

A recognition-enhanced colorimetric sensor based on molecularly imprinted CoFe LDH nanozymes for detection of enrofloxacin in real water samples

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Research Article

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Abstract

The widespread occurrence of enrofloxacin (ENR) in aquatic environments poses potential risks to ecosystems and human health. To develop a simple and selective method for ENR detection, a colorimetric sensor was constructed by integrating molecularly imprinted polymers (MIPs) with cobalt iron layered double hydroxides (CoFe LDH). The MIPs@CoFe LDH composite was prepared using chitosan (CS) as the functional monomer and glutaraldehyde (GA) as the crosslinking agent. The imprinted cavities on MIPs@CoFe LDH selectively captured ENR molecules, thereby blocking the diffusion pathway of H_2O_2 to the active sites of CoFe LDH and inhibiting the generation of reactive oxygen species (ROS). As a result, the catalytic oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) was suppressed, generating a quantifiable colorimetric response. The proposed sensor exhibited a linear detection range of 0.19–50 μM for ENR with a detection limit of 0.03 μM . The method was further applied to river water samples, achieving recoveries with acceptable relative standard deviations (RSDs). This study provides a molecularly imprinted nanozyme-based approach for the selective detection of ENR in complex aquatic environments.

1 Introduction

Enrofloxacin (ENR) exhibits broad-spectrum antibacterial activity against Gram-positive, Gram-negative, and anaerobic microorganisms, and is widely used in the clinical treatment of respiratory tract, urinary tract, and skin and soft tissue infections [1]. However, its extensive production and excessive use have led to its emergence as an important pharmaceutical contaminant. In intensive livestock farming, the large-scale use of ENR results in elevated concentrations in animal manure and wastewater, while the direct discharge of untreated aquaculture effluents has become an important source of environmental pollution [2, 3]. Owing to its high environmental persistence and slow biodegradation, ENR can accumulate throughout the food chain, thereby posing potential risks to human health [4, 5]. Therefore, the development of a rapid and direct method for ENR determination in wastewater is of considerable importance. At present, chromatographic techniques are the primary methods for the quantitative analysis of trace ENR in wastewater [6]. Although these techniques provide favorable analytical accuracy, their reliance on sophisticated instrumentation, highly trained personnel, and labor-intensive, time-consuming procedures inevitably results in high analytical costs [7]. Consequently, developing a cost-effective and simplified strategy for ENR detection in river water samples is highly desirable.

Recently, nanozymes, as artificial enzyme mimics, have attracted widespread attention because of their cost-effectiveness, tunable catalytic activity, and excellent stability. They have been extensively applied to the detection of ions [8, 9], glucose [10–12], organophosphorus pesticides [13, 14], and other analytes [15, 16]. However, these sensing strategies generally suffer from poor selectivity toward structural analogues and often require analyte-specific recognition mechanisms, which substantially limits their broader application. Therefore, achieving highly selective detection while expanding the range of detectable analytes has become a critical challenge. The development of molecular imprinting technology (MIT) provides a promising solution to this issue. MIT is a biomimetic strategy for fabricating

molecularly imprinted polymers (MIPs) by mimicking antigen-antibody recognition [17–19]. After removal of the template molecules, three-dimensional imprinted cavities complementary to the template in shape and size are generated, endowing the MIPs with high molecular recognition selectivity [20–22]. Furthermore, integrating MIPs with colorimetric sensors offers a novel strategy to achieve selective recognition while theoretically broadening the scope of target analytes. Su et al. [23] integrated a Fe_3O_4 -Cu nanozyme with a chitosan (CS) based imprinted layer to achieve the selective detection of ketoprofen in animal-derived foods while effectively discriminating structurally similar compounds, including ibuprofen (IBU), bisphenol A (BPA), and dexamethasone (DXMS). Therefore, the integration of nanozymes with MIPs provides an effective approach for the highly selective detection of target analytes in complex matrices.

Inspired by these advances, we integrated MIT with nanozyme technology to achieve the selective detection of ENR in this study. The proposed colorimetric sensing strategy not only simplifies the analytical procedure but also provides an alternative strategy for selective nanozyme-based sensing. First, CoFe LDH with excellent peroxidase-like activity were synthesized via a hydrothermal method. Subsequently, using CoFe LDH as the support, ENR as the template molecule, and CS together with glutaraldehyde (GA) as the functional monomer and crosslinking agent, respectively, a molecularly imprinted layer was constructed. The resulting ENR-imprinted nanozyme (MIPs@CoFe LDH) possessed abundant imprinted cavities, which not only served as mass transfer channels for substrate diffusion toward the active sites of the nanozyme but also functioned as highly specific recognition sites for ENR. Upon selective capture of ENR, steric hindrance blocked the contact between H_2O_2 and the active sites of CoFe LDH, thereby suppressing the peroxidase-like catalytic reaction. Furthermore, the high selectivity of the proposed sensor toward ENR was systematically verified. Overall, its favorable performance in the analysis of river water samples demonstrates its considerable potential for the selective detection of ENR in complex environmental matrices.

2 Experimental section

2.1 Synthesis of CoFe LDH

CoFe LDH was synthesized via a hydrothermal method with slight modifications to a previously reported procedure [24]. Briefly, 600.6 mg of urea, 148 mg of NH_4F , 145.5 mg of $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, and 202.0 mg of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ were sequentially dissolved in 30 mL of deionized water. The resulting solution was stirred at room temperature for 10 min and then transferred into a 50 mL Teflon-lined stainless-steel autoclave. The hydrothermal reaction was conducted at 120°C for 6 h. After naturally cooling to room temperature, the product was collected by vacuum filtration, thoroughly washed with deionized water, freeze-dried at 60°C for 12 h, and finally collected as CoFe LDH.

2.2 Fabrication of MIPs@CoFe LDH

MIPs@CoFe LDH was fabricated using CoFe LDH, CS, and ENR as the support, functional monomer, and template molecule, respectively [25]. Briefly, 125 mg of CoFe LDH was ultrasonically dispersed in 25 mL of 2% acetic acid (HAc) solution containing 0.25 g of CS. Subsequently, 2.5 mL of methanol solution containing 79.5 mg of ENR was added to the suspension, followed by stirring at room temperature for 2 h. Next, 1.25 mL of 50% GA was slowly added dropwise to the mixture, which was further stirred for 1 h to obtain a gel material. The template ENR was then removed from the gel using a methanol/ HCl eluent ($V_{\text{MeOH}}:V_{\text{HCl}} (0.1 \text{ M}) = 1:1$) until the characteristic UV–vis absorption peak of ENR was no longer detected in the supernatant. Finally, the obtained MIPs@CoFe LDH was thoroughly washed with methanol, vacuum-dried at 60°C, and ground into powder for subsequent characterization. Non-imprinted polymers supported on CoFe LDH (NIPs@CoFe LDH) were prepared using the same procedure, except that ENR was omitted during the synthesis.

2.3 Evaluation of the peroxidase-like activity and adsorption behavior of CoFe LDH and MIPs@CoFe LDH

The peroxidase-like activities of CoFe LDH and the MIPs@CoFe LDH composites were evaluated using TMB and H_2O_2 as the reaction substrates. A typical catalytic reaction was carried out in a total volume of 2 mL containing 400 μL of CoFe LDH dispersion ($500 \text{ mg}\cdot\text{L}^{-1}$), 200 μL of NaAc-HAc buffer (0.2 M, pH 4.5), 100 μL of TMB solution (10 mM), and 100 μL of H_2O_2 solution (500 mM). After incubation at room temperature for 10 min, the reaction mixture was centrifuged, and the supernatant was collected for UV–vis spectral analysis over the wavelength range of 500–800 nm [11]. Quantitative analysis was performed based on the molar extinction coefficient of oxidized TMB (OxTMB) at 652 nm ($39,000 \text{ M}^{-1} \text{ cm}^{-1}$) [26].

Steady-state kinetic experiments were performed by varying the concentration of one substrate while maintaining the concentration of the other substrate constant. Specifically, the concentration of TMB was varied from 0.1 to 1.0 mM at a fixed H_2O_2 concentration of 25 mM, whereas the concentration of H_2O_2 was varied from 10 to 50 mM at a fixed TMB concentration of 0.5 mM. All other experimental conditions were identical to those used for the routine catalytic activity assay described above.

