

RESEARCH ARTICLE

A colorimetric detection of dopamine in urine and serum based on the CeO₂@ZIF-8/Cu-CDs laccase-like nanozyme activity

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Abstract

This study reports a sensitive and selective colorimetric approach for the analysis of dopamine (DA) based on CeO₂@ZIF-8/Cu-CDs laccase-like nanozymes activity. The CeO₂@ZIF-8/Cu-CDs was synthesized using cerium oxide (CeO₂) and copper-doped carbon dots (Cu-CDs) with 2-methylimidazole by a facile hydrothermal approach. The CeO₂@ZIF-8/Cu-CDs exhibited excellent laccase-like nanozymes activity and can oxidize the colorless substrate (DA) to red product with 4-aminoantipyrine as the chromogenic agent. The Michaelis–Menten constant (K_m) and the maximal velocity (V_{max}) of CeO₂@ZIF-8/Cu-CDs are 0.20 mM and 1.48 μ M/min, respectively. The detection method has a linear range of 0.05–7.5 μ g/mL and a detection limit as low as 8.5 ng/mL with good reproducibility. The developed colorimetric sensor was applied to rapid and precise quantitative evaluation of DA levels in serum and urine samples. This study presents a new approach for detecting biological molecules by utilizing the controlled regulation of nanozymes' laccase-like activity.

KEYWORDS

CeO₂@ZIF-8/Cu-CDs nanozymes, colorimetric detection, dopamine

1 | INTRODUCTION

Dopamine (DA) is an essential neurotransmitter of the central nervous system (CNS) [1, 2]. It can control a wide range of brain functions and mental activities of the brain's CNS, including perception, emotion, behavior, and movement [3]. Disorders of DA in the body can lead to diseases such as schizophrenia and Parkinson's disease [4–6]. The determination of DA concentrations is critical for the study of physiological processes and the application of clinical research [7, 8]. Furthermore, the use of hard drugs such as opium, cocaine, heroin, codeine, and morphine, as well as doping agents, alcohol and tobacco, has been shown to increase DA release in the human body [9–12]. Hence, it is imperative to promptly determine the concentration of DA in human body fluids.

The currently reported assays include ultraviolet (UV)-visible spectrophotometry [13], high-performance liquid chromatography (HPLC) [14], electrochemical methods [15], capillary electrophoresis [16], chemiluminescence [17], and so forth. Although these techniques are accurate, they are often time-consuming, lack sensitivity, or require complex equipment. Hence, the development of a cost-effective, rapid and reliable technique for the quantification of minute quantities of DA in body fluids, such as blood and urine, continues to pose a significant obstacle.

When Gao et al. [18] first discovered intrinsic enzymes that mimic the activity of ferromagnetic nanoparticles, nanomaterials with enzyme-like properties were referred to as 'nanoenzymes.' Currently, a variety of nanomaterials have been discovered to have enzyme-like properties. These include noble metal nanoparticles [19], metal oxides [20], carbon nanotubes [21], graphene and its derivatives [22], metal-organic frameworks [23–25], and several others. Nanozymes

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are a type of artificial enzyme with outstanding characteristics such as high catalytic activity, high thermal and chemical stability, easy production, and long stability [26]. Because of their multiple advantages, nanozymes are widely considered feasible alternatives to natural enzymes in a variety of applications, including sensors, imaging, therapies, and disease diagnosis [27].

The catalytic activity of nanozymes with laccase-mimicking capabilities is widely recognized to be similar to that of natural laccase. These nanozymes exhibit the capacity to catalyze the oxidation of diverse aryl diamines, phenols, and polyamines in the absence of hydrogen peroxide, thus confirming their designation as environmentally friendly catalysts [28, 29]. Laccase-like nanozymes, being regarded as environmentally friendly catalysts, exhibit tremendous potential in a variety of industries, including textiles, environmental remediation, and pollutant detection [30–32]. The field of nanozyme research is now growing and presents a unique and promising approach for further investigation.

