

A sulfur-doped bimetallic nanozyme for colorimetric detection of chlorogenic acid in homologous medicinal and edible plants

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Abstract

This study developed a colorimetric detection platform based on a sulfur-doped bimetallic nanozyme (FePd_2S_4) for the detection of chlorogenic acid (CGA) in medicinal plants. FePd_2S_4 demonstrate peroxidase-like activity, which enables it to oxidize 3,3',5,5'-tetramethylbenzidine (TMB) into a blue oxidation product (oxTMB). The introduction of CGA enhances the redox characteristics of the nanozyme, strengthening the blue signal of oxTMB and significantly increasing the corresponding ultraviolet-visible (UV-vis) absorption intensity. Based on this phenomenon, an UV-vis detection model was established for CGA, achieving a linear range of 1–50 $\mu\text{g/mL}$, with a detection of limit (LOD) of 0.15 $\mu\text{g/mL}$. To further enhance practicality, a smartphone-integrated colorimetric sensing platform for the detection of CGA was constructed with RGB values by the "Color Grab" application. This model is also applicable to the detection of CGA within the linear range of 1–50 $\mu\text{g/mL}$, with a LOD of 0.45 $\mu\text{g/mL}$. Furthermore, the dual-modal detection platform was successfully employed in the actual detection of CGA in herbs. In comparison with the UV-vis detection mode, the smartphone-integrated colorimetric platform presents the advantages of straightforward operation, portability, rapidity, and low cost. This work provide a, a novel colorimetric approach for utilizing a sulfur-doped bimetallic nanozyme as a precise tool for the detection of CGA, which has a significant potential for application in food quality and pharmaceutical analysis.

Introduction

Chlorogenic acid (CGA), a phenylpropanoid ester of caffeic and quinic acids, is widely distributed in homologous medicinal and edible plants such as *Lonicerae japonica* (honeysuckle flower), *Chrysanthemum morifolium* (chrysanthemum) and *Eucommia ulmoides*, as well as common fruits and vegetables (Gu et al., 2010; Lu et al., 2020). It exhibits diverse bioactivities including antioxidation (Rice-Evans et al., 1996), free radical scavenging (Liang et al., 2016), anti-cancer (Huang et al., 2010), antiviral (Cheng et al., 2013), and liver protection (Hemmerle et al., 1997), while excessive intake may induce oxidative stress, allergies, and liver or kidney damage (Alagumalai et al., 2022). As a key quality marker in pharmaceutical management, existing CGA detection methods include high performance liquid chromatography (HPLC) (Amakura et al., 2000), UV–Vis spectrometry (Yang et al., 2018), chemiluminescence (Cui et al., 1999), capillary electrophoresis (Li et al., 2010; Tong et al., 2009), infrared spectrometry (Fábián et al., 1994) and electrochemical methods (Moccelini et al., 2008; Yardım et al., 2013). Most of these traditional methods suffer from high cost, complex operation, tedious sample preparation, long detection time and low efficiency. Thus, developing efficient, simple, low-cost and eco-friendly detection methods is crucial for food quality and safety supervision.

Among various existing rapid detection methods, enzyme-catalyzed colorimetric detection has attracted widespread attention due to its significant advantages of simplicity, low cost, and intuitive detectability (Kumar et al., 2022; Ye et al., 2021). However, natural enzymes have inherent drawbacks such as poor stability. In contrast, nanozymes, with their high catalytic stability, low cost, and sustainability, have emerged as ideal alternative materials to natural enzymes (Wu et al., 2019). To date, various types of

nanozymes have been reported, including noble metals, transition metal oxides, and carbon-based materials (Chen and Song, 2022; Huang et al., 2022; Liu et al., 2020; Wang et al., 2018). To further improve the catalytic performance of nanozymes, heteroatom doping has been proven to be an effective modification strategy, among which carbon (C)/nitrogen (N) doping is the mainstream direction in current research. In comparison, research on sulfur (S) doping is relatively scarce but exhibits unique advantages. Specifically, the advantages of S doping are mainly reflected in three aspects: first, it can enrich the catalytic active sites of nanozymes and enhance substrate adsorption capacity and electron transfer efficiency (Chen et al., 2021; Wu et al., 2023); second, it can precisely regulate the electronic structure of active sites and accelerate the catalytic reaction process by optimizing oxygen adsorption energy (Abdeta et al., 2022; Zhang et al., 2025); third, the co-doping of S with metals, N, and other elements can produce a synergistic effect, which not only further improves catalytic activity but also endows nanozymes with multi-enzymatic cascade activity (Jiang et al., 2025; Wang et al., 2023). These unique advantages indicate that the S doping strategy provides an important idea for the design of high-performance nanozymes and has broad application prospects in related fields.

Smartphone-assisted platforms have witnessed rapid development in the field of detection owing to their diverse functions. These platforms can not only streamline intricate experimental operation procedures but also function as communication media, advanced converters (Roda et al., 2016), and mobile biosensors (Dutta, 2019; Quesada-Gonzalez and Merkoci, 2017). To fulfill the requirement for rapid on-site analysis, smartphones have been incorporated into the field of analytical chemistry and employed as portable devices, with relevant application cases being gradually implemented. For example, Peng et al. (2022) developed two smartphone-based colorimetric devices that exploited the catalytic characteristics of cobalt hydroxide nanosheets to accomplish colorimetric detection of ascorbic acid. Wu et al. (2023) carried out the qualitative and quantitative visual detection of tetrachloro-p-benzoquinone by constructing an analytical model using system RGB values and a sensor-coated fluorescent test paper based on a smartphone. Shrivastava et al. (2020) developed a chromaticity meter paper device integrated with a smartphone, utilizing sucrose-modified gold nanoparticles as probes to conduct on-site analysis of arsenic (III). The core technical principle involves capturing the target image with the smartphone camera, identifying the RGB values via application software, and then establishing an analytical model in conjunction with the analyte concentration. It is noteworthy that, to the best of our knowledge, no reports have been published regarding the combination of UV-Vis spectrophotometers with smartphones for the detection of CGA in herbs.

Based on this foundation, this study developed a dual-mode detection and analysis method for CGA. This method is based on the enhancement of the peroxidase-like activity of iron-palladium bimetallic sulfide (FePd_2S_4) in a colorimetric reaction. In the colorimetry reaction, this sulfide can catalyze the oxidation of colorless 3,3',5,5'-tetramethylbenzidine (TMB) to blue oxidized TMB (oxTMB) in the presence of hydrogen peroxide (H_2O_2). When CGA is introduced into the system, its peroxidase-like catalytic activity is notably enhanced. The signal change of this chromogenic system can be simultaneously detected in dual modes using a UV-Vis spectrophotometer and smartphone color extraction. This

method was employed for the detection of CGA in three real herbal samples, which further verified the detection reliability and practical applicability of the method (Scheme 1).

“Please insert Scheme 1 here”

Experimental

Reagents and materials

Ferric nitrate nonahydrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$), palladium nitrate ($\text{Pd}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$), L-cysteine, ethylene glycol, TMB, o-phenylenediamine (OPD), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), furfural (FA), tert-butanol (t-BuOH), thiourea (TH), p-benzoquinone (PBQ), DL-histidine (DL-His), sodium nitrate (NaNO_3), calcium chloride (CaCl_2), sodium chloride (NaCl), potassium chloride (KCl), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium sulfate (Na_2SO_4), lactose, fructose, maltose, urea, citric acid, DL-malic acid were purchased from Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). Methanol, ethanol, isopropanol (IPA), hydrogen peroxide (30%), acetic acid (HAc), sodium acetate (NaAc), ascorbic acid (AA), anhydrous glucose (Glucose) were purchased from Kolon Chemical Reagent Co., Ltd. (Chengdu, China). Glutathione (GSH), proline (Pro), arginine (Arg) were purchased from Chongqing Taisin Chemical Co., Ltd. (Chongqing, China). The CGA standard was purchased from Chengdu Zhibiao Huachun Biotechnology Co., Ltd. The bamboo leaves were procured from Zhejiang Academy of Forestry (Hangzhou, China). The chrysanthemum was acquired from the planting base of Zhejiang Tongxiang Lv Kang Chrysanthemum Industry Co., Ltd. (Tongxiang, China); the honeysuckle flower was obtained from Hunan Jicaotang Honeysuckle Technology Development Co., Ltd. (Chenzhou, China).

Preparation of FePd_2S_4

A total of 0.4040 g of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, 0.4609 g of $\text{Pd}(\text{NO}_3)_2 \cdot x\text{H}_2\text{O}$, and 0.96938 g of L-cysteine were precisely weighed in a 100 mL beaker at a molar ratio of 1:2:8. Subsequently, 25 mL of deionized water and 25 mL of ethylene glycol were introduced into the beaker and stirred at room temperature for 30 minutes. The obtained mixed solution was then transferred to a 100 mL polytetrafluoroethylene-lined stainless steel autoclave and remained at 160°C for 8 hours. After allowing the autoclave to cool naturally to room temperature, the product was washed three times with deionized water and ethanol, respectively, and then dried under vacuum at 60°C to yield FePd_2S_4 .

The Enzyme-Mimetic Properties of FePd_2S_4

Enzyme classification and functional characteristics

The peroxidase-like and oxidase-like activities of FePd₂S₄ were assessed through the catalysis of the oxidation of the colorless TMB substrate into its blue oxidized form (oxTMB), which exhibits a characteristic absorption peak at 652 nm. The experimental protocol was as follows: 50 µL of FePd₂S₄ (500 mg/L), 50 µL of hydrogen peroxide (2 mM), and 50 µL of TMB (3 mM) were sequentially added to 850 µL of sodium acetate buffer (0.2 M, pH 3.5). Subsequently, the absorption spectrum of the reaction solution was documented within the wavelength range of 300 ~ 800 nm. Thereafter, TMB was substituted with OPD and ABTS, and the procedure was replicated in accordance with the aforementioned steps.

Steady-state kinetics analysis

The steady-state kinetic parameters of FePd₂S₄ nanocomposites were investigated by varying the concentrations of H₂O₂ and TMB. The steady-state kinetic experiments of FePd₂S₄ were carried out in the following manner: A solution of 0.1 mM H₂O₂ was mixed with TMB solutions of different concentrations (ranging from 0.05 to 1.0 mM), and the absorbance of oxTMB was measured at 652 nm every 10 seconds for a total of 30 measurements. Similarly, a solution of 0.15 mM TMB was mixed with H₂O₂ solutions of different concentrations (ranging from 0.05 to 1.8 mM), and the absorbance of oxTMB was measured at 652 nm every 10 seconds for a total of 30 measurements. The data were fitted using the Michaelis-Menten equation $V = V_{\max} [S] / (K_m + [S])$, where V represents the reaction rate, $V_{\max}$ represents the maximum reaction rate, $[S]$ represents the substrate concentration, and K_m represents the Michaelis constant.