2.4 Colorimetric sensing performance of MIPs@CoFe LDH toward ENR

A total of 1.6 mL of ENR solution with concentrations ranging from 0 to 100 μM was added to a 5 mL centrifuge tube containing 200 μL of NaAc-HAc buffer (0.2 M, pH 4.5) and 2 mg of MIPs@CoFe LDH. After incubation at room temperature for 30 min, 100 μL of TMB solution (10 mM) and 100 μL of H_2O_2 solution (500 mM) were sequentially added to each tube. The reaction mixtures were further incubated for 20 min and then centrifuged. The supernatants were collected, and their UV–vis absorption spectra were recorded over the wavelength range of 500–800 nm [11].

2.5 Detection of ENR in real water samples

To evaluate the practical applicability of the proposed colorimetric sensing method, various environmental water samples were collected and analyzed. The real water samples included water from the Yangtze River system (collected from Danjiangkou Reservoir, Nanyang, China), water from the Yellow River (collected from Xiaolangdi Reservoir, Luoyang, China), water from Longyuan Lake (collected near Henan Polytechnic University, Jiaozuo, China), livestock wastewater sample 1 (collected from No. 3 Farm of Henan Muyuan Breeding Pig Breeding Co., Ltd., Nanyang, China), livestock wastewater sample 2 (collected from Qixian Muyuan Agriculture and Animal Husbandry Co., Ltd., Kaifeng, China), medical wastewater sample 1 (collected from Jiaozuo People's Hospital, Jiaozuo, China), and medical wastewater sample 2 (collected from The First Affiliated Hospital of Henan Polytechnic University, Jiaozuo, China). Prior to analysis, all water samples were filtered through a 0.22 μm membrane. The analytical procedure followed the colorimetric sensing protocol described in Section 2.6, except that the pretreated water samples were used in place of deionized water.

3 Results and discussion

3.1 Fabrication and characterization of MIPs@CoFe LDH

MIPs@CoFe LDH with selective recognition capability toward ENR was synthesized via a two-step strategy. In the first step, CoFe LDH with peroxidase-like catalytic activity was synthesized through a hydrothermal method (Fig. 1A). In the second step, ENR, CS, and GA were employed as the template molecule, functional monomer, and crosslinking agent, respectively, to graft an ENR-imprinted layer onto the surface of CoFe LDH via MIT at room temperature (Fig. 1B). After the template ENR was removed, specific imprinted cavities complementary to the spatial structure of ENR were generated on the surface of the MIPs@CoFe LDH composite. These cavities served as physical mass-transfer channels, facilitating the diffusion of substrate molecules (e.g., H_2O_2) to the active sites of the nanozyme.

The morphologies of CoFe LDH and the MIPs@CoFe LDH composite were characterized by SEM. As shown in Fig. 2A, CoFe LDH exhibited a uniform spherical morphology assembled from stacked layered nanosheets, with particle sizes ranging from 5–8 μm . After grafting the ENR-imprinted layer onto the surface of CoFe LDH, the particle contours became less distinct, accompanied by a noticeable increase in surface roughness and a slight increase in particle size to 7–10 μm (Fig. 2B). Following removal of the template ENR, the surface roughness of MIPs@CoFe LDH decreased slightly (Fig. 2C), which was mainly attributed to extraction of ENR from the imprinted layer by the organic solvent, thereby exposing the CoFe LDH surface within the imprinted cavities. These cavities not only provide physical mass-transfer channels for the diffusion of substrate molecules (e.g., H_2O_2) to the active sites of CoFe LDH but also serve as highly specific recognition sites through precise site matching and spatial confinement for ENR molecules.

The microstructures of CoFe LDH, MIPs@CoFe LDH, and NIPs@CoFe LDH were further characterized by transmission electron microscopy (TEM), and the corresponding results are presented in Fig. S1. As shown in Fig. S1A and B, CoFe LDH exhibited a well-defined stacked lamellar architecture, characteristic

of layered double hydroxides. After coating the CoFe LDH surface with an ENR-templated molecularly imprinted polymer layer, the originally distinct layered morphology became blurred (Fig. S1C and D), while a uniform polymer shell was formed around the CoFe LDH substrate, accompanied by increased surface roughness and particle size, confirming the successful grafting of the imprinted layer. In contrast, the non-imprinted material, NIPs@CoFe LDH (Fig. S1E and F), also possessed a uniform polymer coating but lacked the specific cavities generated by template removal. Compared with MIPs@CoFe LDH, the surface of NIPs@CoFe LDH was smoother and contained no recognition cavities. After removal of the template ENR, numerous specific imprinted cavities were exposed on the surface of MIPs@CoFe LDH. These cavities not only establish efficient physical mass-transfer pathways for the diffusion of substrate molecules (e.g., H_2O_2) to the active sites of CoFe LDH but also provide spatially confined binding sites with favorable size matching for the selective recognition of ENR.

The particle size distributions of CoFe LDH and the MIPs@CoFe LDH composites were analyzed using a laser particle size analyzer (Fig. 2D–F). The equivalent particle size of CoFe LDH was determined to be 300–800 nm, whereas SEM observations revealed spherical aggregates with diameters of 4–7 μm assembled from layered nanosheets. This discrepancy arises because the porous layered structure leads to an underestimation of particle size by the light-scattering algorithm [27]. Moreover, the low polydispersity index ($\text{PDI} < 0.2$) indicates excellent size uniformity and negligible aggregation after dispersion in the aqueous phase [28]. For MIPs@CoFe LDH, the SEM-observed particle size was 5–8 μm , whereas the equivalent particle size determined by laser diffraction ranged from 400 to 1600 nm. The overall increase in particle size can be attributed to the formation of a CS–GA–ENR imprinted layer with an estimated thickness of approximately 100–800 nm on the particle surface. Its slightly higher PDI than that of CoFe LDH is associated with minor variations in the thickness of the imprinted layer. Similarly, NIPs@CoFe LDH exhibited a particle size of 5–8 μm by SEM and an equivalent particle size of 600–1700 nm by laser diffraction, intermediate between those of CoFe LDH and MIPs@CoFe LDH. Owing to the absence of ENR-derived imprinted cavities, the CS–GA non-imprinted layer possessed a relatively higher density and greater thickness (approximately 300–1100 nm).

The surface charges of CoFe LDH and the MIPs@CoFe LDH composites were characterized by zeta potential analysis. CoFe LDH exhibited a positive surface charge, which can be attributed to the intrinsic positive charge originating from the high-valence metal cations ($\text{Co}^{2+}/\text{Fe}^{3+}$) within the LDH layers [29]. In contrast, both NIPs@CoFe LDH and MIPs@CoFe LDH displayed weak negative surface charges, which can be ascribed to the deprotonation of surface hydroxyl groups under alkaline conditions ($\text{Co/Fe-OH} \rightarrow \text{Co/Fe-O}^-$) [30]. As shown in Fig. S2, CoFe LDH exhibited the highest positive zeta potential ($\zeta > 17$ mV), indicating superior colloidal stability in aqueous media. In contrast, both MIPs@CoFe LDH and NIPs@CoFe LDH showed zeta potentials close to 0 mV with weak negative charges ($|\zeta| < 5$ mV). This behavior can be attributed to the CS coating, whose partially protonated amino groups neutralize part of the surface charge of LDH, while the organic polymer layer further reduces the surface charge density [30]. The nearly identical zeta potentials of the two composites indicate that the surface charge is

predominantly governed by the CS–GA imprinted layer rather than by the presence or absence of the ENR template.