In this research, $\text{CeO}_2\text{@ZIF-8/Cu-CDs}$ nanomaterial was synthesized by a three-step hydrothermal method. The prepared $\text{CeO}_2\text{@ZIF-8/Cu-CDs}$ nanoparticles exhibited superior laccase-mimicking performance. Subsequently, in the presence of 4-aminoantipyrine (4-AP), a colorimetric biosensor for detection of DA based on $\text{CeO}_2\text{@ZIF-8/Cu-CDs}$ nanoparticles was used for the detection of trace amounts of DA in serum and urine samples with satisfactory results.

2 | EXPERIMENTAL

2.1 | Chemicals

Cerium nitrate hydrate ($\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$), copper chloride ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$), zinc nitrate hydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$), hydrogen peroxide, citrate, dopamine hydrochloride were purchased from Macklin Company (Shanghai, China). Aqueous ammonia, 4-AP, glucose, urea, uric acid, 2-methylimidazole, and cholesterol were obtained from Xi Long Scientific Company (Shantou, China). All reagents were purchased from a local vendor and they were all of analytical grade. They were used without undergoing any additional pretreatment. Sodium phosphate and sodium dihydrogen phosphate solutions were combined following a standard procedure to prepare a phosphate-buffered saline (PBS) with a pH 7.7. The preparation of all solutions and the washing procedures in the experiments were carried out using double distilled water. Furthermore, all experiments were conducted under standard temperature and pressure conditions.

2.2 | Instruments

The colorimetric detection of DA was conducted using a Shimadzu UV-2550[®] spectrophotometer (Shimadzu, Kyoto, Japan) equipped with a quartz cuvette having an optical path length of 10 mm. The Fourier-transform infrared (FTIR) spectrum was acquired using

the TENSOR-27[®] FTIR spectrometer (Bruker, Karlsruhe, Germany). The field emission transmission electron microscopy (FE-TEM, JSM-7900F; Japan) was utilized to acquire scanning electron microscopy (SEM) images and energy-dispersive X-ray spectroscopy (EDS) data. The crystal structure of nanomaterials was characterized using X-ray diffraction (XRD), specifically the Bruker D8 advanced model. X-ray photoelectron spectroscopy (XPS) was conducted with a PHI5000 Versa Probe-II[®] (ULVAC-PHI[™]; Japan). Electron spin resonance (ESR) analysis was proved with a JES-FA200 instrument (JEOL, Tokyo, Japan). Leici PHS-3[®] digital pH meter (Shanghai, China) was utilized to measure the pH. A PE lambda 750 UV-visible-near infrared spectrophotometer (PerkinElmer Instrument, Waltham, MA, USA) was used to survey the UV-visible diffuse reflectance spectroscopy (DRS).

2.3 | Preparation of cerium oxide (CeO_2)

Initially, $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (1.22 g) was completely dissolved in deionized water (75 mL). After that, 2.5 mL of hydrogen peroxide (30%, v/v) and ammonium hydroxide (30%, 7.5 mL) were successively introduced into the mixture, followed by vigorous stirring. Subsequently, upon raising the temperature to 100°C and maintaining for 1 h, a pale-yellow dispersion was acquired. The reaction mixture underwent centrifugation and was washed many times with deionized water and methanol until a neutral pH.

2.4 | Preparation of copper-doped carbon dots (Cu-CDs)

Briefly, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.1 g) and dopamine hydrochloride (30.9 mg) were measured and dissolved in 30 mL of deionized water. Then 50 μL of ethylenediamine and 2.1 g of citric acid were introduced into the aforementioned solution. Following 20 min of magnetic stirring, the mixture was subsequently transferred to a Teflon reactor, where it underwent a reaction at 180°C for 6 h. After the reactor was cooled to room temperature, the product was centrifuged at 10,000 rpm for 15 min and was filtered through a 0.22 μm filter membrane and copper-doped carbon dots (Cu-CDs) were obtained.