Specific activity determination

The specific activity (SA, U mg⁻¹) of nanozymes is defined as the quantity of units per unit mass of nanozymes. The experimental procedure is described as follows: FePd₂S₄ (500 mg/L) was introduced into the buffer solution containing TMB (3 mM) (pH 3.5), and the reaction was conducted at 25°C. The change in absorbance at 652 nm was measured every 10 seconds up to 300 seconds.

The calculation formula is presented as follows: $b_{\text{nanozyme}} = V/(\epsilon \times l) \times (\Delta A/\Delta t)$ and $SA = b_{\text{nanozyme}}/m$. In this context, b_{nanozyme} denotes the nanozyme activity at 25°C, which refers to the quantity of TMB converted into the product per minute (1 µmol/min). V represents the total volume of the reaction solution (1000 µL), ϵ stands for the molar absorptivity of TMB (39000 M⁻¹ cm⁻¹), l indicates the optical path (1 cm), $\Delta A/\Delta t$ is the slope value of the initial change in absorbance at 652 nm (652 nm/min), and m represents the amount of nanozyme utilized (mg).

Detection of CGA

To construct the standard curve of CGA, 50 µL of FePd₂S₄ solution at a concentration of 500 mg/L, 50 µL of 3 mM TMB solution, 50 µL of 2 mM hydrogen peroxide solution, and CGA solutions of varying

concentrations were added to a 0.2 M sodium acetate-acetic acid (NaAc-HAc) buffer solution with a pH of 3.5. The total volume of the solution was then adjusted to 1 mL. Subsequently, the mixture was transferred to a microplate reader for absorbance measurement. By plotting the relationship curve between the change in absorbance (ΔA , where $\Delta A = A_0 - A$; A_0 represents the absorbance at 652 nm in the absence of CGA, and A represents the absorbance at 652 nm in the presence of CGA) and the concentration of CGA, the standard curve of CGA was generated. Quantitative analysis of CGA in the samples was performed based on the established standard curve. Color images of the final reaction solution were captured using a smartphone, and the RGB values of the sample solution were measured with the Color Grab software to construct the colorimetric standard curve.

Real Sample Testing

Sample processing

Initially, 0.5 g each of honeysuckle flower, bamboo leaves, and chrysanthemum was precisely weighed and placed into different beakers. Subsequently, 50 mL of 75% methanol was added to each beaker at a liquid-to-solid ratio of 100 mL/g. The mixtures were then thoroughly blended and subjected to ultrasonication (500 W, 40 kHz) for 40 minutes. After that, the mixtures were filtered through filter paper to obtain the extracts, which were stored in a refrigerator at 4°C for subsequent use.

Determination of CGA content in samples

50 μ L of the sample stock solution was added to a mixed solution containing varying concentrations of CGA, FePd_2S_4 (50 μ L, 500 mg/L), TMB (50 μ L, 3 mM), hydrogen peroxide (50 μ L, 2 mM), and 750 μ L of sodium acetate buffer (0.2 M, pH 3.5). Subsequently, the ultraviolet absorbance of the solution at 652 nm was measured.

HPLC analysis

The analysis by HPLC was performed on a C18 column (4.6 mm $\times$ 150 mm, 5 μ m) according to the 2025 Chinese Pharmacopoeia method with some adjustments. The mobile phase consisted of acetonitrile (A) and 0.1% formic acid (B) with an isocratic elution proportion of 12:88 (v/v). The flow rate was 1.0 mL/min, and the column temperature was 35 °C. The detection DAD wavelength was performed at 327 nm, and the injection volume was 20 μ L. The concentration of CGA was calculated by the regression curves, where Y is the peak area of these indicative compounds and X is the concentration ($\mu\text{g mL}^{-1}$) of the CGA, respectively. Their equation was expressed as $Y = 13706 X + 10322$ ($R^2 = 0.9992$, $n = 7$) with the linear range between 10.6 and 95.4 $\mu\text{g mL}^{-1}$.

Results and Discussion

Characterization of FePd_2S_4

FePd₂S₄ nanoparticles were synthesized via a one-step hydrothermal method, with L-cysteine employed as an auxiliary agent. In this process, L-cysteine fulfilled two crucial roles. Firstly, owing to the presence of –SH, –NH₂, and –COOH groups in the L-cysteine molecule, it was capable of forming coordination bonds with metal ions, rendering it an ideal template for the preparation of metal sulfides (Zhang et al., 2014). Secondly, during the preparation process, the –SH group at the end of L-cysteine decomposed to generate S²⁻ ions, suggesting that L-cysteine functioned as a reducing agent in the formation of FePd₂S₄ nanoparticles (Zhang et al., 2014). Meanwhile, FePd₂O₃ nanoparticles were prepared without the addition of L-cysteine as a control. Scanning electron microscopy (SEM) images revealed that the microsphere morphologies of FePd₂S₄ and FePd₂O₃ differed (Fig. S1a, b), and the particles of FePd₂S₄ were notably larger than those of FePd₂O₃. From the EDS (Fig. S1c), Fe, S, and Pd were detected in FePd₂S₄, indicating that S was successfully incorporated into the nanoparticles. Transmission electron microscopy (TEM) characterization further demonstrated that the FePd₂S₄ material had a granular structure (Fig. 1d), and the high-resolution transmission electron microscopy (HRTEM) image indicated that the crystal plane structure of FePd₂S₄ was not distinct, which was consistent with its XRD results.

Further characterization was conducted using XRD and XPS. The XRD patterns of the samples are presented in Fig. S2. Except for a minor shift in peak positions, the diffraction peaks of FePd₂S₄ at 19.91°, 39.68°, 45.91°, 49.81° (Fig. S2a) are in full agreement with the (011), (013), (220), and (311) crystal-plane diffraction peaks of spinel Pd₃S (PDF# 97-002- 4347). Additionally, the diffraction peak at 28.73° is consistent with the (111) crystal-plane diffraction peak of FeS₂ (PDF No. 97-000-0316). This indicates that some Pd ions in Pd₃S were substituted by Fe ions to form FePd₂S₄, while the crystal structure of Pd₃S was maintained (Xu et al., 2013). In contrast, the diffraction peaks of the undoped oxide FePd₂O₃ are largely consistent with those of Fe₂O₃ (PDF#97-002-2505). Moreover, these peaks are sharper and exhibit higher crystallinity compared to those of the S-doped samples (Fig. S2b).

Figure S3a and Fig. S3b respectively present the N₂ adsorption-desorption isotherms and pore size distributions of the relevant particles, suggesting that both types of particles possess micropores, mesopores, and macropores. Furthermore, the pore size distribution of the S-doped particles is broader than that of the undoped particles. As presented in Table S1, the Brunauer-Emmett-Teller (BET) specific surface area of the sulfur-doped nanoparticles is 11.555 m²/g, accompanied by a pore volume of 0.034 cm³/g and a particle size of 3.296 nm. This value is lower than that of the FePd₂O₃ particles.

To determine the elemental composition and oxidation state, XPS analysis was carried out. As depicted in Fig. S4, within the XPS full spectrum of FePd₂S₄, elements including Fe, Pd, S, O, N, and C can be discerned. In contrast, in the XPS full spectrum of FePd₂O₃, only the elements Fe, Pd, O, and C can be identified. The oxidation states in FePd₂S₄ were determined through Gaussian fitting of the high-resolution Fe 2p spectrum (Fig. S5a). Peaks at 710.56 and 725.17 eV were ascribed to Fe²⁺, and the peak at 714.03 eV was attributed to Fe³⁺ (Wang et al., 2024; Xu et al., 2013). Similarly, in the Pd 3d region (Fig. S5b), peaks at 336.79 and 342.08 eV were attributed to Pd²⁺ (Wang et al., 2024), whereas the peaks at 335.58 and 339.43 eV corresponded to two vibration satellite peaks. In the S 2p spectrum presented in Fig. S5c, the binding energies of 162.39 and 163.5 eV corresponded to S 2p_{3/2} and S 2p_{1/2}, respectively.

The peak at 164.17 eV was attributed to the metal-S bond (Ma et al., 2016; Tang et al., 2017), and the peak at 169.2 eV corresponded to a vibration satellite peak. Consequently, FePd_2S_4 contains Fe^{2+} , Fe^{3+} , Pd^{2+} , and S^{2-} .

Figure S6 presents a comparison of the Raman spectra of FePd_2S_4 and FePd_2O_3 . The Raman spectrum of FePd_2S_4 displays distinct characteristic peaks at 322.8 cm^{-1} and 459.45 cm^{-1} , which are within the typical Raman vibration range of metal-sulfur (M-S) bonds. These two characteristic peaks respectively correspond to the characteristic Raman shifts of Fe-S bonds and Pd-S bonds and serve as the core structural identifiers of the FePd_2S_4 sulfide. This directly reflects the structural characteristics of the sulfide. In contrast, the Raman spectrum of FePd_2O_3 reveals significant structural disparities compared to that of FePd_2S_4 . The spectrum at 636.34 cm^{-1} features a characteristic peak absent in the spectrum of FePd_2S_4 , and the wavelength of this peak falls within the characteristic Raman vibration range of Fe-O/Pd-O bonds. It is a characteristic Raman signal of the iron-palladium oxide. The types and wavelengths of the characteristic peaks in the Raman spectra of the two substances exhibit clear and substantial phase differences. FePd_2S_4 demonstrates typical Raman characteristics of M-S bonds in sulfides and lacks characteristic peaks associated with Fe-O/Pd-O bonds. This result fully validates the successful synthesis of FePd_2S_4 and clearly differentiates its phase structure from that of the iron-palladium oxide FePd_2O_3 .

Peroxidase-like Properties of FePd_2S_4

Employing TMB, OPD, and ABTS as chromogenic substrates, the catalytic activities of FePd_2S_4 , functioning as an enzyme-like oxidase and peroxidase, were comprehensively evaluated. The peroxidase-like activity was specifically examined via the TMB oxidation system mediated by H_2O_2 . As depicted in Fig. 1a, subsequent to a 5-minute incubation of TMB, H_2O_2 , and FePd_2S_4 , the solution displayed a distinct blue color, and the absorbance at 652 nm (A_{652}) notably increased. In contrast, when only FePd_2S_4 was present with TMB, the color change was negligible, and the A_{652} value was minimal, suggesting that FePd_2S_4 possesses high peroxidase-like activity and extremely feeble catalytic activity as an oxidase. This conclusion can be further corroborated by utilizing OPD and ABTS substrates (Fig. 1b, c). When both OPD and ABTS were combined with H_2O_2 and FePd_2S_4 , they respectively manifested obvious yellow and green color alterations. However, when only FePd_2S_4 was mixed with these substrates, the color change was insignificant, and the absorbance value was low, further validating that FePd_2S_4 has high intrinsic peroxidase-like activity and extremely weak oxidase activity.