The pore structures and specific surface areas of CoFe LDH and the MIPs@CoFe LDH composites were investigated by N₂ adsorption–desorption measurements. As shown in Fig. 2G–I, all three materials exhibited typical type IV adsorption–desorption isotherms with pronounced hysteresis loops at $P/P_0 > 0.4$, indicating the presence of mesoporous structures. The pore size distributions were mainly concentrated within the mesoporous range (2–50 nm), with dominant pore diameters of 2–5 nm (20–50 Å), confirming the formation of mesoporous channels by the stacked layered architecture [31]. CoFe LDH possessed a specific surface area of $11.3719 \text{ m}^2 \text{ g}^{-1}$, with its pore volume and pore size mainly originating from interlayer spacing and interparticle voids, resulting in a relatively compact surface. In contrast, the specific surface area of MIPs@CoFe LDH increased significantly to $21.0312 \text{ m}^2 \text{ g}^{-1}$, approximately twice that of CoFe LDH, accompanied by an increased pore volume. This enhancement is attributed to the formation of ENR-specific imprinted cavities (approximately 2–5 nm, matching the molecular dimensions of ENR) after template removal, together with the porous organic framework. The enlarged specific surface area provides abundant recognition sites for the selective adsorption of ENR while simultaneously facilitating the mass transfer of substrate molecules to the active sites of CoFe LDH [31]. NIPs@CoFe LDH exhibited a specific surface area of $17.1108 \text{ m}^2 \text{ g}^{-1}$, intermediate between those of CoFe LDH and MIPs@CoFe LDH. Its CS–GA crosslinked layer generated only randomly distributed pores without specific recognition cavities, resulting in a broader pore size distribution (12–18 nm) and a lower pore volume. These results demonstrate that the ENR template-induced imprinted cavities are primarily responsible for the enhanced specific surface area of MIPs@CoFe LDH. Although both composites retained the mesoporous characteristics of CoFe LDH, partial filling of the mesoporous channels by the organic polymer layer led to a slight decrease in overall porosity.

To confirm the successful fabrication of the prepared materials, FT-IR spectroscopy was employed to characterize their functional groups (Fig. 3A). For CoFe LDH, a broad and intense absorption band centered at 3420 cm^{-1} was observed, which was assigned to the O–H stretching vibrations of hydroxyl groups (Co/Fe–OH) in the LDH layers and interlayer water molecules, confirming the typical hydrated layered double hydroxide structure [32, 33]. In addition, a prominent absorption peak at approximately 1350 cm^{-1} was attributed to the asymmetric stretching vibration of CO_3^{2-} , indicating that atmospheric CO_2 was incorporated into the interlayer galleries during synthesis to form carbonate-intercalated LDH (CO_3^{2-} -LDH) [24]. In the low-frequency region ($< 600 \text{ cm}^{-1}$), the continuous absorption bands at 557 and 466 cm^{-1} were assigned to Co/Fe–O lattice vibrations and M–O–M bending vibrations, respectively, confirming the formation of the layered framework. Based on the successful synthesis of CoFe LDH, the FT-IR spectra of MIPs@CoFe LDH and NIPs@CoFe LDH were further analyzed. The spectrum of NIPs@CoFe LDH retained the characteristic absorption bands of the Co/Fe–O lattice vibration, M–O–M bending vibration, asymmetric stretching vibration of CO_3^{2-} , and O–H stretching vibration arising from Co/Fe–OH groups and interlayer water molecules. In addition, characteristic absorption bands of CS

corresponding to C–H and C–O stretching vibrations appeared at 2900 and 1062 cm^{-1} , respectively [32–34]. Furthermore, the absorption peak at 1630 cm^{-1} was assigned to the C = N bond formed via the Schiff base reaction between CS and GA [35], demonstrating the successful fabrication of NIPs@CoFe LDH. Compared with NIPs@CoFe LDH, the FT-IR spectrum of MIPs@CoFe LDH contained all of the characteristic peaks observed for NIPs@CoFe LDH, indicating that the two materials possessed similar chemical compositions and functional groups [36]. Moreover, additional characteristic absorption bands at 1700, 1500, and 1300 cm^{-1} , corresponding to the C = O, C = C, and C–F bonds of ENR, respectively, were observed in the spectrum of MIPs@CoFe LDH, confirming the successful incorporation of the template molecule ENR into the molecularly imprinted layer on the CoFe LDH surface.

The crystal structure of CoFe LDH was characterized by X-ray diffraction (XRD) (Fig. 3B). As shown in the diffraction pattern, characteristic diffraction peaks appeared at 2θ values of 11.7°, 23.5°, 33.5°, 38.2°, 45.9°, and 58.0°, which correspond well to the (003), (006), (012), (015), (018), and (110) crystal planes of hexagonal cobalt–iron hydroxide carbonate ($\text{Co}_{5.84}\text{Fe}_{2.16}(\text{OH})_{16}(\text{CO}_3)_{1.08} \cdot 0.32\text{H}_2\text{O}$, PDF#00-050-0235) [24, 37]. The well-resolved diffraction peaks corresponding to the (003) and (006) planes indicate that the synthesized CoFe LDH possessed a highly ordered layered crystal structure [38]. XRD analysis of MIPs@CoFe LDH and the non-imprinted counterpart (NIPs@CoFe LDH) further demonstrated that both composites retained the characteristic diffraction peaks of CoFe LDH, indicating that the molecular imprinting modification did not disrupt the crystalline framework of the LDH substrate. XPS was subsequently employed to investigate the surface elemental composition, chemical valence states, functional groups, and elemental distribution of the prepared materials. According to the XPS results (Fig. 3C–G), the coexistence of $\text{Co}^{2+}/\text{Co}^{3+}$ and $\text{Fe}^{2+}/\text{Fe}^{3+}$ species was confirmed in CoFe LDH. The deconvolution of the O 1s spectrum into three components further verified the integrity of the layered structure, with the three peaks corresponding to lattice metal–oxygen bonds, hydroxyl oxygen, and carbonate oxygen, respectively. In the C 1s spectrum, the peaks located at approximately 284.8 eV and 288.8 eV were assigned to adventitious carbon and carbonate carbon, respectively, further confirming the presence of interlayer carbonate anions [37, 39, 40]. The thermal stability of the prepared materials was evaluated by TGA (Fig. 3H). The results demonstrated that CoFe LDH exhibited excellent thermal stability, with a mass loss of less than 5% when the temperature increased to 200°C.

3.2 Evaluation of the peroxidase-like activity and adsorption behavior of CoFe LDH and MIPs@CoFe LDH

First, the peroxidase-like properties of CoFe LDH and MIPs@CoFe LDH were evaluated based on the catalytic oxidation of colorless TMB to blue OxTMB in the presence of H_2O_2 . The peroxidase-mimicking performance of the materials was systematically investigated according to the ability of the colorless TMB substrate to be catalytically oxidized into its blue oxidation product in the presence of H_2O_2 [41]. As shown in Fig. 4A, when only TMB or both TMB and H_2O_2 were present in the reaction system, the UV–vis absorption of the solution was negligible, and the solution remained colorless. After CoFe LDH was introduced into the system containing TMB and H_2O_2 , a distinct absorption peak corresponding to

OxTMB appeared at 652 nm (Fig. 4A), accompanied by an obvious blue color visible to the naked eye (inset of Fig. 4A). This phenomenon was attributed to the partial oxidation of TMB into its blue product OxTMB under the catalysis of peroxidase-like CoFe LDH [35].

To further elucidate the catalytic behavior of CoFe LDH, its steady-state kinetics toward H_2O_2 were systematically investigated under steady-state conditions. A typical Michaelis–Menten curve was obtained by varying the H_2O_2 concentration while maintaining a constant TMB concentration (Fig. 4B). The kinetic parameters were further analyzed using a Lineweaver–Burk plot (Fig. 4C). For H_2O_2 , the K_m value of CoFe LDH was lower than that of natural horseradish peroxidase (HRP) [42], indicating that CoFe LDH exhibited a higher apparent affinity toward H_2O_2 under the tested conditions. This behavior may be associated with the favorable interfacial environment provided by the Fe/Co bimetallic sites for H_2O_2 adsorption and activation. The V_{max} of CoFe LDH was slightly higher than that of HRP, suggesting a higher maximum reaction rate. For the chromogenic substrate TMB, the K_m value of CoFe LDH was also lower than that of HRP, demonstrating its stronger apparent affinity toward TMB, which provides a favorable basis for constructing a sensitive colorimetric detection system.