2.5 | Preparation of $\text{CeO}_2\text{@ZIF-8/Cu-CDs}$

A mixture of 0.2975 g of $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 1 mL of Cu-CDs aqueous solution and 0.6568 g of 2-methylimidazole in 40 mL of methanol was combined with 0.172 g of cerium oxide (CeO_2) dissolved in the same volume of methanol. Then the solution was thoroughly mixed at 60°C, followed by washing with methanol and centrifugation at 3000 rpm three times. Finally, the $\text{CeO}_2\text{@ZIF-8/Cu-CDs}$ nanozymes were dispersed in 10 mL of pure water to obtain a solution with a concentration of 50 mg/mL. Figure 1 displays the flowchart

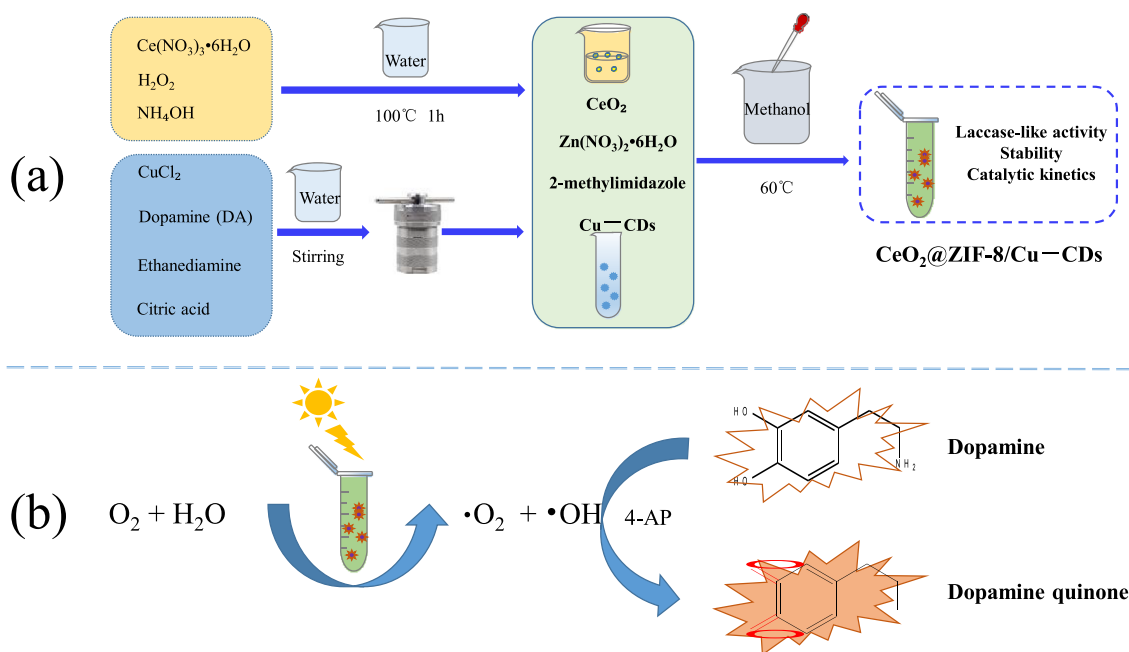


FIGURE 1 A schematic illustrating the synthesis and DA detection of $\text{CeO}_2@ZIF-8/\text{Cu-CDs}$.

representation for the synthesis of the $\text{CeO}_2@ZIF-8/\text{Cu-CDs}$. The non- CeO_2 polymer (ZIF-8/Cu-CDs) was prepared similarly in the absence of the surface-coated CeO_2 .

2.6 | Sample preparation

Before analysis, human serum and urine samples were collected from healthy adult volunteers. The samples were stored at 3°C for subsequent use within 15 days. Prior to use, 1.0 mL of serum was added to 3 mL of absolute ethanol, which was then vortexed for 30 s and centrifuged for 1 min. Next, 400 μL of the supernatant was extracted into a centrifuge tube and subsequently subjected to a hot water bath to eliminate the organic solvent. The residue can be used for colorimetric detection of DA after being dissolved with 0.4 mL of PBS (pH 7.7). Without additional pretreatment, 0.4 mL of urine sample was placed in a colorimetric tube for DA detection.