The peroxidase-like activity of FePd_2O_3 was compared under identical experimental conditions. As depicted in Fig. 1d, the FePd_2O_3 material also demonstrated peroxidase-like activity; however, it was weaker than that of FePd_2S_4 . Subsequent to S doping, its activity increased by a factor of 2.5. These results suggest that the doping of S exerts a certain enhancing influence on the peroxidase-like activity of FePd_2S_4 materials. This phenomenon can be attributed to the energy difference between the 2p energy level of oxygen in transition metal oxides and the 3p energy level of sulfur, which results in higher conductivity and catalytic activity of metal sulfides compared to their corresponding oxides (Zhu et al., 2022). The doping of S not only creates active sites to catalyze the decomposition of H_2O_2 to generate

•OH (as will be discussed later), but also improves its conductivity and facilitates the charge transfer between the active sites and reactants (Fig. 1d). In summary, the doping of S enables the synergistic effect of Fe and Pd to achieve a higher peroxidase-like catalytic activity.

As the reaction time is prolonged, the quantity of oxTMB produced by the FePd₂S₄ catalyst increases, and the A₆₅₂ value rises (Fig. 2a). After approximately 20 minutes of the reaction, the increase in the absorbance of oxTMB at 652 nm decelerates, suggesting that the catalytic reaction has essentially reached an equilibrium state. To minimize the error in subsequent analysis and detection, 30 minutes was chosen as the final reaction time. Additionally, as depicted in Fig. 2b, the peroxidase-like activity of FePd₂S₄ significantly enhances with the increase in its mass concentration. The more rapid the catalytic reaction of TMB, the stronger the peak of oxTMB at 652 nm. The inset figure in Fig. 2b presents a point-and-line graph of the mass concentration of FePd₂S₄ and A₆₅₂, which shows a nearly linear correlation within the concentration range of 1–25 µg mL⁻¹, indicating that FePd₂S₄ exhibits a high peroxidase-like activity.

In the colorimetric system, both the pH value and the temperature of the reaction solution can influence the catalytic activity of the peroxidase-like enzyme. Generally, the optimal reaction medium for the oxidation of TMB is acidic, and the majority of the reported optimal reaction pH values are 3.5 (Wang et al., 2024; Wang and Wang, 2025). Figure 2d indicates that FePd₂S₄ demonstrates a strong catalytic capacity for TMB color development at a pH of 3.5, which is consistent with the previously reported results. Therefore, pH 3.5 was chosen as the final pH for the subsequent reactions.

Generally speaking, an elevation in the catalytic temperature will augment the collision frequency between reactants and the active sites on the enzyme surface, facilitating the thermodynamic process of TMB oxidation and consequently enhancing the reaction efficiency (He et al., 2025). As depicted in Fig. 2c, the rise in reaction temperature exerts minimal influence on the peroxidase-like activity. This phenomenon differs from the optimal temperature mechanism of natural enzymes. Nanomaterial enzymes have expanded the reaction temperature range, offering a means to construct peroxidase-like enzymes that are adaptable to a broader temperature environment. Taking into account the simplicity of application, 25°C was chosen as the final temperature for subsequent exploration, analysis, and detection.

In addition to the catalytic activity of nanoenzymes, the stability of nanoenzymes also serves as a crucial parameter for assessing the superior performance and practicality of nanoenzymes. Therefore, we evaluated the batch-to-batch differences in the synthesis of FePd₂S₄ nanoenzymes and their storage stability. In Fig. 2e, the relative standard deviation (RSD) of the peroxidase-like activity of the five synthesized batches of FePd₂S₄ was merely 3.01%, indicating that the nanoenzymes can be synthesized stably and the peroxidase-like activity exhibits good reproducibility. Figure 2f reveals that the peroxidase-like catalytic activity of FePd₂S₄ nanoenzymes did not display a significant decline even after being stored in an aqueous solution for 30 days, demonstrating excellent storage stability.

As depicted in Fig. 3a-c, under the optimal reaction conditions, the specific activity (SA) of FePd₂S₄ and FePd₂O₃ was measured. As the quantity of FePd₂S₄ and FePd₂O₃ increased, the catalytic oxidation kinetic process of TMB accelerated (Fig. 3a). In accordance with the standardized SA calculation procedure, FePd₂S₄ demonstrated higher peroxidase-like activity, with its SA approximately 0.000242 U mg⁻¹ (Fig. 3b), which was 1.57 times that of FePd₂O₃ (SA = 0.000154 U mg⁻¹, Fig. 3c). This finding emphasizes the crucial role of S doping in regulating the peroxidase-like activity of FePd₂S₄. In the electrochemical impedance spectroscopy (EIS) Nyquist plots (Fig. 3d), the radius of the semicircle for FePd₂S₄ was significantly smaller than that of FePd₂O₃, and the equivalent resistance of FePd₂S₄ (6363Ω) was also lower than that of FePd₂O₃ (8127Ω). Therefore, the doping of S enhanced the electron transfer ability of the bimetallic Fe-Pd.

Steady-state kinetics analysis

To conduct a more in-depth investigation into the catalytic activity of FePd₂S₄ nanoenzymes, steady-state kinetic experiments were carried out, and the kinetic parameters were calculated. Figures 4a and c present the Michaelis-Menten kinetic curves for TMB and H₂O₂. Following double reciprocal processing, the K_m values and the V_{max} values were determined and compared with those of natural horseradish peroxidase (HRP) and other representative peroxide-mimicking enzymes reported previously. The experimental results are in full agreement with the typical Michaelis-Menten curve. Utilizing the Lineweaver-Burk formula of the double reciprocal curve, the K_m value of the FePd₂S₄ nanoenzyme for TMB was found to be 0.204 mM, and the V_{max} value was 2.78×10^{-6} M s⁻¹. This was compared with other oxide-based nanoenzymes reported earlier. As shown in Table S2, the K_m value of FePd₂S₄ for TMB was lower than those of other reported oxide-based nanoenzymes. A lower K_m value implies a higher affinity of the FePd₂S₄ material for TMB. Additionally, when compared to other oxide-based nanoenzymes, the FePd₂S₄ nanoenzyme exhibited a smaller K_m for H₂O₂ (0.0504 mM) and a larger V_{max} value (1.03×10^{-6} M s⁻¹), indicating its superior catalytic performance. This outcome suggests that FePd₂S₄ has a stronger affinity for TMB and H₂O₂.

Enzyme Mechanism

To elucidate the intrinsic enzyme-like catalytic mechanism of FePd₂S₄, Fig. 5a presents a summary of the experimental results regarding the capture of reactive oxygen species (ROS) by scavengers at different concentrations. TH• is capable of eliminating •OH, p-BQ can eliminate superoxide anion free radicals (O₂^{•-}), AA can serve as a scavenger for both hydroxyl radicals (•OH) and O₂^{•-}, and DL-His can eliminate singlet oxygen (¹O₂). The results indicate that DL-His has a relatively minor influence on the response of the FePd₂S₄ reaction system, suggesting that only a small quantity of ¹O₂ participated in the catalytic process. Conversely, O₂^{•-} and •OH play a predominant role in the oxidation of TMB, as the addition of p-BQ and AA results in a substantial decrease in the absorbance of the FePd₂S₄ + TMB system.

Furthermore, PTA was used to detect the generation of $\cdot\text{OH}$ through the formation of fluorescent 2-hydroxy p-phenylenediamine. As depicted in Fig. 5b, in comparison with the PTA + FePd_2S_4 system without H_2O_2 , a distinct fluorescence emission peak emerged at 450 nm subsequent to the addition of H_2O_2 . Moreover, upon the addition of isopropanol (IPA, a $\cdot\text{OH}$ scavenger), the intensity of the fluorescence emission peak decreased instantaneously, further validating the presence of $\cdot\text{OH}$ in the system (Fig. 5b). This suggests that $\cdot\text{OH}$ exists in the FePd_2S_4 + TMB system, which is in accordance with the results of the free-radical scavenging experiment. Finally, electron paramagnetic resonance (ESR) was employed to verify the generation of $^1\text{O}_2$, $\cdot\text{OH}$, and $\text{O}_2^{\cdot-}$. As shown in Fig. 5c, when DMPO was utilized as a specific capture agent for $\cdot\text{OH}$, the characteristic signal peaks of $\cdot\text{OH}$ exhibited an intensity ratio of 1:2:2:1. When TEMP was used as a specific capture agent for $^1\text{O}_2$ and $\text{O}_2^{\cdot-}$, the corresponding characteristic signals were also detected, offering reliable evidence for the generation of $^1\text{O}_2$, $\cdot\text{OH}$, and $\text{O}_2^{\cdot-}$, too.

To further elucidate the enhanced peroxidase-like activity of the FePd_2S_4 nanoenzyme, the Zeta potential of FePd_2S_4 and FePd_2O_3 suspensions in deionized water and an acetic acid-sodium acetate buffer solution (0.2 M, pH 3.5) was measured. As shown in Fig. 5d, in comparison with FePd_2O_3 , the surface of FePd_2S_4 carried more negative charges in both water and the buffer solution. For the positively charged TMB, the negatively charged nanoenzyme enhances the affinity between the substrate and the nanoenzyme (Zhu et al., 2023), thereby increasing the catalytic activity towards TMB. Furthermore, subsequent to the addition of H_2O_2 , FePd_2S_4 exhibited more negative charges in the buffer solution. Conversely, it carried positive charges in water, which is consistent with the findings regarding the optimal pH value. The reaction is appropriate for acidic conditions, and the activity declines as the pH increases. The Zeta potential demonstrated macroscopically that FePd_2S_4 possesses a stronger electrostatic adsorption capacity for TMB.

Colorimetric Method for the Detection of CGA

The colorimetric sensing mechanism of CGA is depicted in Fig. 6a. Specifically, curve a represents the kinetic curve of the FePd_2S_4 + TMB + H_2O_2 system, while curve c represents the kinetic curve when CGA is added at 150 seconds into the reaction. It is evident that the A_{652} value increases instantaneously upon the addition of CGA, suggesting that CGA can directly oxidize TMB. Curve b is the kinetic curve of the FePd_2S_4 + TMB + H_2O_2 system with CGA added at the onset of the reaction. In comparison with curve a, the oxidation of TMB is expedited, indicating that CGA can promote the oxidation of TMB by augmenting ROS. CGA solutions with concentrations of 5, 10, 15, 25, 30, 35, 40, 45, and 50 $\mu\text{g mL}^{-1}$ were added to the FePd_2S_4 + TMB + H_2O_2 system and incubated to obtain the linear regression equation of CGA. As can be observed from the inset of Fig. 6b, as the concentration of CGA increases, the blue color gradually intensifies, and the A_{652} value rises (Fig. 6c). From Fig. 6c, it can be inferred that a colorimetric sensor for detecting CGA can be established by utilizing the colorimetric reaction catalyzed by the FePd_2S_4 nanozyme for the oxidation of CGA. There exists a linear relationship between ΔA_{652} and the concentration of CGA within the range of 1–50 $\mu\text{g mL}^{-1}$ ($R^2 = 0.9818$, $\Delta A_{652} = 0.0086C + 0.0071$), with a detection limit of 0.15 $\mu\text{g mL}^{-1}$ and a quantitative limit of 0.40 $\mu\text{g mL}^{-1}$.