To identify the ROS involved in the CoFe LDH-catalyzed oxidation of TMB, the effects of different radical scavengers on TMB oxidation were investigated and further verified by electron paramagnetic resonance (EPR) spectroscopy (Fig. 4D–E). Methanol (MeOH) exhibits strong quenching ability toward both $\text{SO}_4^{\cdot-}$ ($k = 2.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$) and $\cdot\text{OH}$ ($k = 9.7 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$), whereas tert-butanol (TBA) mainly scavenges $\cdot\text{OH}$ radicals ($k = 3.8\text{--}7.6 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$) [43]. As shown in Fig. 4D, the TMB oxidation rate decreased to 67.5% in the presence of MeOH, while the addition of TBA further reduced the oxidation rate to 56.9%. The corresponding kinetic rate constants (Fig. S3) decreased from 0.0204 min^{-1} to 0.0075 min^{-1} and 0.0065 min^{-1} , respectively. These results indicate that $\cdot\text{OH}$ plays a more important role than $\text{SO}_4^{\cdot-}$ in TMB oxidation. Considering that furfuryl alcohol (FFA) has a strong scavenging effect toward $^1\text{O}_2$ ($k = 1 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$) [44], the effect of FFA addition on the TMB oxidation rate was further examined. In the presence of FFA, the oxidation rate decreased to 70%, and the k value decreased to 0.0079 min^{-1} . These results suggest that the activity order of radical species in the radical pathway follows $\cdot\text{OH} > ^1\text{O}_2 > \text{SO}_4^{\cdot-}$. When p-benzoquinone (p-BQ) was used as a scavenger for $\text{O}_2^{\cdot-}$ ($k = 1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) [44], the oxidation rate decreased to 81.7%, and the k value decreased to 0.0167, indicating that $\text{O}_2^{\cdot-}$ also participated in the TMB oxidation process. To further verify the ROS present in the catalytic system, EPR spectroscopy was introduced (Fig. 4E). DMPO and TEMP were used as spin traps for radical species ($\text{SO}_4^{\cdot-}$, $\cdot\text{OH}$, and $\text{O}_2^{\cdot-}$) and non-radical species ($^1\text{O}_2$), respectively. As shown in Fig. 4E, the DMPO– $\cdot\text{OH}$ adduct displayed a characteristic signal intensity ratio of 1:2:2:1. The TEMP– $^1\text{O}_2$ adduct exhibited a distinct triplet signal with an intensity ratio of 1:1:1. These results confirm the involvement of $\cdot\text{OH}$ and $^1\text{O}_2$ in the catalytic system.

After clarifying the intrinsic enzyme-like activity and catalytic mechanism of CoFe LDH, the influence of the molecularly imprinted layer on the catalytic behavior was further investigated. To directly compare the differences in peroxidase-like activity among the materials, the UV-vis spectra of reaction systems containing CoFe LDH, MIPs@CoFe LDH (before ENR elution), MIPs@CoFe LDH (after ENR elution), and NIPs@CoFe LDH were recorded (Fig. 4F). When MIPs@CoFe LDH before ENR elution was used instead of CoFe LDH as the catalyst, no obvious blue color was observed by the naked eye, although a weak absorption peak at 652 nm could be detected by UV-vis spectrophotometry; however, the colorimetric intensity was much lower than that of CoFe LDH. After ENR was eluted from the surface of MIPs@CoFe LDH, the reaction system recovered an obvious blue color, and the colorimetric intensity increased significantly, although it remained lower than that of CoFe LDH (Fig. 4F). This phenomenon can be attributed to the fact that the active CoFe LDH component in MIPs@CoFe LDH was covered by the molecularly imprinted layer, while the imprinted channels and cavities generated after ENR elution allowed the substrates (H_2O_2) to access the active CoFe LDH core, thereby retaining partial catalytic activity. Based on these observations, it can be concluded that the essential function of the imprinted cavities is to act as physical mass-transfer channels for H_2O_2 diffusion toward the active sites of CoFe LDH. Once the target ENR is specifically adsorbed and occupies these cavities, the mass-transfer pathway of H_2O_2 to the active sites is physically blocked, resulting in a sharp decrease in the generation of $\cdot\text{OH}$ and $^1\text{O}_2$ radicals catalyzed by CoFe LDH and ultimately leading to significant inhibition of the TMB oxidation color reaction (Fig. 4G). This mechanism is consistent with the conclusion from the radical scavenging experiments (Fig. 4D–E), in which $\cdot\text{OH}$ and $^1\text{O}_2$ were identified as the dominant reactive species. To verify this conclusion, NIPs@CoFe LDH without ENR in the molecularly imprinted layer was prepared as a control. In sharp contrast, NIPs@CoFe LDH, lacking imprinted channels and cavities that enable substrate diffusion to the active sites, could barely initiate the chromogenic reaction of TMB, and its catalytic activity was negligible. Overall, the peroxidase-mimicking activities of the four materials followed the order: CoFe LDH > MIPs@CoFe LDH (after ENR elution) > MIPs@CoFe LDH (before ENR elution) > NIPs@CoFe LDH. In addition, to verify that the decrease in absorbance was indeed caused by ENR occupying the imprinted cavities and blocking the substrate mass-transfer channels, the UV-vis spectral changes induced by MIPs@CoFe LDH after ENR elution before and after incubation in ENR solution were independently compared (Fig. S4). The results further support the proposed mass-transfer blocking mechanism. Based on the above observations, the core conclusion can be drawn that the imprinted cavities on the surface of MIPs@CoFe LDH are not only specific recognition sites for ENR but also mass-transfer channels for H_2O_2 to access the active centers of CoFe LDH.

The adsorption process of the materials toward the target analyte was investigated through dynamic adsorption experiments. The results showed that the adsorption capacity Q_t gradually increased with increasing adsorption time. However, when the adsorption time reached 30 min, the growth rate of Q_t markedly slowed and eventually approached a stable adsorption equilibrium (Fig. S5A). In addition, comparative results showed that the adsorption capacity of MIPs@CoFe LDH was significantly higher than that of NIPs@CoFe LDH. This difference was attributed to the presence of specific imprinted

cavities on the surface of MIPs@CoFe LDH that are complementary to the spatial structure of the template molecule ENR, thereby endowing the material with stronger selective adsorption capability toward the target analyte.

To further clarify the underlying mechanism of the adsorption process, the dynamic adsorption data were fitted using the pseudo-first-order kinetic model (Fig. S5B) and pseudo-second-order kinetic model (Fig. S5C). The corresponding kinetic equations are shown in Equations 1 and 2. According to the correlation coefficients (R^2), the adsorption processes of both MIPs@CoFe LDH and NIPs@CoFe LDH fitted well with the pseudo-second-order kinetic model. This result indicates that the adsorption of the target analyte by both materials is dominated by chemisorption, and that the adsorption rate is mainly controlled by chemical interactions between the adsorbent and the adsorbate.

$$\ln(Q_e - Q_t) = \ln Q_e - k_1 t$$

1

$$\frac{t}{Q_t} = \frac{1}{k_2 Q_e^2} + \frac{1}{Q_e} t$$

2

Q_e and Q_t represent the adsorption capacities at equilibrium and at different adsorption times, respectively, while k_1 and k_2 denote the rate constants of the pseudo-first-order and pseudo-second-order kinetic models, respectively.

The adsorption performance of MIPs@CoFe LDH and NIPs@CoFe LDH toward the template molecule ENR was systematically investigated and compared through static adsorption experiments. The results showed that the equilibrium adsorption capacity (Q_e) of both materials gradually increased with increasing ENR concentration. When the ENR concentration reached 200 μM , the Q_e of MIPs@CoFe LDH reached its maximum value of 230 $\mu\text{M g}^{-1}$. NIPs@CoFe LDH exhibited a similar adsorption trend to MIPs@CoFe LDH, but its equilibrium adsorption capacity was significantly lower, with a maximum value of only 47 $\mu\text{M g}^{-1}$. The Q_e values of MIPs@CoFe LDH were consistently higher than those of NIPs@CoFe LDH, mainly owing to the presence of specific imprinted cavities on the surface of MIPs@CoFe LDH that are spatially complementary to ENR molecules. These cavities selectively bind ENR through molecular recognition, thereby substantially enhancing the adsorption capacity. The static adsorption results confirm that MIPs@CoFe LDH exhibits superior adsorption performance compared with NIPs@CoFe LDH, providing an important foundation for constructing a highly selective colorimetric sensor for ENR.

