-ZnO-NiO) nanocomposite (0.01-0.07 μM) (Chufamo Jikamo, et al., 2023). The LOD for this approach was lower than 23 nM, and the sensitivity was preferable compared to the CEC-integrated UiO-66-NH₂-IMER (121.08 nM) (Liu et al., 2022), CuNZs (148 nM) (Wu, et al., 2024), and aptamers-decorated AuNPs (1480 nM) (Li, et al., 2023). Although the LOD for the Zr-CoOOH nano-enzymes assay was slightly higher than for the FeCo-NC dual-atom nano-enzymes (FeCo-NC DAzyme) (Ge, et al., 2022), since the detection of MAL without acid phosphatase, the developed method presented the merits of lower cost and higher stability.

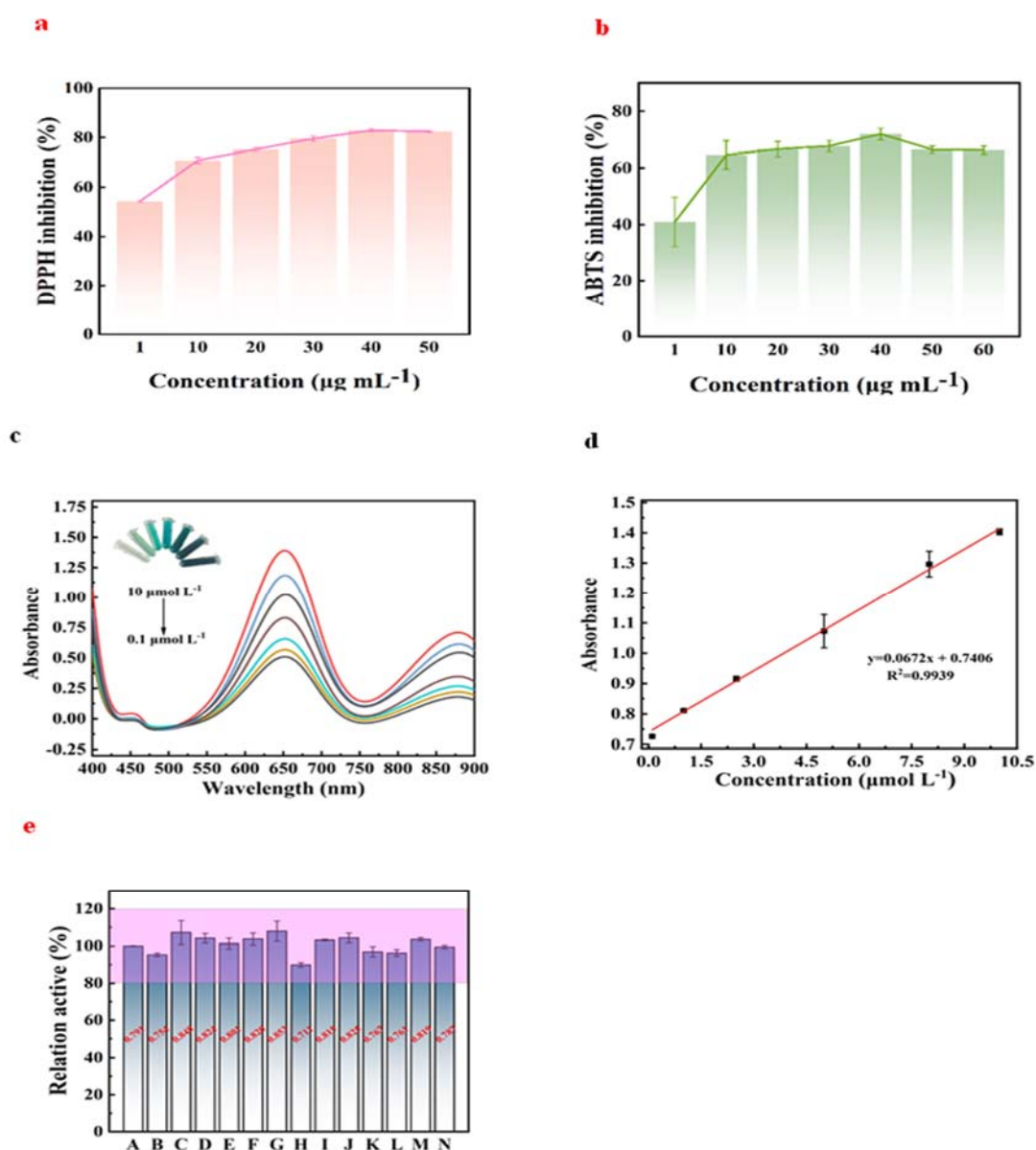


Fig. 4. (a) The DPPH Radical Scavenging Capacity of Zr-CoOOH nano-enzymes. (b) The ABTS Radical Scavenging Capacity of Zr-CoOOH nano-enzymes. (c) Absorption spectrum of Zr-CoOOH+TMB+MAL system in the presence of MAL at various concentrations. (d) Plots of the absorbance at 652 nm as a function of MAL concentration. (e) UV-vis absorption peak responses towards 100 $\mu\text{mol L}^{-1}$ MAL in the absence (A) or presence of possible interferents, (B) K⁺, (C) Na⁺, (D) Zn²⁺, (E) Cl⁻, (F) SO₄²⁻, (G) Glu, (H) GLY, (I) Chl, (J) AA, (K) GSH, (L) MP, (M) TCF and (N) all.

3.9. Anti-interference and stability of the Zr-CoOOH nano-enzymes

The anti-interference of the Zr-CoOOH+TMB+MAL colorimetric sensing system was conducted by discussing the influence of matrix disturbance by the existence of several potential interferers. These interferers included inorganic ions (K^+ , Na^+ , Zn^{2+} , Cl^- , SO_4^{2-}) at 100 times their concentration, organic matter (glucose, ascorbic acid, glutathione) at 10 times its concentration, and structural analogs (glyphosate, chlorpyrifos, methyl parathion, trichlorfon) at the same concentration, all of which were subject to controlled trials. The Relative Standard Deviation (RSD) of all interferences on the detected characteristic peak signals was only 5.38% (Fig. 4e), indicating that Zr-CoOOH nano-enzymes is capable of detecting MAL in complex substrates and that this method is characterized by good anti-interference.