During the color detection process utilizing smartphones, it was noted that the color intensity of ox TMB increased with the incremental addition of CGA (Fig. 6d). The value of the (R + G)/2B channel gradually declined as the quantity of CGA added increased (Fig. 6d). Through the establishment of the relationship between the concentration of CGA and the R, G, and B values, precise quantitative analysis was accomplished. Among various quantitative models, the (R + G)/2B channel exhibited the most favorable linear relationship with the CGA concentration within the range of 1 $\mu\text{g mL}^{-1}$ to 50 $\mu\text{g mL}^{-1}$ ($R^2 = 0.9862$). The derived linear equation was $IR = -0.0048X + 0.9166$, and the calculated detection limit was 0.45 $\mu\text{g mL}^{-1}$, whereas the quantification limit was 0.70 $\mu\text{g mL}^{-1}$.

Furthermore, the developed method was compared with several recently reported methods, and the relevant results are presented in Table 1. At present, the detection of CGA using nanoenzymes primarily relies on fluorescence, electrochemistry, and SERS. It is evident that this study exhibits a moderate detection performance. The results confirm that a straightforward and precise quantitative analysis method can be integrated with smartphone RGB colorimetry, suggesting that the current multi-modal detection platform can be considered an outstanding sensor for detecting CGA.

Table 1
Comparison of the current method with reported methods

Sensing system	Methods	Linear range	LOD	References
water-soluble carbon dots (CDs)	fluorescent	0.15–60 μM	45 nM	(Yang et al., 2018)
DPA-Au/CuNCs	fluorescent	12.5–200 $\mu\text{g mL}^{-1}$	3.75 $\mu\text{g mL}^{-1}$	(Zhao et al., 2025)
microneedle electrode	electrochemical	0.1–78.01 $\mu\text{g mL}^{-1}$	0.34 $\pm$ 0.2 $\mu\text{g mL}^{-1}$	(Dhanjai et al., 2020)
Gold Nanoparticles	SERS	10 μM –350 μM	60 μM	(Pavlova et al., 2024)
N,S-CDs	fluorescent	0.33–29.70 $\mu\text{g mL}^{-1}$	0.12 $\mu\text{g mL}^{-1}$	(Liu et al., 2021)
Co-L@RGO(1:1)	electrochemical sensors	0.1–2 μM and 2–20 μM	7.24 nM	(Zhao et al., 2023)
boron-doped diamond electrode	electrochemical	5.0–45.0 mg mL^{-1}	0.448 mg mL^{-1} (1.26 10^{-6} M)	(Zhao et al., 2023)
FePd ₂ S ₄	Colorimetry / Smartphone-assisted RGB color	1–50 / 1–50 $\mu\text{g mL}^{-1}$ (2.82–141.12 μM)	0.15 / 0.45 $\mu\text{g mL}^{-1}$ (0.423/1.269 μM)	This work

To investigate the selectivity of this colorimetric detection method, the anti-interference ability of the method was tested using several potential interfering substances (Fig. 7). In the reaction system, inorganic salt ions (K^+ , Na^+ , Ca^{2+} , HPO_4^{2-} , $H_2PO_4^-$, SO_4^{2-}), possible interfering substances and additives in food such as sucrose, glucose, fructose, lactose, citric acid, DL-malic acid, and biological molecules such as glutathione (GSH), arginine (Arg), proline (Pro), uric acid (UA), and citric acid were introduced. The concentration of each interfering substance was set at 1 mM. By comparing with the blank group where no interfering substances were added, it was observed that the absorbance of the group with added interfering substances did not exhibit a significant change. This finding indicates that the colorimetric detection method based on the peroxidase activity of $FePd_2S_4$ nanoenzymes demonstrates good selectivity.

Real Sample Analysis

Firstly, the labeled recovery method was employed to verify the analysis accuracy of the two detection methods in quantifying the CGA content in actual samples. To this end, the CGA concentrations in three herbal extracts of honeysuckle flower, chrysanthemum and bamboo leaves were measured. As presented in Table 2, the recovery rate of CGA in actual samples ranged from 91.6% to 106.8%, and the relative standard deviation (RSD) was between 0.38% and 3.45%. In addition, the routine HPLC analysis was applied to compare with our methods for determining CGA contents in the real samples. As listed in Table 3, it can be seen that CGA contents in honeysuckle flower, chrysanthemum and bamboo leaves were in good agreement with the results obtained by HPLC method. The RSD values were less than 3% in our UV-vis spectrophotometry and smartphone RGB colorimetry, indicating the satisfactory precision for CGA determination in actual samples. These findings suggest that the proposed dual-mode methods are reliable for the determination of CGA in herbal extracts and have good practical application potential.

Table 2
Results of CGA determination in the actual samples

Methods	Sample	Spiked ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Recovery (%)	RSD (% n = 3)
UV-vis spectrophotometry	Honeysuckle flower	20	20.104 $\pm$ 0.531	100.5	2.57
		30	32.053 $\pm$ 0.395	106.8	1.07
		40	40.694 $\pm$ 0.424	101.7	0.99
		50	52.710 $\pm$ 1.132	105.4	1.71
	Bamboo leaves	20	18.905 $\pm$ 0.171	94.5	0.81
		30	28.582 $\pm$ 0.155	95.3	0.54
		40	38.733 $\pm$ 0.148	96.8	0.38
		50	46.733 $\pm$ 0.484	93.5	1.04
	Chrysanthemum	20	19.825 $\pm$ 0.175	99.6	0.89
		30	30.335 $\pm$ 0.683	99.4	2.22
		40	41.147 $\pm$ 1.059	99.8	2.50
		50	50.327 $\pm$ 1.389	98.5	2.70
	Smartphone RGB colorimetry	20	18.677 $\pm$ 0.641	93.4	3.45
		30	28.482 $\pm$ 0.520	94.9	1.83
		40	36.754 $\pm$ 0.957	91.9	2.60
		50	49.066 $\pm$ 0.907	98.1	1.81
	Bamboo leaves	20	18.728 $\pm$ 0.225	93.6	1.19

Methods	Sample	Spiked ($\mu\text{g mL}^{-1}$)	Found ($\mu\text{g mL}^{-1}$)	Recovery (%)	RSD (% n = 3)
	Chrysanthemum	30	27.796 $\pm$ 0.511	92.7	1.75
		40	36.634 $\pm$ 0.464	91.6	1.23
		50	46.786 $\pm$ 0.383	93.6	0.84
		20	19.224 $\pm$ 0.475	96.1	2.40
		30	30.816 $\pm$ 0.571	102.7	1.82
		40	38.048 $\pm$ 0.566	95.1	1.49
		50	48.123 $\pm$ 1.273	96.3	2.54

Table 3
Comparison of our methods with HPLC for CGA determination in the real samples

Sample	HPLC (mg g^{-1})	RSD (%, n = 3)	UV-vis spectrophotometry (mg g^{-1})	RSD (%, n = 3)	Smartphone RGB colorimetry (mg g^{-1})	RSD (%, n = 3)
Honeysuckle flower	24.884 $\pm$ 0.050	0.199	24.581 $\pm$ 0.066	0.270	24.543 $\pm$ 0.358	1.460
Bamboo leaves	0.335 $\pm$ 0.009	2.797	0.329 $\pm$ 0.006	1.809	0.320 $\pm$ 0.006	1.881
Chrysanthemum	2.165 $\pm$ 0.021	0.972	2.132 $\pm$ 0.018	0.855	2.142 $\pm$ 0.015	0.707

Conclusion

In this study, FePd_2S_4 nanoparticles were successfully synthesized via the hydrothermal method. L-cysteine concurrently fulfilled the dual functions of a coordination template and a S source reducing agent. Structural characterization revealed that S doping preserved the Pd_3S crystal structure of FePd_2S_4 , resulting in the formation of a multi-level pore structure composed of micropores, mesopores, and macropores. Moreover, mixed valence states of $\text{Fe}^{2+}/\text{Fe}^{3+}$ and Pd^{2+} were present on the surface. In comparison with FePd_2O_3 , FePd_2S_4 demonstrated more outstanding peroxidase-like activity (with an activity enhancement of 2.5 times), featuring a specific activity of $0.000242 \text{ U mg}^{-1}$, which is 1.57 times that of FePd_2O_3 . Additionally, it exhibited good batch reproducibility and 30-day storage stability. The

steady-state kinetic analysis demonstrated that the K_m value of FePd₂S₄ for TMB was 0.204 mM, which was lower compared to that of most reported nanoenzymes. This lower value indicates a higher affinity of FePd₂S₄ for the substrate. The investigation into the catalytic mechanism verified that the system accomplished substrate oxidation through the generation of ¹O₂, ·OH, and O₂^{·-}. Moreover, the negative surface potential induced by S doping enhanced the electrostatic interaction with TMB. Leveraging this catalytic activity, the quantification of protoanemonin in herbal extracts was carried out using both a UV-Vis spectrophotometer and a smartphone. A satisfactory linear relationship was established within the concentration range of 1 ~ 50 µg mL⁻¹, with coefficients of determination (R²) of 0.9818 and 0.9862, respectively. The LODs were found to be as low as 0.15 and 0.45 µg mL⁻¹. This analytical method exhibited high selectivity towards common interfering substances, and the spiked recovery rates in actual samples, including honeysuckle flower, bamboo leaves and chrysanthemum, ranged from 91.6% to 106.8% (RSD ≤ 3.45%). In conclusion, the FePd₂S₄ nanoenzyme, characterized by its high catalytic activity, stability, and selectivity, offers a novel approach for the rapid and accurate detection of. It exhibits substantial potential for quantitative determination of CGA in homologous medicinal and edible plants and related pharmaceutical or nutraceutical products.

Declarations

CRedit authorship contribution statement

Jiayi Jiang: Conceptualization, Data curation, Investigation, Software, Writing - original draft, Methodology. **Yifeng Zhou:** Validation, Project administration, Writing - review & editing, Supervision. **Liling Wang:** Methodology, Validation, Supervision, Writing – review & editing, Funding acquisition, Visualization.

Declaration of competing interest

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.

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Data Availability

The data will be made available on request.

Supporting information

The online version contains supplementary material available at <https://doi.org/10.1007/XXX>.