3.3 Optimization of MIPs@CoFe LDH performance

To further improve the specific recognition capability and sensing response of MIPs@CoFe LDH toward ENR, the material synthesis parameters and sensing conditions were systematically optimized according

to the method described in Section 2.5. First, the thickness of the imprinted layer was directly regulated by adjusting the amount of CS. When the CS amount was insufficient, the resulting imprinted layer was too thin, allowing the substrate H_2O_2 to readily penetrate the imprinted layer and reach the active CoFe LDH core, thereby reducing the selectivity of the nanozyme-catalyzed reaction. Conversely, excessive CS produced an overly thick imprinted layer, which not only hindered the recognition and binding of the target molecule ENR but also restricted its mass transfer to the active sites. In addition, an excessively thick imprinted layer severely impeded the diffusion of substrate molecules (e.g., H_2O_2) to the active centers of CoFe LDH, resulting in an extremely low background signal (A_0) in the absence of ENR. Under this condition, the ΔA lost an effective comparison baseline and response sensitivity because A_0 was too small. The experimental results showed that when the optimal mass ratio of CoFe LDH to CS was 1:2.5, the absorbance difference between the MIPs@CoFe LDH + H_2O_2 + TMB systems with and without ENR reached its maximum (Fig. S6A). Since the amount of the template molecule ENR is closely related to the number of specific cavities in the imprinted layer, its influence on material performance was further investigated. As the mass ratio of CoFe LDH to ENR increased, the number of specific cavities and mass-transfer channels formed in the imprinted layer increased, and the peroxidase-like catalytic activity of MIPs@CoFe LDH gradually improved. However, when the ENR amount was further increased, the thickness of the imprinted layer also increased, making complete template removal difficult and consequently reducing the specific recognition capability and catalytic activity of MIPs@CoFe LDH toward ENR (Fig. S6B). The optimal mass ratio of CoFe LDH to ENR was therefore determined to be 4:1 and was used for the subsequent preparation of MIPs@CoFe LDH. To achieve highly sensitive and selective ENR detection, the sensing conditions were further optimized. The results showed that the absorbance difference was most pronounced when the MIPs@CoFe LDH dosage was 0.1 mg mL^{-1} (Fig. S6C). To balance sensing sensitivity with analysis time, the incubation time between the imprinted nanozyme and the substrates was set to 8 min (Fig. S6D). For pH optimization, considering the complex composition and limited buffering capacity of natural river water samples, as well as the need for operational simplicity in portable on-site detection, the selected pH should balance catalytic efficiency with practical environmental applicability. In addition, CoFe LDH nanozymes generally exhibit optimal peroxidase-like activity under weakly acidic conditions (pH 4.5–5.5). Based on these considerations, the effect of pH on the sensing response was systematically investigated. Similar to other peroxidase mimics [45, 46], the MIPs@CoFe LDH-catalyzed chromogenic reaction occurred most effectively under moderately acidic conditions (pH 5.0), where the most sensitive response toward ENR was obtained (Fig. S6E). For temperature optimization, although an appropriate increase in temperature can enhance molecular diffusion and catalytic reaction rates, an excessively high reaction temperature is unfavorable for rapid on-site analysis of real samples and may increase both operational energy consumption and nonspecific background reactions. By comparing the absorbance differences between the two systems at different ambient temperatures, the reaction temperature was finally set to 35°C (Fig. S6F).

3.4 Evaluation of the colorimetric detection performance of MIPs@CoFe LDH toward ENR

MIPs@CoFe LDH prepared under the optimized conditions was used as a colorimetric sensor for the specific detection of ENR (Fig. 5A). The response of the sensing system toward ENR was evaluated using the ΔA , where A_0 and A represent the absorbance values in the absence and presence of ENR, respectively [47]. Under the optimal conditions determined in Section 3.3, the response of the TMB + H_2O_2 + MIPs@CoFe LDH system toward different ENR concentrations was systematically investigated.

The experimental observations and spectral data showed that the blue color of the system gradually faded with increasing ENR concentration, and the color change could be readily distinguished by the naked eye (Fig. 5B). Consistently, the UV-vis absorption signal of the system gradually decreased as the ENR concentration increased (Fig. 5B). Quantitative analysis revealed a good linear relationship between the absorbance at 652 nm and the ENR concentration over the range of 0.19–50 μM (Fig. 5C). Based on the signal-to-noise criterion of $S/N = 3$, the limit of detection (LOD) of the colorimetric sensor for ENR was calculated to be 0.03 μM . Accordingly, a colorimetric method for the quantitative detection of ENR was established. Compared with previously reported ENR detection methods [48–54], the proposed colorimetric sensor exhibited a relatively wide linear range. Although the detection limit of this method is not superior to those of certain fluorescence- and mass spectrometry-based techniques, it fully satisfies the requirements for detecting ENR residues in real river water samples, demonstrating promising practical applicability.

CIP, DIF, TTC, and FLO were selected as structural or functional analogues of ENR, and their chemical structures are shown in Table 1. The selectivity of MIPs@CoFe LDH toward ENR was systematically evaluated by investigating the effects of these analogues on the absorbance of the colorimetric sensing system (Fig. 5D). At the same concentration (10 μM), ENR and its analogues exerted relatively minor effects on the CoFe LDH-based colorimetric sensing system. However, after introduction of the molecularly imprinted layer, the ΔA induced by ENR was significantly higher than those induced by the other analogues, owing to the presence of specific imprinted cavities on the surface of MIPs@CoFe LDH that matched the molecular size and spatial structure of ENR. This result indicates that the molecularly imprinted layer not only enhanced the response sensitivity toward ENR but also endowed MIPs@CoFe LDH with highly selective recognition capability. In contrast, in the colorimetric sensing system constructed with NIPs@CoFe LDH, no significant difference in ΔA was observed between ENR and the other analogues, further confirming the critical role of the imprinted cavities. The underlying mechanism can be explained as follows: ENR is selectively adsorbed and bound within the imprinted cavities of MIPs@CoFe LDH through molecular recognition, thereby blocking the mass-transfer pathway of H_2O_2 toward the nanozyme active centers and ultimately decreasing the absorbance of the system. In contrast, the other analogues, whose molecular structures and sizes do not match the imprinted cavities, are difficult to access the cavities and thus exert minimal influence on the absorbance response. These results demonstrate the excellent selectivity of MIPs@CoFe LDH for complex sample analysis. In addition, the specificity of the MIPs@CoFe LDH colorimetric sensor was assessed by introducing common interfering substances into the system (Fig. 5E). Although the concentrations of the interfering substances were set at ten times that of ENR, their effects on the ΔA were significantly lower than that

of ENR itself. This result further confirms the excellent specific recognition capability of MIPs@CoFe LDH toward ENR and supports its practical application in complex sample matrices. Moreover, the regeneration and reusability of MIPs@CoFe LDH were investigated. An HAc–H₂O solution (5:95, v/v) was used as the eluent to efficiently remove the adsorbed ENR from the material surface, enabling regeneration of the molecularly imprinted nanozyme. After five regeneration cycles, MIPs@CoFe LDH still retained its capability for specific recognition and detection of ENR without significant deterioration in sensing performance (Fig. 5F), confirming its good stability and reusability and further enhancing its practical application value.

3.5 Evaluation of detection performance in real samples

To demonstrate the practical applicability of the proposed method, the ENR concentrations in river water, livestock wastewater, and medical wastewater samples were determined (Table 1). The method exhibited excellent performance for the determination of ENR in both native and spiked samples, with low relative standard deviations (RSDs) (0.36%–3.43%) and satisfactory recoveries (98.6%–104%). These results demonstrate the high accuracy and reliability of the proposed colorimetric sensing method. Figure 5G compares the performance of the proposed method with those of the current mainstream methods for trace ENR determination, demonstrating its strong competitiveness.

Table 1
ENR detection results in various environmental water samples.

Samples	Spiked (μM)	Found (μM)	Recovery (%)	RSD (%)
River water sample	0	-	-	-
	0.5	0.498 ± 0.012	98.6	3.2
	5	4.96 ± 0.16	99.7	2.99
	50	50.8 ± 0.36	102.6	0.87
Livestock wastewater sample	0	-	-	-
	0.5	0.492 ± 0.013	98.4	2.65
	5	4.97 ± 0.16	99.4	3.22
	50	51.5 ± 0.22	103	0.43
Medical wastewater sample	0	-	-	-
	0.5	0.498 ± 0.012	99.6	2.41
	5	4.95 ± 0.17	99.0	3.43
	50	50.7 ± 0.20	101.4	0.39

4 Conclusions

In this study, MIPs were integrated with a CoFe LDH nanozyme under mild conditions to develop a highly selective colorimetric sensing platform for ENR detection. CS and GA were employed as the functional monomer and crosslinking agent, respectively, to fabricate MIPs@CoFe LDH. The resulting molecularly imprinted layer preserved the intrinsic peroxidase-like activity of CoFe LDH while introducing abundant imprinted cavities. These cavities not only specifically recognized ENR but also served as mass-transfer channels between the chromogenic substrates and the active sites of CoFe LDH. Upon specific recognition of ENR, the occupied imprinted cavities physically blocked the diffusion pathway of H_2O_2 toward the active sites of CoFe LDH through a steric exclusion effect, thereby suppressing the nanozyme-catalyzed colorimetric reaction. Based on this mechanism, highly selective discrimination of ENR from its structural analogues was achieved. The constructed MIPs@CoFe LDH-based colorimetric sensor exhibited a linear detection range of 0.19–50 μM with a LOD of 0.03 μM . The sensor was applied to the analysis of real water samples, yielding satisfactory recoveries and low RSDs. The colorimetric sensor developed in this work provides a promising strategy for the selective detection of ENR in river water samples and offers a novel perspective for developing selective sensing platforms for other environmental contaminants.