2.7 | Colorimetric detection of dopamine (DA)

A colorimetric sensor based on $\text{CeO}_2@ZIF-8/\text{Cu-CDs}$ laccase-like activity for the detection of DA. Briefly, the solution of prepared serum and urine sample was added to 25 μL of 2% 4-AP solution and 200 μL of $\text{CeO}_2@ZIF-8/\text{Cu-CDs}$ (50 mg/mL) solution was added to reach an overall volume of 3 mL with deionized water. Each solution was mixed and reacted for 30 min and centrifuged. The absorbance of the supernatant was detected at 500 nm.

To evaluate the reaction kinetics of $\text{CeO}_2@ZIF-8/\text{Cu-CDs}$, the initial reaction rates of these solutions were assayed. The Michaelis-

Menten constant (K_m) and the maximal velocity ($V_{\max}$) were obtained by the Michaelis-Menten equation:

$$v = \frac{V_{\max} + [S]}{K_m + [S]}$$

2.8 | Selectivity

The DA sensor's selectivity was tested in the presence of various substances including NH_4^+ , K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , SO_4^{2-} as well as interfering substances such as uric acid, urea, glucose, ascorbic acid, and cholesterol. The interfering substances were dispersed into 25 $\mu\text{g/mL}$ DA aqueous solution. Under the presence of 4-AP, $\text{CeO}_2@ZIF-8/\text{Cu-CDs}$, and PBS (pH 7.7), 50 $\mu\text{g/mL}$ of the earlier analogue were added into the solution for 30 min, detecting the absorbance at 500 nm.

3 | RESULTS

3.1 | Characterization of $\text{CeO}_2@ZIF-8/\text{Cu-CDs}$

SEM was used to analyze the nanostructural properties of $\text{CeO}_2@ZIF-8/\text{Cu-CDs}$. The SEM images in Figure 2a show the fine amorphous structure of CeO_2 . TEM micrographs (Figure 2b,c) show that ZIF-8 was a multilayered lamellar structure and Cu-DACDs was spherical aggregates with a diameter of about 10 nm, which were