References

1. Abdeta AB, Wu Q, Kuo D, Li P, Zhang H, Zhang J, Yuan Z, Lin J, Chen X (2022) Activated carbon-supported AgMoOS bimetallic oxysulfide as a catalyst for the photocatalytic hydrogen evolution and pollutants reduction. *J Colloid Interface Sci* 913:165287. <https://doi.org/10.1016/j.jallcom.2022.165287>
2. Alagumalai K, Shanmugam R, Chen S, Arumugam B, Chen T, Yu J, Liu X, Ramaraj SK (2022) A portable advanced electrocatalyst for polyphenolic chlorogenic acid evaluation in food samples. *Chem Eng J* 435:134796. <https://doi.org/10.1016/j.cej.2022.134796>
3. Amakura Y, Okada M, Tsuji S, Tonogai Y (2000) Determination of phenolic acids in fruit juices by isocratic column liquid chromatography. *J Chromatogr A* 891(1):183–188. [https://doi.org/10.1016/S0021-9673\(00\)00625-7](https://doi.org/10.1016/S0021-9673(00)00625-7)
4. Chen F, Song T (2022) AuPt bimetallic nanozymes for enhanced glucose catalytic oxidase. *Front Chem* 10:854516. <https://doi.org/10.3389/fchem.2022.854516>
5. Chen X, Sun H, Kuo D, Abdeta AB, Zelekew OA, Guo Y, Zhang J, Yuan Z, Lin J (2021) Spherical nanoflower-like bimetallic (Mo,Ni)(S,O)_{3-x} sulfo-oxide catalysts for efficient hydrogen evolution under visible light. *Appl Catal B Environ* 287:119992. <https://doi.org/10.1016/j.apcatb.2021.119992>
6. Cheng J, Sun N, Zhao X, Niu L, Song M, Sun Y, Jiang J, Guo J, Bai Y, He J, Li H (2013) In vitro screening for compounds derived from traditional chinese medicines with antiviral activities against porcine reproductive and respiratory syndrome virus. *J Microbiol Biotechnol* 23(8):1076–1083. <https://doi.org/10.4014/jmb.1303.03074>
7. Cui H, He C, Zhao G (1999) Determination of polyphenols by high-performance liquid chromatography with inhibited chemiluminescence detection. *J Chromatogr A* 855(1):171–179. [https://doi.org/10.1016/S0021-9673\(99\)00670-6](https://doi.org/10.1016/S0021-9673(99)00670-6)
8. Dhanjai Mugo SM, Lu W (2020) Modified stainless steel microneedle electrode for polyphenolics detection. *Anal Bioanal Chem* 412(26):7063–7072. <https://doi.org/10.1007/s00216-020-02836-w>
9. Dutta S (2019) Point of care sensing and biosensing using ambient light sensor of smartphone: critical review. *Trac Trends Anal Chem* 110:393–400. <https://doi.org/10.1016/j.trac.2018.11.014>
10. Fábíán Z, Izvekov V, Salgó A, örsi F (1994) Near-infrared reflectance and fourier transform infrared analysis of instant coffee mixtures. *Anal Proc* 31(9):261–263. <https://doi.org/10.1039/ai9943100261>
11. Gu X, Xu R, Yuan G, Lu H, Gu B, Xie H (2010) Preparation of chlorogenic acid surface-imprinted magnetic nanoparticles and their usage in separation of traditional chinese medicine. *Anal Chim Acta* 675(1):64–70. <https://doi.org/10.1016/j.aca.2010.06.033>
12. He Y, Feng M, Zhang X, Huang Y (2025) MOF-derived bundle-like Mn doped NiO with rich oxygen vacancy as oxidase mimic for the determination of total antioxidant capacity. *Sens Actuat B: Chem* 428:137227. <https://doi.org/10.1016/j.snb.2025.137227>

13. Hemmerle H, Burger HJ, Below P, Schubert G, Rippel R, Schindler PW, Paulus E, Herling AW (1997) Chlorogenic acid and synthetic chlorogenic acid derivatives: Novel inhibitors of hepatic glucose-6-phosphate translocase. *J Med Chem* 40(2):137–145. <https://doi.org/DOI 10.1021/jm9607360>
14. Huang N, Wen J, Yi D, Wei Z, Long Y, Zheng H (2022) Colorimetric detection of ATP by inhibiting the Peroxidase-like activity of carbon dots. *Spectrochim Acta A* 268:120658. <https://doi.org/10.1016/j.saa.2021.120658>
15. Huang W, Cai Y, Zhang Y (2009) Natural phenolic compounds from medicinal herbs and dietary plants: Potential use for cancer prevention. *Nutr Cancer* 62(1):1–20. <https://doi.org/10.1080/01635580903191585>
16. Jiang C, Sun M, Wang Y, Dong C, Yu Y, Wang G, Lu Y, Chen Z (2025) Coordination engineering in Fe-Mn dual-atom nanozyme: yielding ros storm to efficiently promote wound healing. *Adv Funct Mater* 35(29):2424599. <https://doi.org/10.1002/adfm.202424599>
17. Kumar A, Virender Saini M, Mohan B, Shayoraj Kamboj M (2022) Colorimetric and fluorescent schiff base sensors for trace detection of pollutants and biologically significant cations: a review (2010–2021). *Microchem J* 181:107798. <https://doi.org/10.1016/j.microc.2022.107798>
18. Li Z, Huang D, Tang Z, Deng C, Zhang X (2010) Fast determination of chlorogenic acid in tobacco residues using microwave-assisted extraction and capillary zone electrophoresis technique. *Talanta* 82(4):1181–1185. <https://doi.org/10.1016/j.talanta.2010.06.037>
19. Liang N, Lu X, Hu Y, Kitts DD (2016) Application of attenuated total reflectance-fourier transformed infrared (ATR-FTIR) spectroscopy to determine the chlorogenic acid isomer profile and antioxidant capacity of coffee beans. *J Agric Food Chem* 64(3):681–689. <https://doi.org/10.1021/acs.jafc.5b05682>
20. Liu Q, Dong Z, Hao A, Guo X, Dong W (2021) Synthesis of highly fluorescent carbon dots as a dual-excitation rationmetric fluorescent probe for the fast detection of chlorogenic acid. *Talanta* 221:121372. <https://doi.org/10.1016/j.talanta.2020.121372>
21. Liu X, Yan L, Ren H, Cai Y, Liu C, Zeng L, Guo J, Liu A (2020) Facile synthesis of magnetic hierarchical flower-like Co_3O_4 spheres: Mechanism, excellent tetra-enzyme mimics and their colorimetric biosensing applications. *Biosens Bioelectron* 165:112342. <https://doi.org/10.1016/j.bios.2020.112342>
22. Lu H, Tian Z, Cui Y, Liu Z, Ma X (2020) Chlorogenic acid: A comprehensive review of the dietary sources, processing effects, bioavailability, beneficial properties, mechanisms of action, and future directions. *Compr Rev Food Sci Food Saf* 19(6):3130–3158. <https://doi.org/10.1111/1541-4337.12620>
23. Ma L, Hu Y, Chen R, Zhu G, Chen T, Lv H, Wang Y, Liang J, Liu H, Yan C, Zhu H, Tie Z, Jin Z, Liu J (2016) Self-assembled ultrathin NiCo_2S_4 nanoflakes grown on Ni foam as high-performance flexible electrodes for hydrogen evolution reaction in alkaline solution. *Nano Energy* 24:139–147. <https://doi.org/10.1016/j.nanoen.2016.04.024>

24. Moccelini SK, Spinelli A, Vieira IC (2008) Biosensors based on bean sprout homogenate immobilized in chitosan microspheres and silica for determination of chlorogenic acid. *Enzyme Microb Technol* 43(4–5):381–387. <https://doi.org/10.1016/j.enzmictec.2008.06.002>
25. Pavlova A, Maleeva K, Moskalenko IV, Belyaev V, Zhukov MV, Kirilenko D, Bogdanov KV, Smirnov E (2024) Self-assembled gold nanoparticles as reusable SERS substrates for polyphenolic compound detection. *Int J Mol Sci* 25(23):12785. <https://doi.org/10.3390/ijms252312785>
26. Peng B, Guo Y, Ma Y, Zhou M, Zhao Y, Wang J, Fang Y (2022) Smartphone-assisted multiple-mode assay of ascorbic acid using cobalt oxyhydroxide nanoflakes and carbon quantum dots. *Microchem J* 175:107185. <https://doi.org/10.1016/j.microc.2022.107185>
27. Quesada-Gonzalez D, Merkoci A (2017) Mobile phone-based biosensing: An emerging diagnostic and communication technology. *Biosens Bioelectron* 92:549–562. <https://doi.org/10.1016/j.bios.2016.10.062>
28. Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* 20(7):933–956. [https://doi.org/10.1016/0891-5849\(95\)02227-9](https://doi.org/10.1016/0891-5849(95)02227-9)
29. Roda A, Michelini E, Zangheri M, Di Fusco M, Calabria D, Simoni P (2016) Smartphone-based biosensors: A critical review and perspectives. *TrAC Trends Anal Chem* 79:317–325. <https://doi.org/10.1016/j.trac.2015.10.019>
30. Shrivastava K, Patel S, Sinha D, Thakur SS, Patle TK, Kant T, Dewangan K, Satnami ML, Nirmalkar J, Kumar S (2020) Colorimetric and smartphone-integrated paper device for on-site determination of arsenic (III) using sucrose modified gold nanoparticles as a nanoprobe. *Microchim Acta* 187:173. <https://doi.org/10.1007/s00604-020-4129-7>
31. Tang S, Zhu B, Shi X, Wu J, Meng X (2017) General controlled sulfidation toward achieving novel nanosheet-built porous square-FeCo₂S₄-tube arrays for high-performance asymmetric all-solid-state pseudocapacitors. *Adv Energy Mater* 7(6):1601985. <https://doi.org/10.1002/aenm.201601985>
32. Tong P, Zhang L, He Y, Chi Y, Chen G (2009) A simple capillary electrophoresis with electrochemical detection method for determination of the hydrolysis rate constant of chlorogenic acid. *Talanta* 77(5):1790–1794. <https://doi.org/10.1016/j.talanta.2008.10.026>
33. Wang B, Liu F, Wu Y, Chen Y, Weng B, Li CM (2018) Synthesis of catalytically active multielement-doped carbon dots and application for colorimetric detection of glucose. *Sens Actuators B: Chem* 255:2601–2607. <https://doi.org/10.1016/j.snb.2017.09.067>
34. Wang L, Wang Y (2025) PdCo@NC-based colorimetric dual-wavelength for highly sensitive Hg²⁺ ions detection. *Microchem J* 215:114367. <https://doi.org/10.1016/j.microc.2025.114367>
35. Wang L, Wang Y, Zhou Y (2024) Bimetallic mof-derived three-dimensional nanoflowers PdCoO_x as peroxidase mimic activity for determining total antioxidant capacity. *Food Chem* 457:140120. <https://doi.org/10.1016/j.foodchem.2024.140120>
36. Wang Y, Feng Q, Liu M, Xue L, Wang G, Zhang S, Hu W (2023) N, P, S codoped carbon nanozymes with enhanced peroxidase-like activity and binding affinity for total antioxidant capacity assay. *ACS*