Declarations

Associated Content

Supporting Information

Additional experimental details, material characterizations, peroxidase-like activity evaluation, sensing-condition optimization, and real-water-sample detection data. Figures S1–S6 (PDF).

CRediT authorship contribution statement

Chunyan Xiao: Conceptualization, Funding acquisition, Writing–review and editing. **Zhihang Guo:** Methodology, Data curation, Formal analysis, Writing–original draft preparation. **Yunlong Jiu:** Investigation. Dongxue Lu: Visualization. **Yubo Yao:** Data curation. **Baihan Li:** Validation. **Dongyang Wang:** Funding acquisition, Formal analysis, Project administration, Writing–review and editing.

Declarations of generative AI and AI-assisted technologies

No generative artificial intelligence or AI-assisted tools were utilized at any stage throughout the preparation of this manuscript. All textual composition, data analysis, graphical illustration design, as well as manuscript revision and refinement work were independently completed by the authors without any AI-based auxiliary support.

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 statement

Data will be made available on request.

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Figures

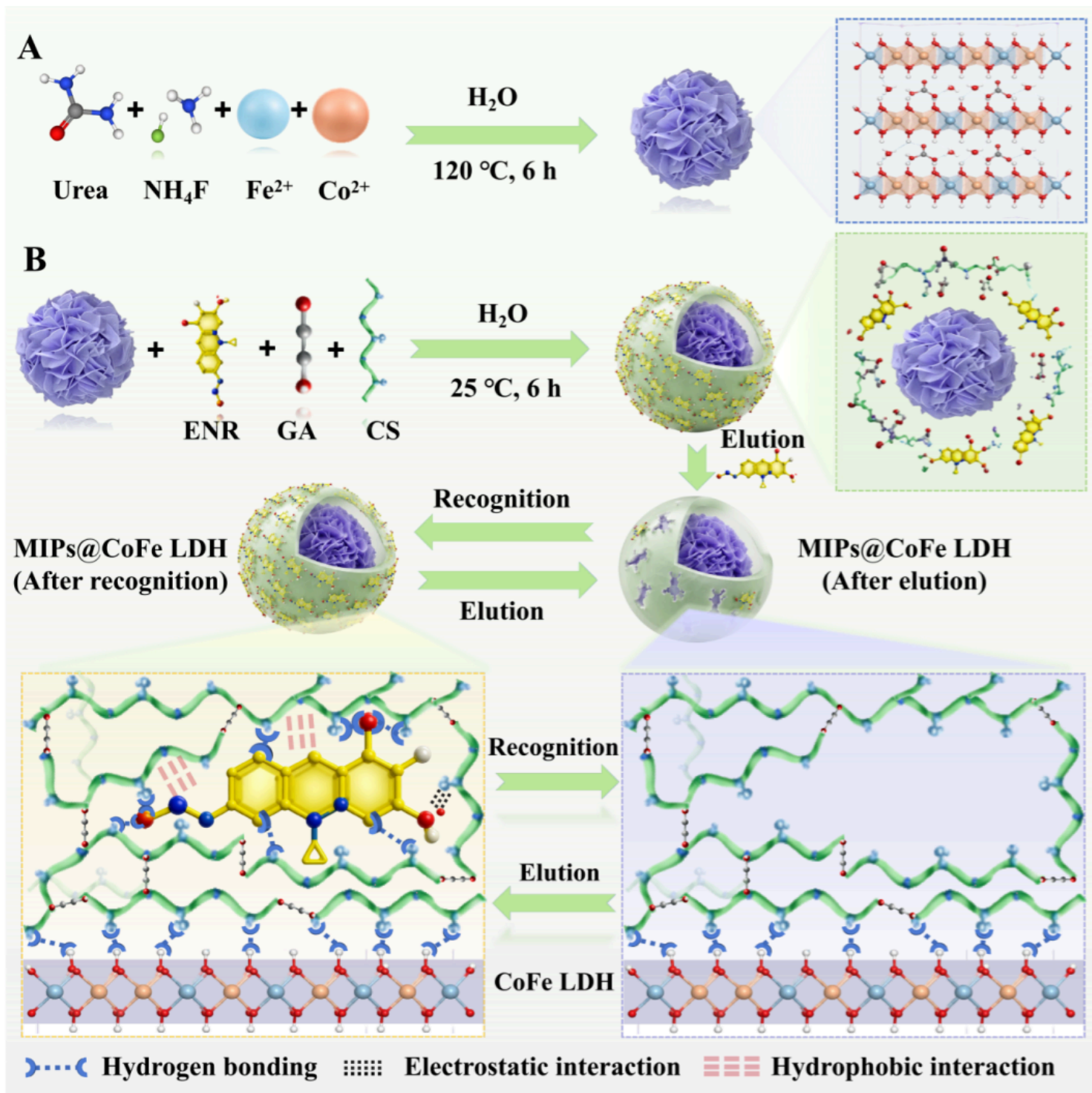


Figure 1

(A) Schematic illustration for the synthesis of CoFe LDH, (B) Schematic diagram of the construction process of MIPs@CoFe LDH.