To evaluate the consistent performance of nano-enzymes from different production batches, the batch stability of Zr-CoOOH nano-enzymes was explored in Fig. S2. When six batches of Zr-CoOOH materials were tested in the same MAL solution, the results indicated that there was no obvious difference between the absorbance values among different batches, confirming the excellent batch stability of Zr-CoOOH nano-enzymes. To check the storage stability of Zr-CoOOH nano-enzymes, 5 μ M MAL was added in a dry environment at 4 °C on days 0, 7, 17, 21, 28, and 35, respectively, and it was found that after 5 weeks of storage, the activity of Zr-CoOOH nano-enzymes retained 71.54% of their initial activity (Fig. S3). This confirms that the method possesses good batch stability and storage stability.

3.10. Analysis of actual samples

To estimate the accuracy of the Zr-CoOOH nano-enzymes colorimetry for detecting MAL, pre-treated apple and cantaloupe fruit samples were subjected to recovery tests. The colorimetric sensing platform exhibited recovery values ranging from 86.02% to 100.67% with an RSD of 3.15%-7.13% (Table 1). This demonstrated that the Zr-CoOOH nano-enzymes colorimetric method provided good accuracy for the detection of MAL.

Table 1 The recovery rate of MAL addition in the actual sample.

Samples	Original level (μ mol L ⁻¹)	Added level (μ mol L ⁻¹)	Found level (μ mol L ⁻¹ , $\pm$ SD)	Recovery ($\pm$ RSD, %)
Apple	0.00	5.00	4.36 $\pm$ 0.14	87.22 $\pm$ 3.15
		1.00	1.01 $\pm$ 0.06	100.63 $\pm$ 5.71
		0.50	0.49 $\pm$ 0.03	97.42 $\pm$ 7.13
		5.00	4.30 $\pm$ 0.24	86.02 $\pm$ 5.67
Cantaloupe	0.00	1.00	1.01 $\pm$ 0.04	100.67 $\pm$ 4.36
		0.50	0.49 $\pm$ 0.02	98.41 $\pm$ 5.04

4. Conclusions

In summary, this study describes the exploitation of a colorimetric sensing platform based on Zr-CoOOH nano-enzymes for sensitized quantification of MAL. Since Zr-CoOOH nano-enzymes exhibits excellent oxygenase-like activity, in the presence of TMB, the system transitions from colorless to blue. The system comprising Zr-CoOOH+TMB+MAL exhibits favorable anti-interference properties and stability of batch for MAL, making it applicable to the quantitative determination of MAL in food samples (e.g. apples and

cantaloupes). The work introduces a direct doping strategy aimed at regulating the catalytic performance of transition metal hydroxides and offers an affordable and effective method based on colorimetric assay for hazards in foods.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (No. 32302215) and Postdoctoral Innovation Project of Shandong Province (No. SDCX-ZG-202400125).