- Appl Nano Mater 6(24):23303–23312. <https://doi.org/10.1021/acsanm.3c04650>
37. Wu J, Wang X, Wang Q, Lou Z, Li S, Zhu Y, Qin L, Wei H (2019) Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes (II). *Chem Soc Rev* 48(4):1004–1076. <https://doi.org/10.1039/c8cs00457a>
38. Wu M, Liu T, Yin C, Jiang X, Sun Q, Gao L, Niu N, Chen L, Gang H (2023) Portable smartphone-assisted RGB-dependent ratiometric sensing platform for the detection of tetrachloro-p-benzoquinone in river samples. *Microchem J* 190:108686. <https://doi.org/10.1016/j.microc.2023.108686>
39. Wu Q, Zhang P, Kuo D, Wu B, Abdeta AB, Su Z, Chen L, Zelekew OA, Lin J, Chen X (2023) Activation of $\text{Cu}_3(\text{MoO}_4)_2(\text{OH})_2$ with the hydrazine-driven cation reduction into a highly efficient catalyst for the reduction of organic dyes and heavy metals ion. *J Environ Chem Eng* 11(3):109974. <https://doi.org/10.1016/j.jece.2023.109974>
40. Xu W, Ni J, Zhang Q, Feng F, Xiang Y, Li X (2013) Tailoring supported palladium sulfide catalysts through H_2 -assisted sulfidation with H_2S . *J Mater Chem A* 1(41):12811–12817. <https://doi.org/10.1039/c3ta12277h>
41. Yang H, Yang L, Yuan Y, Pan S, Yang J, Yan J, Zhang H, Sun Q, Hu X (2018) A portable synthesis of water-soluble carbon dots for highly sensitive and selective detection of chlorogenic acid based on inner filter effect. *Spectrochim Acta A* 189:139–146. <https://doi.org/10.1016/j.saa.2017.07.065>
42. Yardim Y, Keskin E, şentürk Z (2013) Voltammetric determination of mixtures of caffeine and chlorogenic acid in beverage samples using a boron-doped diamond electrode. *Talanta* 116:1010–1017. <https://doi.org/10.1016/j.talanta.2013.08.005>
43. Ye M, Zhu Y, Lu Y, Gan L, Zhang Y, Zhao Y (2021) Magnetic nanomaterials with unique nanozymes-like characteristics for colorimetric sensors: a review. *Talanta* 230:122299. <https://doi.org/10.1016/j.talanta.2021.122299>
44. Zhang L, Wu HB, Yan Y, Wang X, Lou XWD (2014) Hierarchical MoS_2 microboxes constructed by nanosheets with enhanced electrochemical properties for lithium storage and water splitting. *Energy Environ Sci* 7(10):3302–3306. <https://doi.org/10.1039/c4ee01932f>
45. Zhang R, Jia X, Sun M, Liu X, Wang C, Xing Y, Yu X (2025) Synergistic manipulation of sulfur vacancies and palladium doping of In_2S_3 for enhanced photocatalytic H_2 production. *J Colloid Interface Sci* 677:425–434. <https://doi.org/10.1016/j.jcis.2024.07.242>
46. Zhao P, Song Z, Li Y, Liu X, Jiang Z, Zhu Q, Qu J (2025) Rapid and simple fluorescent detection of chlorogenic acid in Aidi injection using aggregation-induced emission (AIE) nanoclusters. *J Pharm Biomed Anal* 254:116570. <https://doi.org/10.1016/j.jpba.2024.116570>
47. Zhao T, Niu X, Pei W, Ma J (2023) Thiocalix[4]arene-based metal-organic framework / reduced graphene oxide composite for electrochemical detection of chlorogenic acid. *Anal Chim Acta* 1276:341653. <https://doi.org/10.1016/j.aca.2023.341653>
48. Zhu C, Yang H, Cao X, Hong Q, Xu Y, Wang K, Shen Y, Liu S, Zhang Y (2023) Decoupling of the confused complex in oxidation of 3,3',5,5'-tetramethylbenzidine for the reliable chromogenic

49. Zhu Q, Xu Q, Du M, Zeng X, Zhong G, Qiu B, Zhang J (2022) Recent progress of metal sulfide photocatalysts for solar energy conversion. Adv Mater 34(45):2202929. <https://doi.org/10.1002/adma.202202929>

Scheme 1

Scheme 1 is available in the Supplementary Files section.

Figures

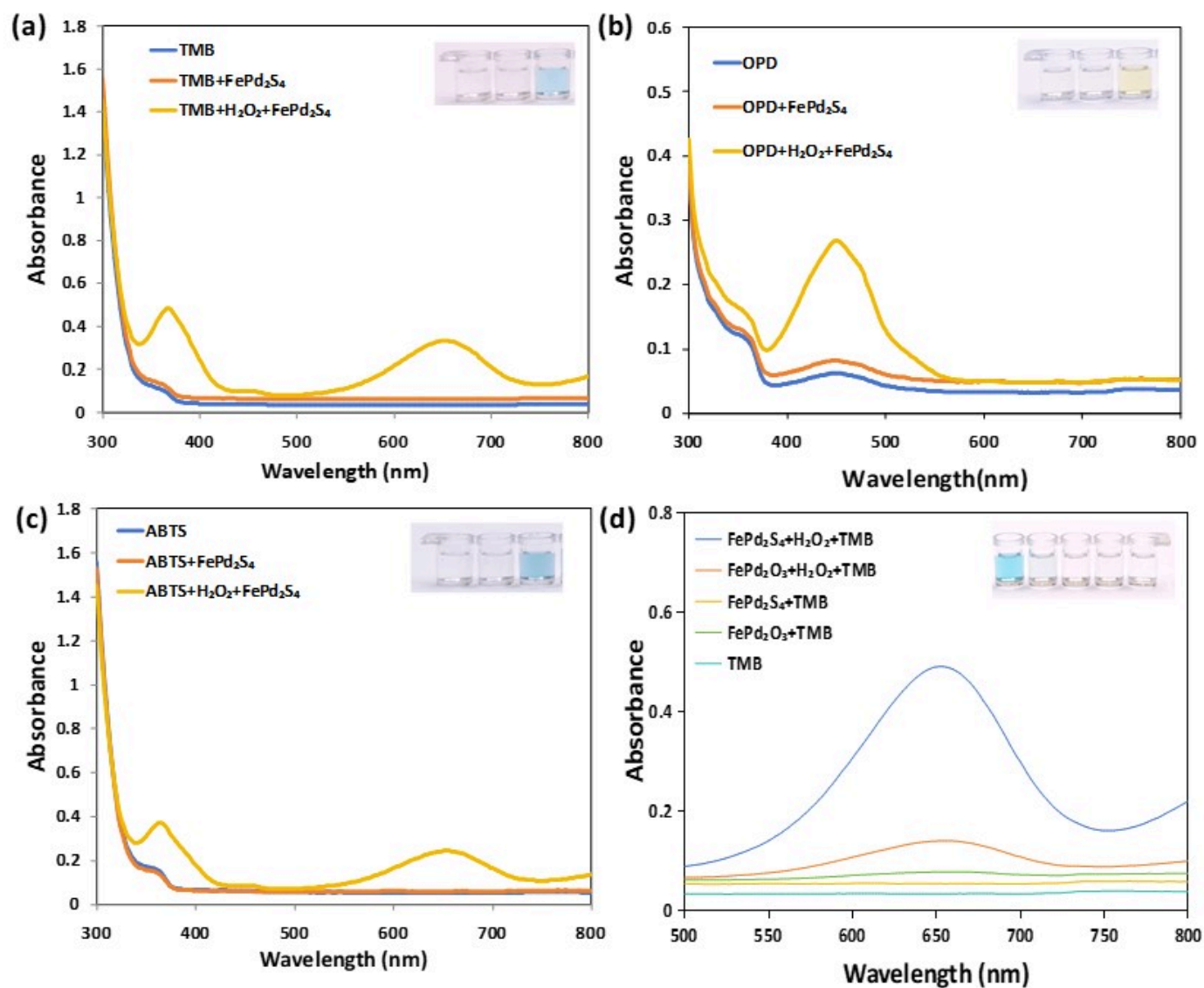


Figure 1

UV-vis absorption spectra of (a)TMB, (b) OPD, (c)ABTS after reaction with FePd₂S₄ in the absence and presence of H₂O₂. (d) UV-vis absorption spectra of TMB after reaction with two nanozyme in the absence

and presence of H_2O_2 (Inset: the photographs of the corresponding solution systems).

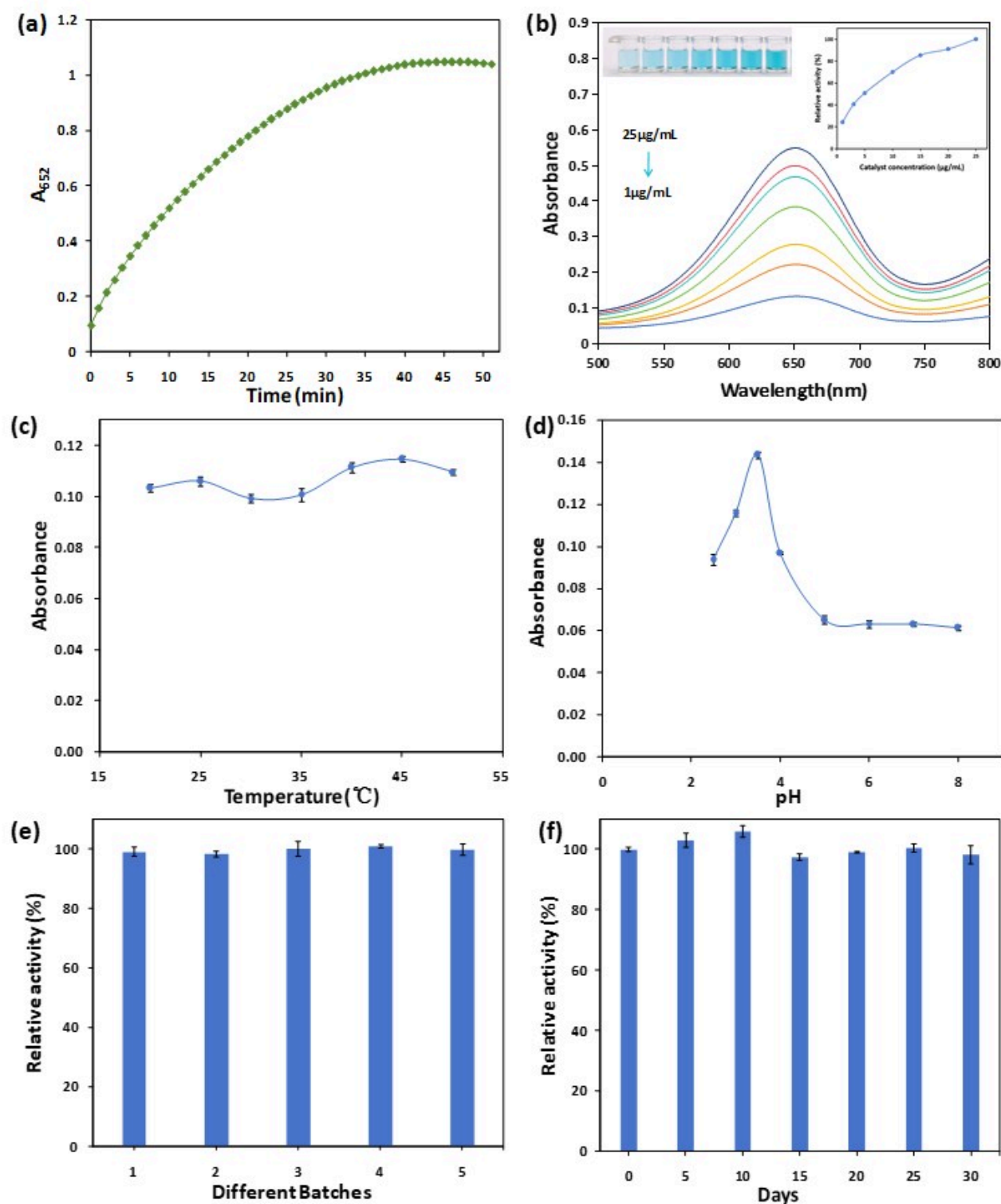


Figure 2
Effect of (a) reaction time, (b) concentration, (c) temperature, (d) pH, (e) different batches and (f) storage days on the POD-like activity of FePd_2S_4 .