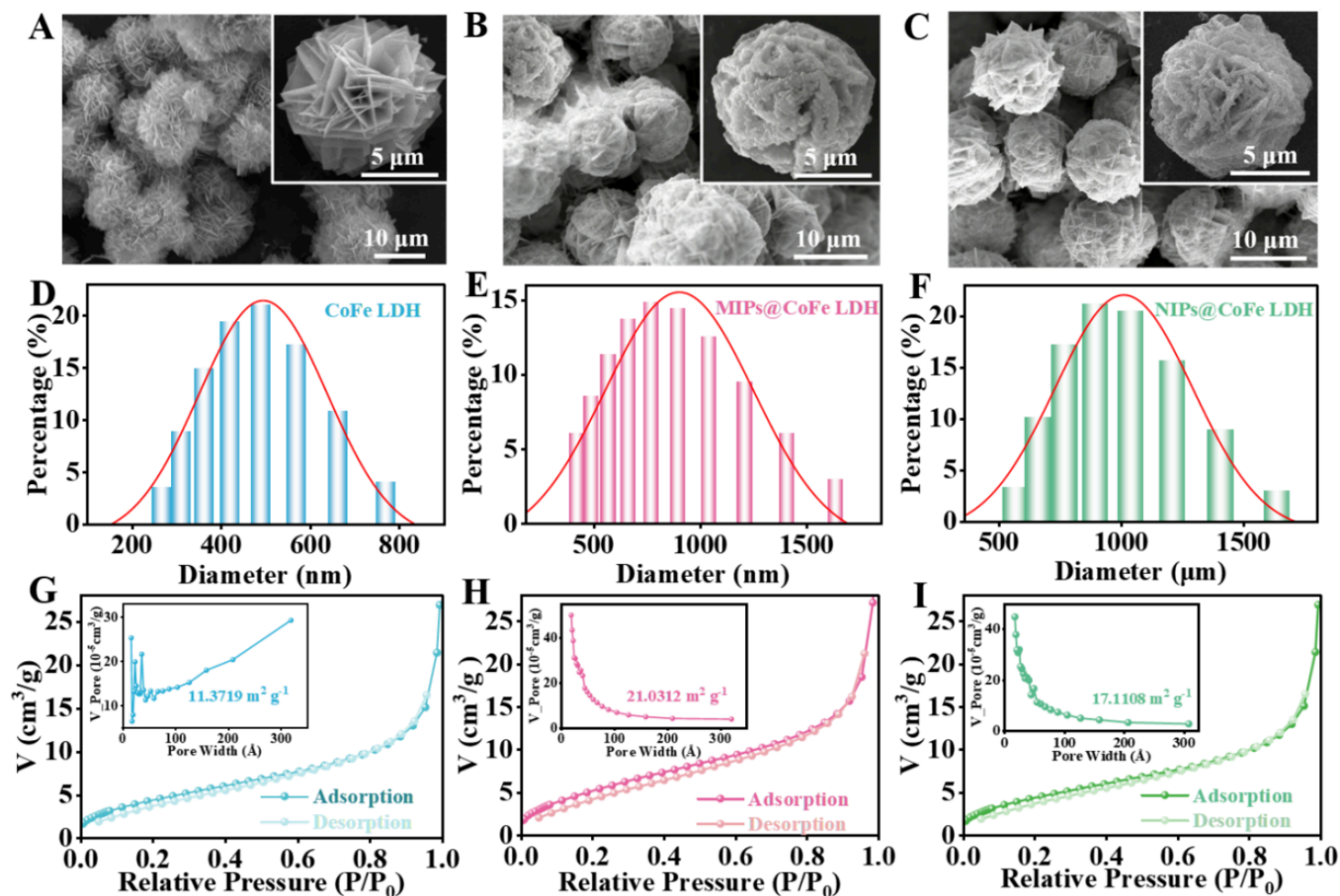


Figure 2

SEM images of (A) CoFe LDH, (B) ENR-loaded MIPs@CoFe LDH and (C) eluted MIPs@CoFe LDH, Particle size distribution curves of (D) CoFe LDH, (E) eluted MIPs@CoFe LDH and (F) NIPs@CoFe LDH, N₂ adsorption–desorption isotherms and corresponding pore size distribution profiles of (G) CoFe LDH, (H) eluted MIPs@CoFe LDH and (I) NIPs@CoFe LDH.

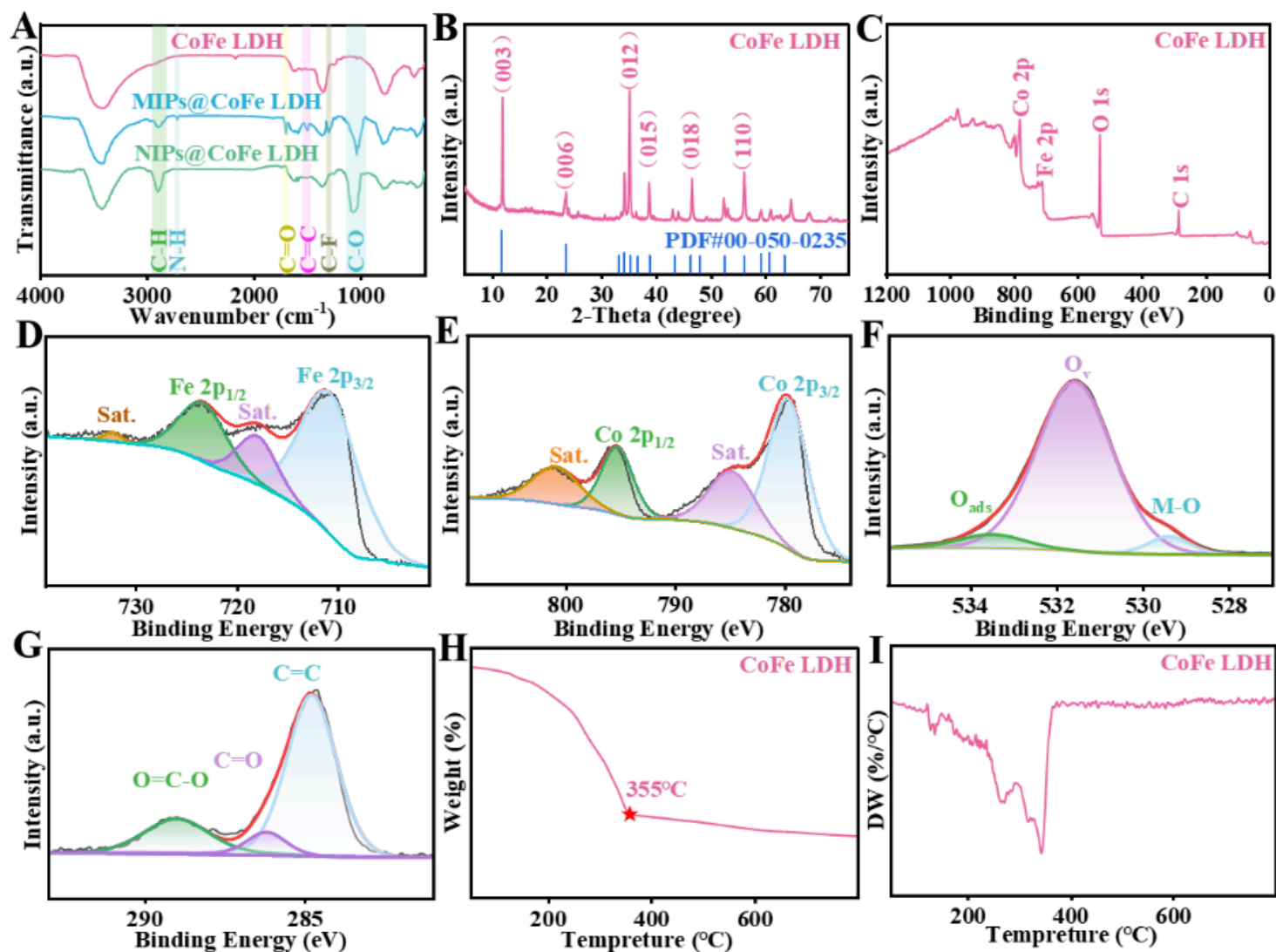


Figure 3

(A) FT-IR spectra of CoFe LDH, MIPs@CoFe LDH, and NIPs@CoFe LDH, For CoFe LDH: (B) XRD pattern, (C) survey XPS spectrum, (D) high-resolution Fe 2p XPS spectrum, (E) high-resolution Co 2p XPS spectrum, (F) high-resolution O 1s XPS spectrum, (G) high-resolution C 1s XPS spectrum, (H) TG curve, and (I) DTG curve.