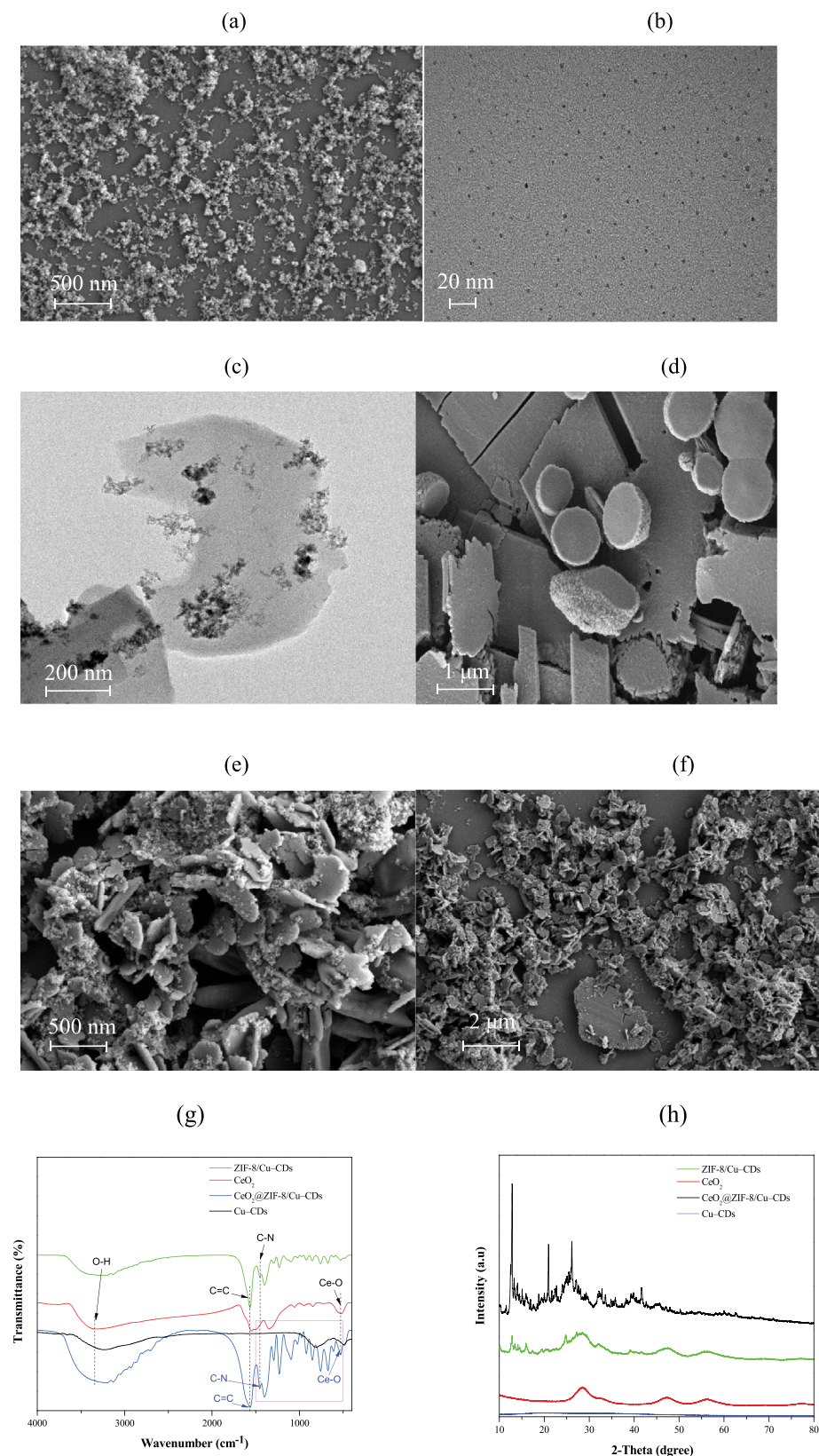


FIGURE 2 (a) SEM image of CeO_2 , (b) TEM image of Cu-CDs, (c) TEM and (d) SEM images of ZIF-8/Cu-CDs, (e, f) SEM images of CeO_2 @ZIF-8/Cu-CDs. (g) FTIR spectra and (h) XRD analysis of CeO_2 , Cu-CDs, ZIF-8/Cu-CDs and CeO_2 @ZIF-8/Cu-CDs.

bound to the surface of ZIF-8 through an in situ self-assembly to finally form ZIF-8/Cu-DACDs. Figure 2d, however, shows the distinct sheet structure of ZIF-8/Cu-CDs. Figure 2e,f shows that when CeO_2 was anchored onto the surface of ZIF-8/Cu-CDs, a core-shell

CeO_2 @ZIF-8/Cu-CDs nanozyme was formed. The possible reason for the formation of CeO_2 @ZIF-8/Cu-CDs is that the chemical reaction between Ce^{3+} and 2-methylimidazole was dominated by the chelating reaction [33].

Surface functional groups of CeO_2 , Cu-CDs, ZIF-8/Cu-CDs, and $\text{CeO}_2@\text{ZIF-8/Cu-CDs}$ were determined using FTIR spectra (Figure 2g). CeO_2 exhibited a characteristic absorption peak at 470 cm^{-1} , which can be attributed to the stretching vibration of the Ce–O bond [34, 35]. The aforementioned absorption peak was also observed in the as-prepared particles. The wide and prominent peak

at 3134 cm^{-1} in the FTIR spectrum of ZIF-8/Cu-CDs suggested the existence of intermolecular stretching vibration originating from the hydrated hydroxyl group of CeO_2 before modification. Similarly, the N–H bond linked to the introduction of DA during the synthesis process of ZIF-8/Cu-CDs was also detected [36]. Importantly, the prominent peaks in the spectrum of $\text{CeO}_2@\text{ZIF-8/Cu-CDs}$ were