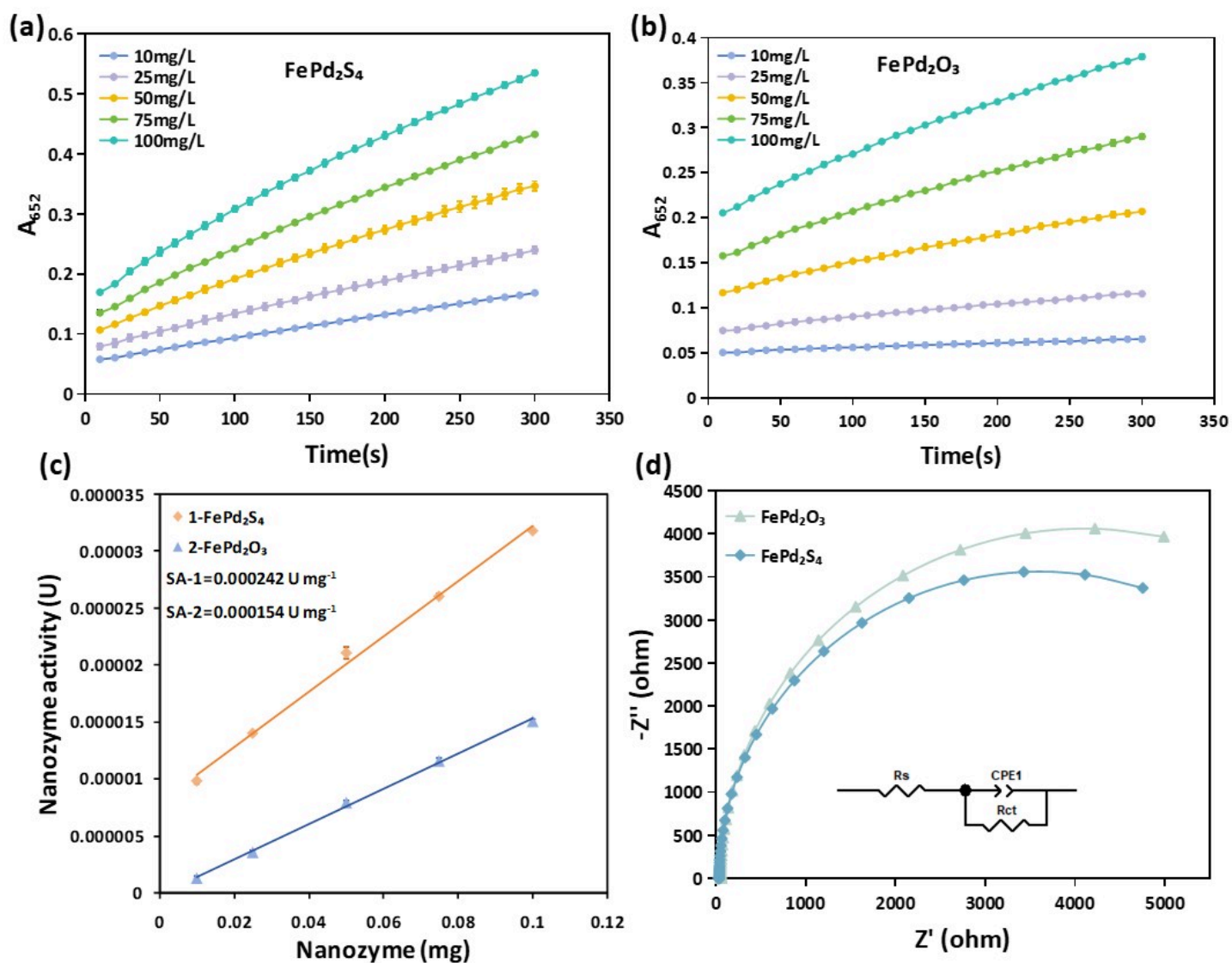


Figure 3

Reaction time curve of FePd₂S₄ (a) and FePd₂O₃ (b) on POD-like activity. (c) SA and (d) EIS curve (Inset: equivalent circuit model) of FePd₂S₄ and FePd₂O₃.

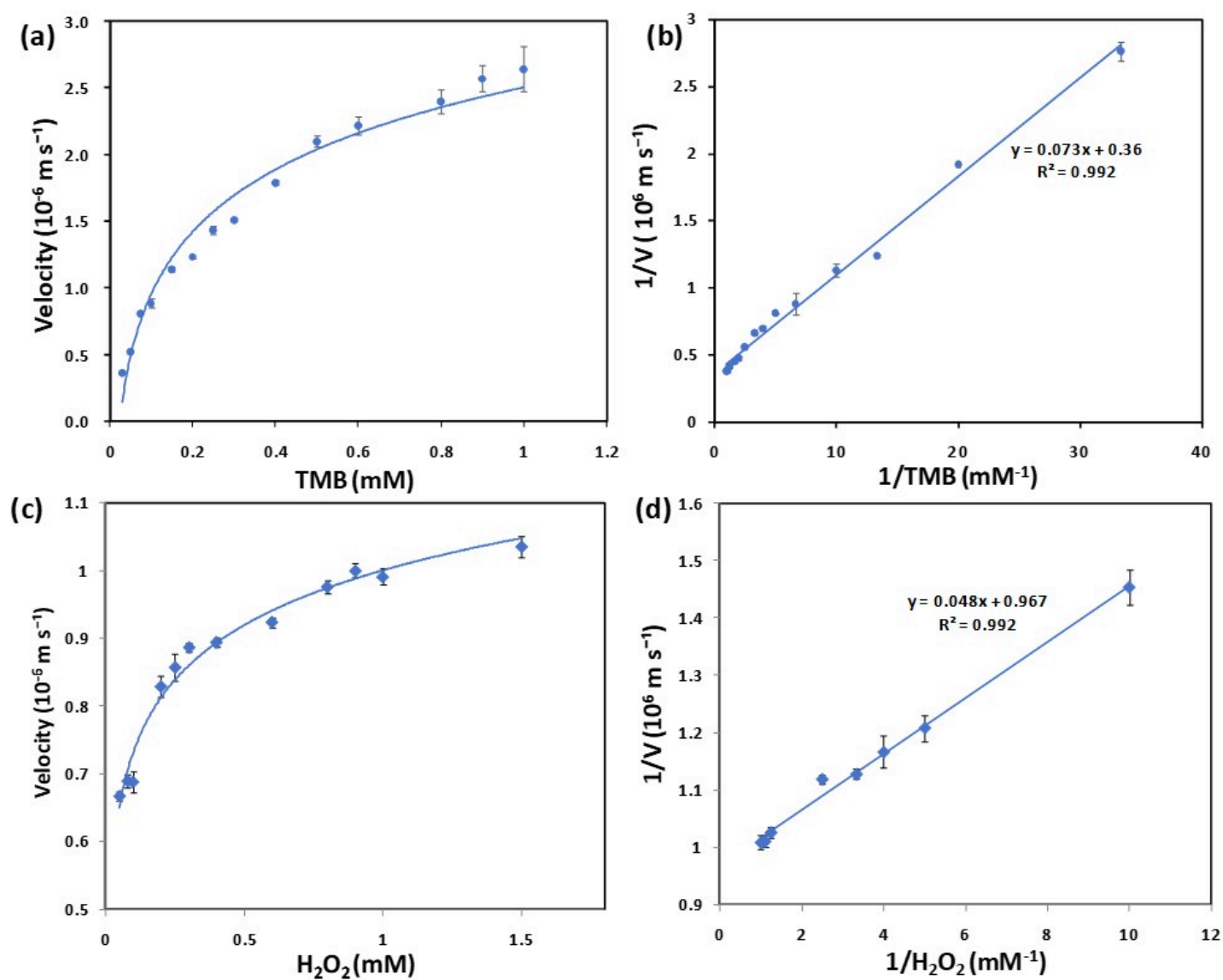


Figure 4

Michaelis-Menten curves of FePd₂S₄ POD-like activity with different concentrations of TMB (a) and H₂O₂ (c); the corresponding double-reciprocal plot with TMB (b) and H₂O₂ (d) as substrate.