References

- [1] Z.A. ALothman, E. Yilmaz, M.A. Habila, et al., Supramolecular solvent microextraction and ultra-performance liquid chromatography-tandem mass spectrometry combination for the preconcentration and determination of malathion in environmental samples, *Desalin. Water Treat.* 144 (2019) 166-171. <https://doi.org/10.5004/dwt.2019.23574>.
- [2] H. Cheng, Y.Y. Chen, M.J. Liu, et al., Theory-guided design of S-doped Fe/Co dual-atom nanozymes for highly efficient oxidase mimics, *Chem. Sci.* 15.36 (2024) 14816-14828. <https://doi.org/10.1039/D4SC03101F>.
- [3] S. Chufamo Jikamo, T. Haile Habtemariam, T. Heliso Dolla., Polyaniline-ZnO– NiO nanocomposite based non-enzymatic electrochemical sensor for malathion detection, *Electroanal.* 35.4 (2023) e202200317. <https://doi.org/10.1002/elan.202200317>.
- [4] Y. Fan, L. Fu, H.H. Su, et al., Membrane separation assisted colorimetric/fluorescent detection of β -galactosidase-positive bacteria in milk and milk powder based on the oxidase-like activity of CoOOH nanosheets, *Food Chem.* 461 (2024) 140946. <https://doi.org/10.1016/j.foodchem.2024.140946>.
- [5] J. Ge, L.K. Yang, Z.H. Li, et al., A colorimetric smartphone-based platform for pesticides detection using Fe-N/C single-atom nanozyme as oxidase mimetics, *J. Hazard. Mater.* 436 (2022) 129199. <https://doi.org/10.1016/j.jhazmat.2022.129199>.
- [6] S.J. Huang, X.Y. Tang, L.Q. Yu, et al., Colorimetric assay of phosphate using a multicopper laccase-like nanozyme, *Microchim. Acta.* 189.10 (2022) 378. <https://doi.org/10.1007/s00604-022-05476-2>.
- [7] R. Jin, Z.H. Xing, D.S. Kong, et al., Sensitive colorimetric sensor for point-of-care detection of acetylcholinesterase using cobalt oxyhydroxide nanoflakes, *J. Mater. Chem. B.* 7.8 (2019) 1230-1237. <https://doi.org/10.1039/C8TB02987C>.
- [8] G. Kang, D. Zhao, H. Wang, et al., Malathion detection based on polydopamine enhanced oxidase-mimetic activity of palladium nanocubes, *Talanta.* 262 (2023) 124730. <https://doi.org/10.1016/j.talanta.2023.124730>.
- [9] B. Li, G.S. Lai, B. Lin, et al., Enzyme-induced biomineralization of cupric subcarbonate for ultrasensitive colorimetric immunosensing of carcinoembryonic antigen, *Sensor Actuat B-chem.* 262 (2018) 789-795. <https://doi.org/10.1016/j.snb.2018.02.049>.
- [10] F. Li, J.M. Jiang, H. Peng, et al., Platinum nanozyme catalyzed multichannel colorimetric sensor array for identification and detection of pesticides, *Sensor Actuat B-chem.* 369 (2022) 132334. <https://doi.org/10.1016/j.snb.2022.132334>.
- [11] H.L. Li, L. Tian, S.N. Yang, et al., Solid-state electrochemiluminescence sensor based on $\text{TiO}_2@ \text{CdSe}$ and $\text{Ru}(\text{bpy})_3^{2+}@ \text{Ag}$ NPs for ultrasensitive detection of malathion, *Food Chem.* 470 (2025a) 142726. <https://doi.org/10.1016/j.foodchem.2024.142726>.
- [12] P. Li, H.N. Zhan, S.J. Tao, et al., Bio-inspired aptamers decorated gold nanoparticles enable visualized detection of malathion, *Front. Bioeng. Biotechnol.* 11 (2023) 1165724. <https://doi.org/10.3389/fbioe.2023.1165724>.
- [13] P.S. Li, J.S. Sun, H.F. Wang, et al., Novel electrochemiluminescence sensing platform for ultrasensitive detection of malathion residue in tea based on SiO_2NSs doped Luminol/AgNPs as a signal amplification strategy, *J. Hazard. Mater.* 477 (2024a) 135358. <https://doi.org/10.1016/j.jhazmat.2024.135358>.