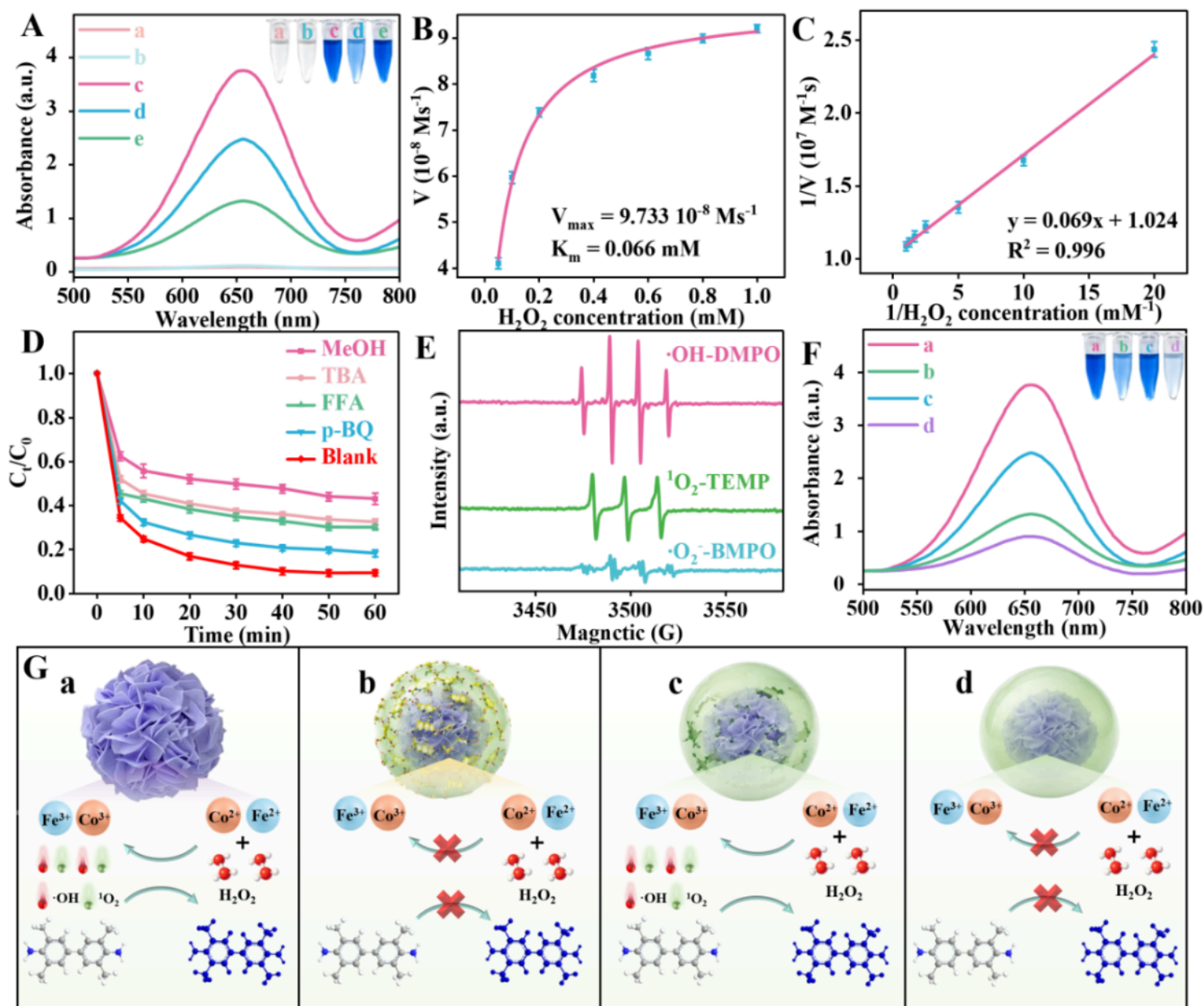


Figure 4

(A) UV-vis absorption spectra of various reaction systems, Inset: digital photographs of corresponding systems, where a: TMB, b: TMB + H₂O₂, c: MIPs@CoFe LDH (ENR-uneluted) + TMB + H₂O₂, d: CoFe LDH + TMB + H₂O₂, e: MIPs@CoFe LDH (ENR-eluted) + TMB + H₂O₂. (B) Michaelis–Menten kinetic curve toward H₂O₂, (C) Lineweaver–Burk plot for H₂O₂, (D) Effects of various radical scavengers on the oxidation efficiency of TMB, CoFe LDH = 100 mg L⁻¹, pH = 7.0, ENR = 100 μM, MeOH = TBA = 40 mM, BQ = FA = 5 mM, (E) EPR spectra of CoFe LDH, (F) UV-vis absorption spectra of different catalytic systems. Inset: digital photographs of the corresponding samples, where a: CoFe LDH + TMB + H₂O₂, b: MIPs@CoFe LDH (ENR-uneluted) + TMB + H₂O₂, c: MIPs@CoFe LDH (ENR-eluted) + TMB + H₂O₂, d: NIPs@CoFe LDH (ENR-eluted) + TMB + H₂O₂, (G) Schematic illustration of the catalytic chromogenic mechanism of TMB oxidation for a: CoFe LDH, b: MIPs@CoFe LDH (ENR-uneluted), c: MIPs@CoFe LDH (ENR-eluted), d: NIPs@CoFe LDH.

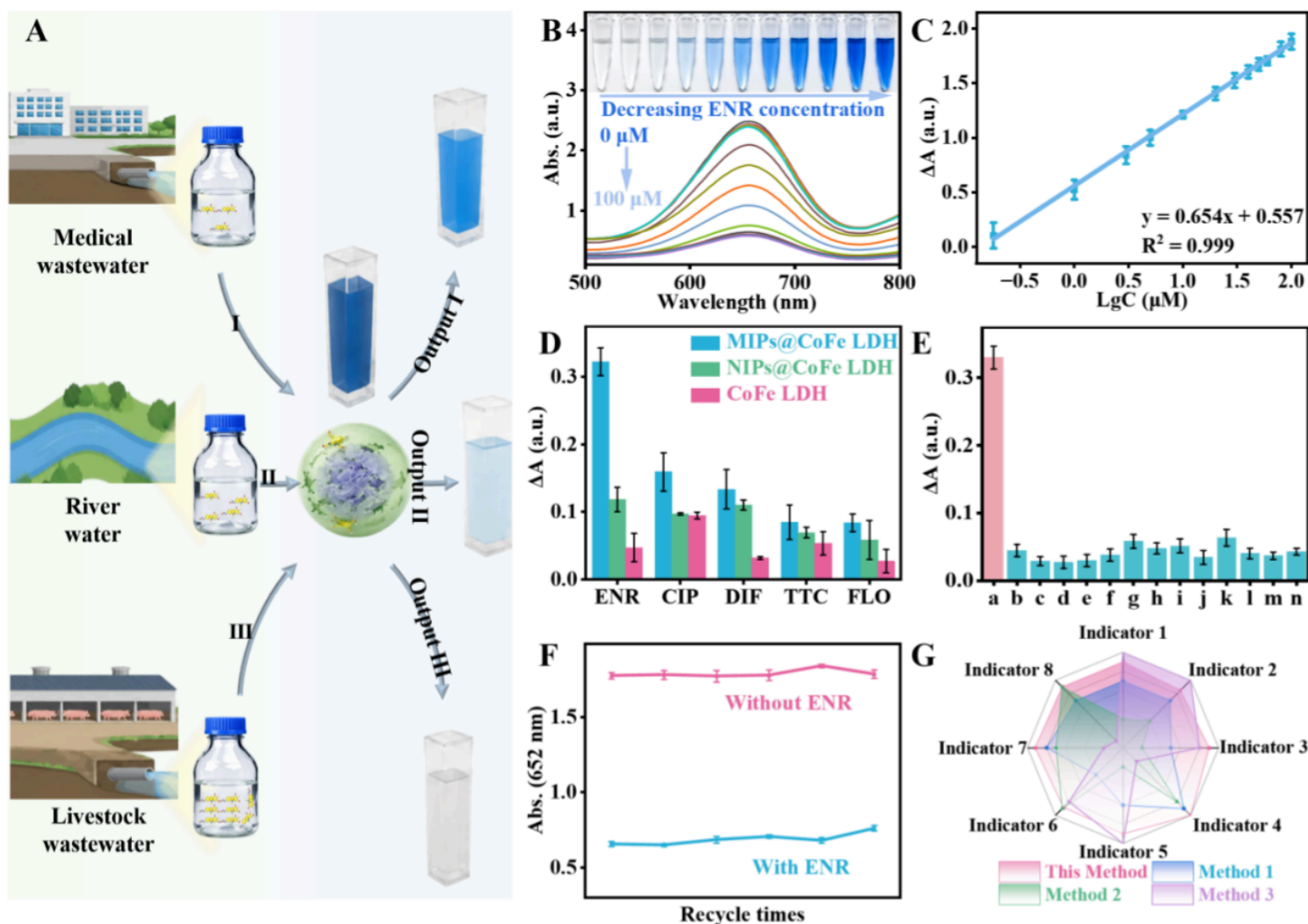


Figure 5

(A) Schematic workflow for real sample analysis, (B) UV-vis absorption spectra of the MIPs@CoFe LDH colorimetric sensing system with various concentrations of ENR, inset: corresponding visual photographs under different ENR concentrations. (C) Absorbance variation curves of oxTMB for the MIPs@CoFe LDH sensing system containing different ENR concentrations, with the inset displaying the linear calibration plot of ΔA versus $\lg C$, (D) ΔA values of the colorimetric system triggered by 10 μM ENR and its structural analogues over MIPs@CoFe LDH, NIPs@CoFe LDH and pristine CoFe LDH catalysts, (E) Anti-interference performance of the sensing assay toward various interfering substances, where a: ENR, b: Na^+ , c: K^+ , d: Ca^{2+} , e: Mg^{2+} , f: Al^{3+} , g: Zn^{2+} , h: Ba^{2+} , i: Cu^{2+} , j: NH_4^+ , k: CO_3^{2-} , l: HCO_3^- , m: SO_4^{2-} , n: Br^- , (F) Recyclable sensing performance of MIPs@CoFe LDH. (G) Comparison diagram of the merits of the proposed colorimetric sensor versus conventional analytical detection methods, where Indicator 1: LOD, Indicator 2: Linear range, Indicator 3: Anti-interference, Indicator 4: Response time, Indicator 5: Selectivity, Indicator 6: Stability, Indicator 7: Sample pretreatment, Indicator 8: Detection cost, Method 1: Conventional UV-vis spectrophotometry, Method 2: LC-MS/MS, Method 3: Electrochemical sensing.

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