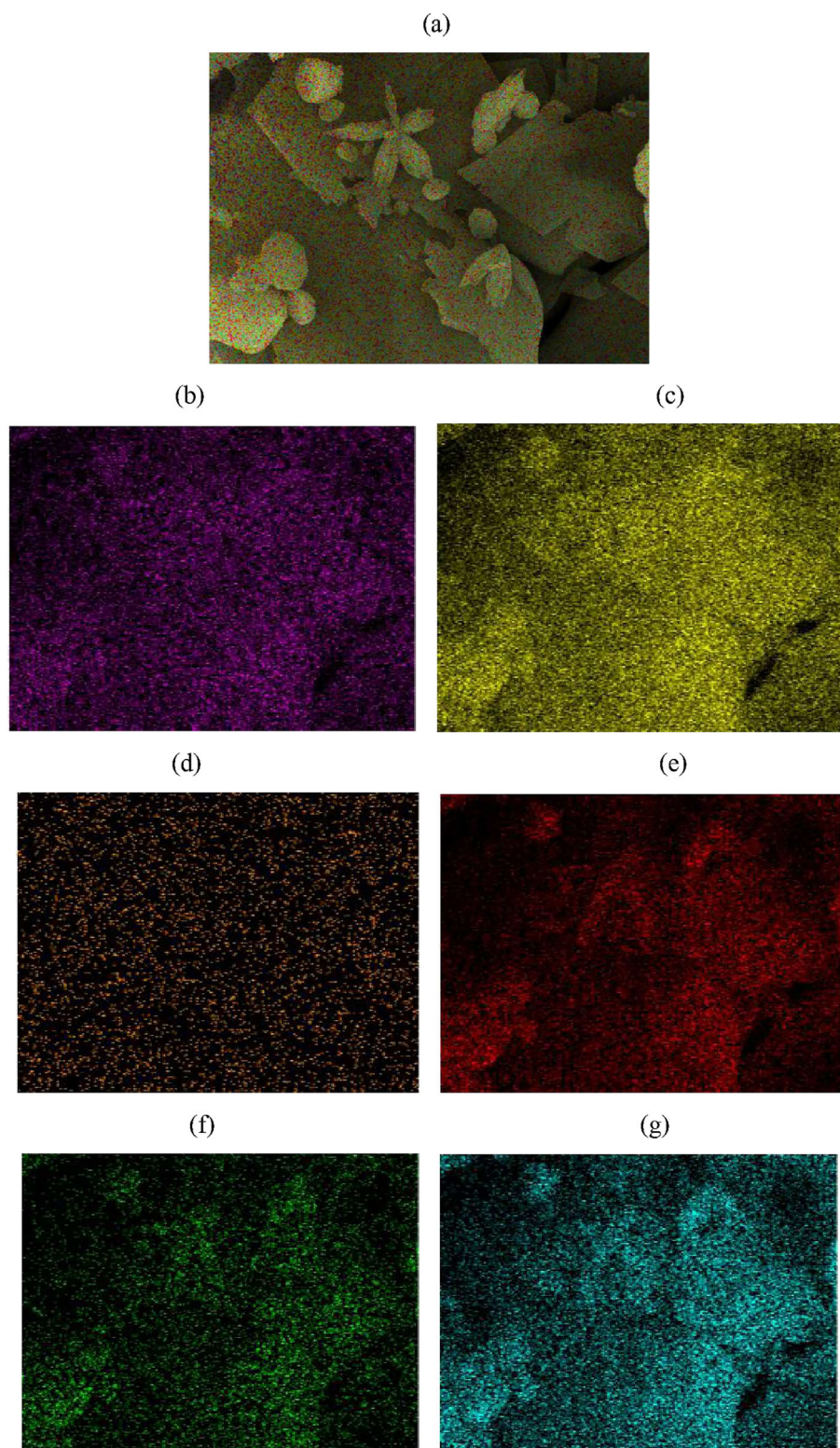


FIGURE 3 EDS mappings for (a) $\text{CeO}_2@\text{ZIF-8/Cu-CDs}$, (b) Cu, (c) Zn, (d) Ce, (e) C, (f) N, and (g) O.

observed at 1566, 1452, and 1096 cm^{-1} , which correspond to the stretching vibrations of the C=O, C-N and C-O bonds [36, 37]. Simultaneously, the strong peaks within the spectral region of 500–1500 cm^{-1} can be ascribed to the stretching and plane vibration of the ZIF-8/Cu-CDs [38]. As a result, CeO_2 @ZIF-8/Cu-CDs was successfully generated and CeO_2 was deposited into the ZIF-8/Cu-CDs surface.