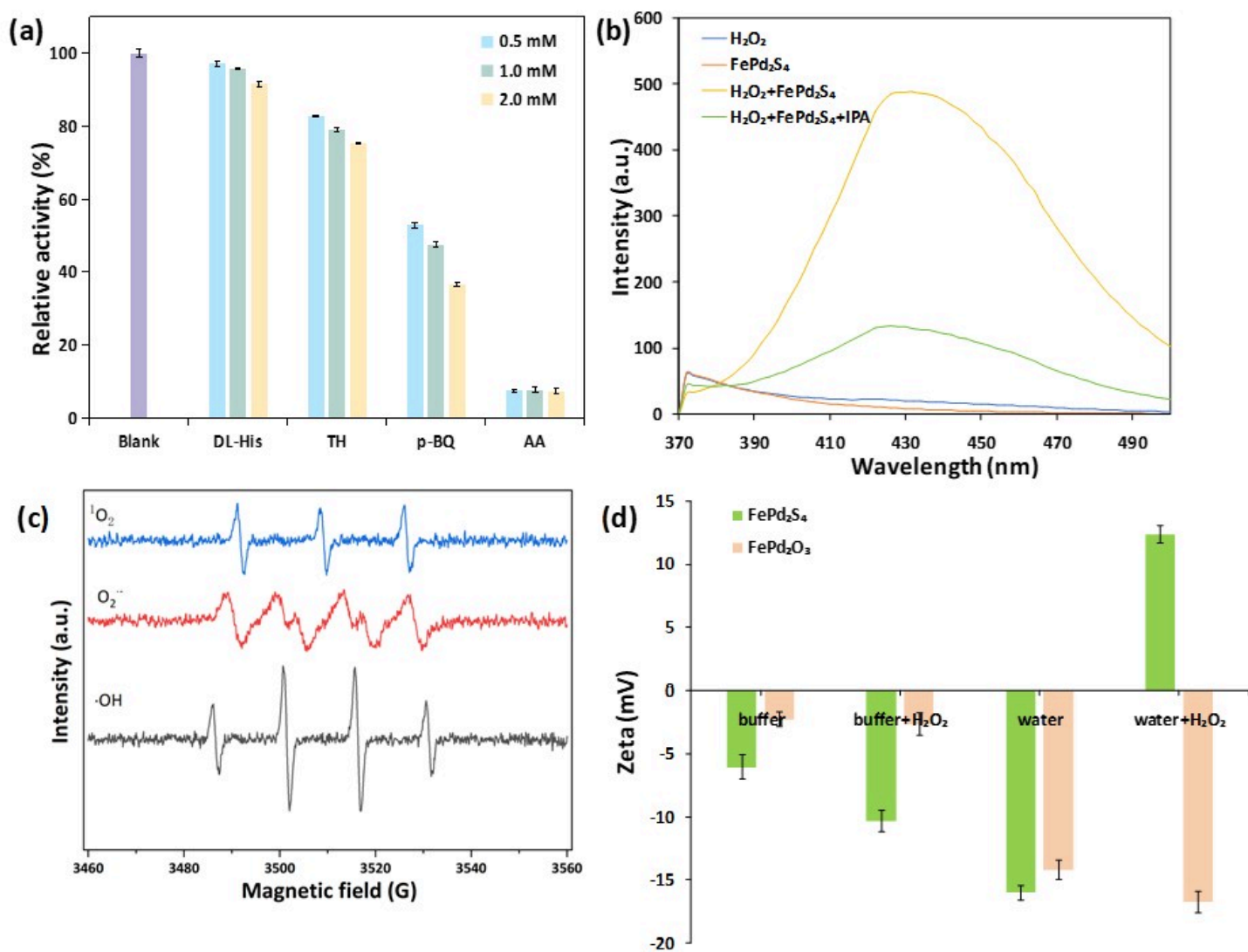


Figure 5

(a) Effect of different ROS scavengers on POD-like activity of FePd₂S₄. (b) Fluorescent spectra of PTA under different reaction systems. (c) The ESR spectrum of DMPO with ¹O₂, O₂^{•-} and •OH. (d) The Zeta potential of FePd₂S₄ and FePd₂O₃ suspensions in different solutions.

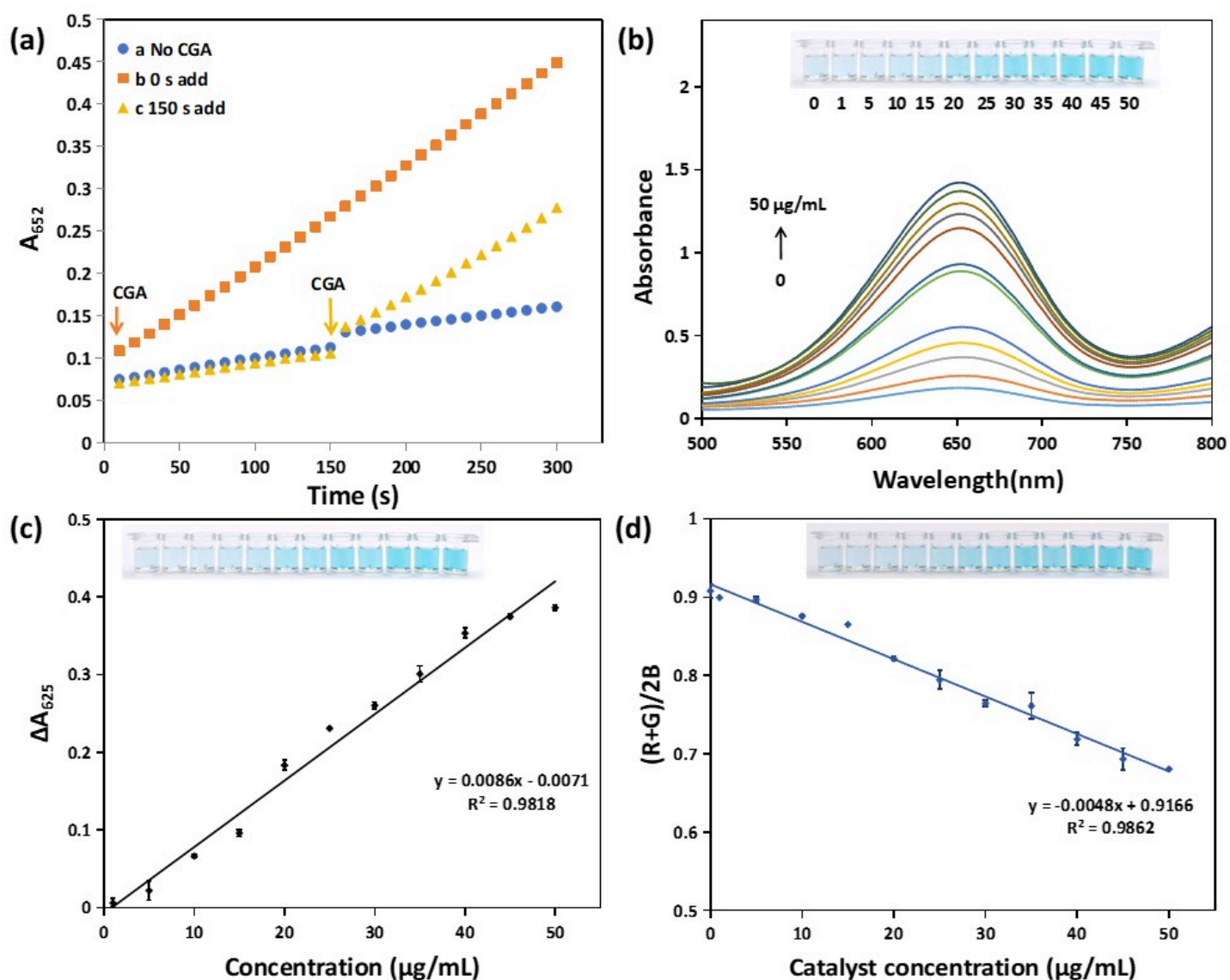


Figure 6

(a) Effect of CGA adding time on POD-like activity of FePd_2S_4 . (b) UV-vis spectra of TMB + FePd_2S_4 + H_2O_2 with different concentration of CGA; (c) Linear fitting curve between ΔA_{652} and CGA concentration; (d) Linear fitting curve between $(R+G)/2B$ values and CGA concentration (Inset: the photographs of the reaction solution with different concentration of CGA).

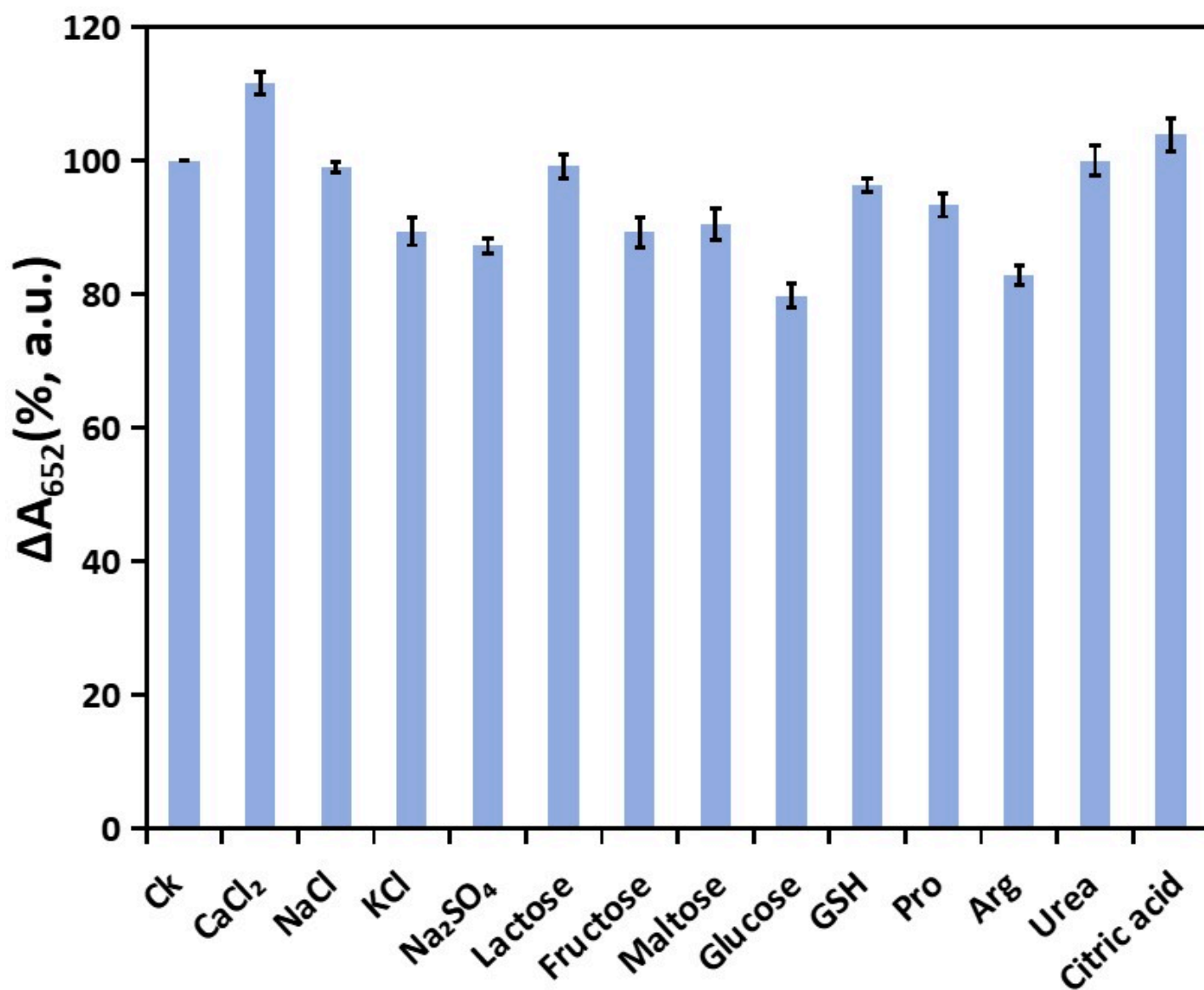


Figure 7

Effects of some coexisting substances on the POD-like activity of FePd₂S₄.

Supplementary Files

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- [2Supplementarymaterial260709.docx](#)
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