- [14] S.F. Li, F.F. Wang, C.H. Wang, et al., B Doped Zn Single-Atom Nanozyme With Enhanced Oxidase-Like Activity Combined CRISPR/Cas13a System for RNA Sensing, *Adv. Funct. Mater.* (2025b) 2418523. <https://doi.org/10.1002/adfm.202418523>.
- [15] Y.H. Li, Q.Q. Ma, H.L. Gong, et al., Superior oxidase-mimetic activity of FeCo-NC dual-atom nanozyme for smartphone-based visually colorimetric assay of organophosphorus pesticides, *Microchim. Acta.* 191.7 (2024b) 368. <https://doi.org/10.1007/s00604-024-06443-9>.
- [16] Z.M. Li, X.L. Zhong, S.H. Wen, et al., Colorimetric detection of methyltransferase activity based on the enhancement of CoOOH nanozyme activity by ssDNA, *Sensor Actuat B-chem.* 281 (2019) 1073-1079. <https://doi.org/10.1016/j.snb.2018.11.085>.
- [17] X. Liang, X.Z. Wang, Y.C. Zhang, et al., Selective inhibition toward dual enzyme-like activities of iridium nanozymes for a specific colorimetric assay of malathion without enzymes, *J. Agric. Food Chem.* 70.12 (2022) 3898-3906. <https://doi.org/10.1021/acs.jafc.1c06954>.
- [18] C.Y. Lin, H.X. Zheng, M. Sun, et al., Highly sensitive colorimetric aptasensor for ochratoxin A detection based on enzyme-encapsulated liposome, *Anal. Chim. Acta.* 1002 (2018) 90-96. <https://doi.org/10.1016/j.aca.2017.11.061>.
- [19] J.T. Liu, J. Tang, Y.Y. Mo, et al., Metal-organic framework-sealed heterogeneous nanoenzyme: A dual-mode core-shell sensor for sensitive determination of the chlorpyrifos residue in environment and bioaccumulation in food, *Chem. Eng. J.* 501 (2024) 157693. <https://doi.org/10.1016/j.cej.2024.157693>.
- [20] R. Liu, G.Y. Yi, B.A. Ji, et al., Metal-Organic Frameworks-Based Immobilized Enzyme Microreactors Integrated with Capillary Electrochromatography for High-Efficiency Enzyme Assay, *Anal. Chem.* 94.17 (2022) 6540-6547. <https://doi.org/10.1021/acs.analchem.1c05586>.
- [21] T.M. Lu, Z.X. Liu, R.N. Qian, et al., Targeted regulation of autophagy using sorafenib-loaded biomineralization nanoenzyme for enhanced photodynamic therapy of hepatoma, *Mater. Today Bio.* 29 (2024) 101270. <https://doi.org/10.1016/j.mtbio.2024.101270>.
- [22] M.L. Mekonnen, E.M. Abda, A. Csáki, et al., Frontiers in Laccase Nanozymes-Enabled Colorimetric Sensing: A review, *Anal. Chim. Acta.* (2024) 343333. <https://doi.org/10.1016/j.aca.2024.343333>.
- [23] X.M. Pang, Y.F. Chen, R. Gao, et al., Single-atom Zr-doped CoOOH with enhanced electrical conductivity as a signal amplifier and detection probe for the indirect non-enzymatic electrochemical determination of malathion in foods, *Food Chem.* 460 (2024) 140563. <https://doi.org/10.1016/j.foodchem.2024.140563>.
- [24] C.L. Shen, L. Zhong, L. Xiong, et al., A novel sandwich-like cytosensor based on aptamers-modified magnetic beads and carbon dots/cobalt oxyhydroxide nanosheets for circulating tumor cells detection, *Sensor Actuat B-chem.* 331 (2021) 129399. <https://doi.org/10.1016/j.snb.2020.129399>.
- [25] V. Shinde, M. Uthayakumar, R. Karthick., Self-assembled cobalt hydroxide micro flowers from nanopetals: Structural, fractal analysis and molecular docking study, *Surf. Interf.* 32 (2022) 102163. <https://doi.org/10.1016/j.surf.2022.102163>.
- [26] T. Tian, D.H. Song, L. Zhang, et al., Facile and selective recognition of sulfonylurea pesticides based on the multienzyme-like activities enhancement of nanozymes combining sensor array, *J. Hazard. Mater.* 469 (2024) 133847. <https://doi.org/10.1016/j.jhazmat.2024.133847>.
- [27] Y.J. Wang, Q.L. Li, Q.H. Xia, et al., A portable and quantitative detection of xanthine and xanthine oxidase based on cascade enzymatic reactions of Fe₃O₄@MWCNTs/Hemin, *J. Mater. Sci.* 58.17 (2023) 7441-7455. <https://doi.org/10.1007/s10853-023-08449-9>.
- [28] C.M. Wu, J.J. Li, J.P. Song, et al., Novel colorimetric detection of oxytetracycline in foods by copper nanozyme, *Food Chem.* 430 (2024) 137040. <https://doi.org/10.1016/j.foodchem.2023.137040>.
- [29] Z.H. Wu, D.Y. Nan, H. Yang, et al., A ratiometric fluorescence-scattered light strategy based on MoS₂ quantum dots/CoOOH nanoflakes system for ascorbic acid detection, *Anal. Chim. Acta.* 1091 (2019) 59-68. <https://doi.org/10.1016/j.aca.2019.09.054>.
- [30] Y.W. Xu, X.P. Jia, S.N. Yang, et al., An aptasensor with colorimetric and electrochemical dual-outputs for malathion detection utilizing peroxidase-like activity of Fe-MOF, *Food Chem. X.* 24 (2024) 101835. <https://doi.org/10.1016/j.fochx.2024.101835>.
- [31] J.J. Zhang, Y.F. Li, X. Gong, et al., Colorimetric detection of total antioxidants in green tea with oxidase-mimetic CoOOH nanorings, *Colloid Surface B.* 218 (2022a) 112711. <https://doi.org/10.1016/j.colsurfb.2022.112711>.