The superior chemical capabilities of CeO_2 @ZIF-8/Cu-CDs were further validated through XRD analysis, as illustrated in Figure 2h, where diffraction peaks of the CeO_2 @ZIF-8/Cu-CDs were found to be identical to those of the standard ZIF-8/Cu-CDs structure. CeO_2 @ZIF-8/Cu-CDs displayed a slight downward shift in 2θ in their

measured diffraction peaks, indicating the successful formation of CeO_2 on the surface of ZIF-8/Cu-CDs. This observation suggests the development of CeO_2 @ZIF-8/Cu-CDs, in contrast to a simple physical adsorption state [39].

The core-shell structure of CeO_2 @ZIF-8/Cu-CDs was further confirmed through EDS elemental mappings (Figure 3a–g). The CeO_2 @ZIF-8/Cu-CDs crystal was composed of ZIF-8/Cu-CDs in its core, with the Ce species uniformly dispersed throughout its surface.

Chemical groups on the surface of CeO_2 @ZIF-8/Cu-CDs were investigated using XPS. Figure 4a illustrates the binding energy of the Cu 2p core level. The two discernible peaks observed at 934.8 and 954.5 eV can be attributed to the Cu $2p_{3/2}$ and Zn $2p_{1/2}$ states,

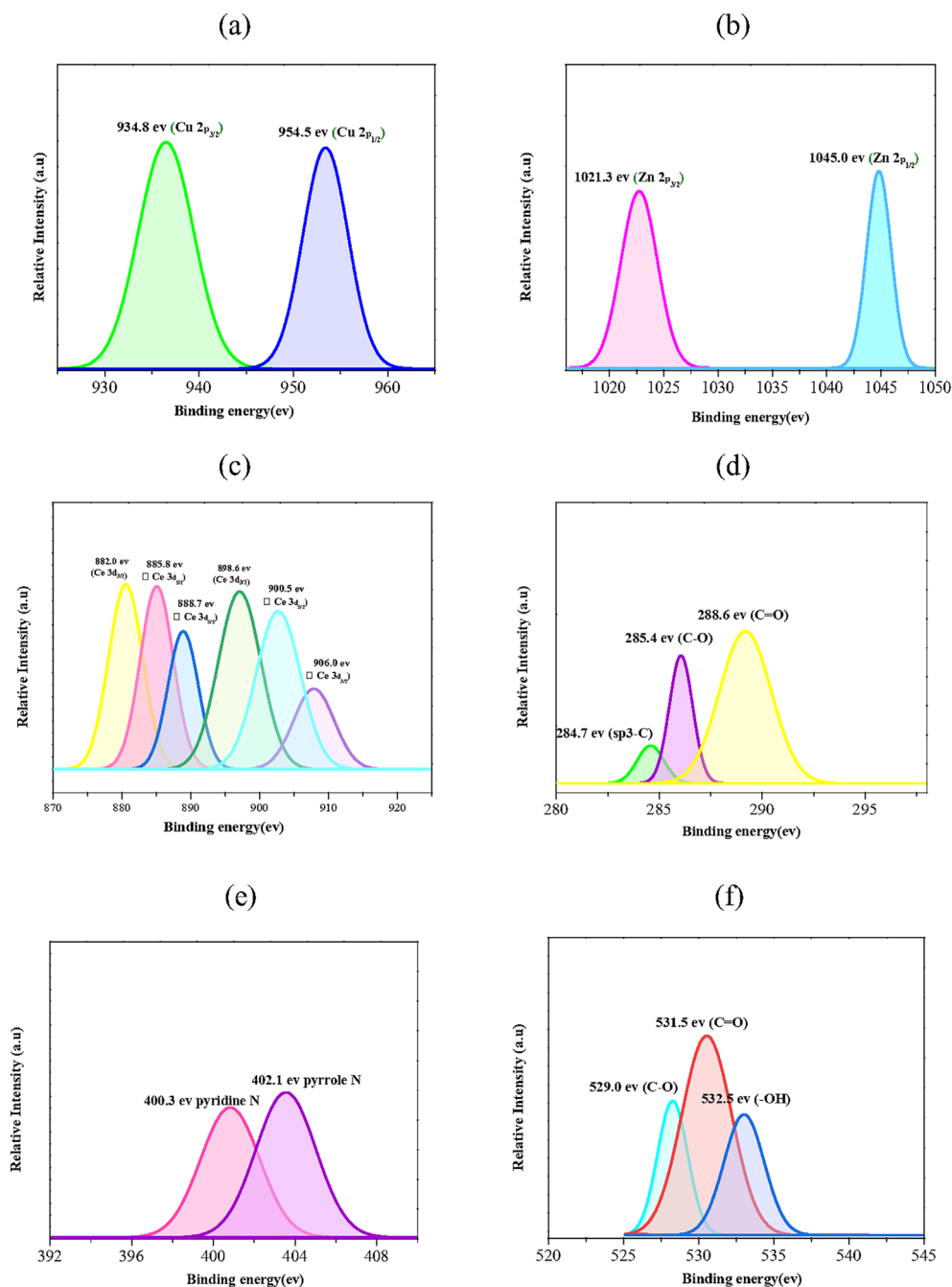


FIGURE 4 XPS for (a) Cu, (b) Zn, (c) Ce, (d) C, (e) N, and (f) O of CeO_2 @ZIF-8/Cu-CDs.

respectively. As depicted in Figure 4b, the Zn 2p spectrum exhibited two distinct peaks, which can be attributed to the Zn 2p_{3/2} peak at 1021.3 eV and the Zn 2p_{1/2} peak at 1045.0 eV. The XPS spectrum displayed two discernible peaks at energy levels of 882.0 