- [32] Y.F. Zhang, L.X. Yang, C. Sun, et al., Aptamer-based sensor for specific recognition of malathion in fruits and vegetables by surface-enhanced Raman spectroscopy and electrochemistry combination, *Anal. Chim. Acta.* 1221 (2022b) 340148. <https://doi.org/10.1016/j.aca.2022.340148>.
- [33] Y.Y. Zou, J.Y. Chen, Q.L. Chai, et al., Stimuli-responsive colorimetric sensor based on bifunctional pyrophosphate-triggered controlled release and enhancing activity of CoOOH nanozyme, *Sensor Actuat B-chem.* 369 (2022) 132215. <https://doi.org/10.1016/j.snb.2022.132215>.
- [34] S. Tahir, M. Iqbal, S. Shad, et al., Biosynthesis of Zr-doped WO₃ nanoparticles: evaluation of antibacterial, antioxidant, and enzymatic activities, *Microb. Pathog.* 198 (2025) 107192. <https://doi.org/10.1016/j.micpath.2024.107192>.
- [35] C. García-Ruiz, G. Álvarez-Llamas, Á. Puerta, et al., Enantiomeric separation of organophosphorus pesticides by capillary electrophoresis: Application to the determination of malathion in water samples after preconcentration by off-line solid-phase extraction, *Anal. Chim. Acta.* 543.1-2 (2005) 77-83. <https://doi.org/10.1016/j.aca.2005.04.027>.
- [36] Y.L. Li, F. Cao, X.Y. Deng, et al., Simultaneous total antioxidant capacity assessment and ascorbic acid accurate quantification utilizing a H₂O₂-independent colorimetric-fluorescent sensor array, *Food Chem.* 499 (2025c) 147257. <https://doi.org/10.1016/j.foodchem.2025.147257>.
- [37] Z. Han, Z.H. Yang, S.H. Zhang, et al., Colorimetric/Photothermal/Fluorescence triple-mode assay based on CWO/NiOOH with oxidase-like activity for accurate detection of H₂O₂ in food, *COLLOID SURFACE A.* 726 (2025) 138000. <https://doi.org/10.1016/j.colsurfa.2025.138000>.
- [38] J. Zhang, R.Y. Li, K.L. Cao, et al., Gold and platinum bimetallic nanoparticles with double enzyme-like properties for the colorimetric detection of glutathione, *J. Mol. Struct.* 1340 (2025a) 142521. <https://doi.org/10.1016/j.molstruc.2025.142521>.
- [39] Z. Hua, S.S. Huang, R.G. Liu, et al., Cascade liver-targeted and ROS-responsive nanoparticles for oral astaxanthin administration in alcoholic liver injury, *Chem. Eng. J.* 521 (2025) 166412. <https://doi.org/10.1016/j.cej.2025.166412>.
- [40] S. Zhang, Z.Y. Liu, Q.Q. Gao, et al., An electrochemical aptamer sensor based on nano-silver-decorated covalent organic framework for the detection of malathion in medicinal herbs, *Microchem. J.* 219 (2025b) 116258. <https://doi.org/10.1016/j.microc.2025.116258>.