and 885.8 eV, as depicted in Figure 4c. These peaks are indicative of the Ce 3d_{3/2} and Ce 3d_{5/2} orbitals, respectively. Furthermore, it was noted that the Ce 3d_{5/2} and Ce 3d_{3/2} orbitals were associated with the binding energy peak at 888.7 and 898.6 eV, respectively. Similarly, the peaks at 900.5 and 906.0 eV were attributed to the Ce 3d_{5/2} and Ce 3d_{3/2} orbitals, respectively. These results demonstrate the presence of Ce in CeO₂@ZIF-8/Cu-CDs. The bonding and chemical environments of CeO₂@ZIF-8/Cu-CDs were determined using the C 1s, O 1s, and N 1s XPS spectra (Figure 4d–f). The aforementioned findings indicate the successful synthesis of CeO₂@ZIF-8/Cu-CDs.

3.2 | Catalytic kinetic parameters

Before sample detection, the laccase activity of CeO₂@ZIF-8/Cu-CDs was investigated by DA as the catalytic reaction substrate. For

detecting the laccase activity of CeO₂@ZIF-8/Cu-CDs, the concentration of DA was fixed individually at 20 µg/mL in pH 7.7 PBS solution. The concentrations of CeO₂, Cu-CDs, ZIF-8/Cu-CDs, and CeO₂@ZIF-8/Cu-CDs were fixed individually at 0.5 mg/mL. As shown in Figure 5a, when DA was present, CeO₂@ZIF-8/Cu-CDs catalyze the oxidation of 4-AP to form a red chemical compound. Compared to CeO₂@ZIF-8/Cu-CDs, CeO₂ did not exhibit any laccase activity, while Cu-CDs and ZIF-8/Cu-CDs had weaker laccase activity. The experiments indicate that the laccase activity can also be strengthened by growing CeO₂ onto ZIF-8/Cu-CDs. Consequently, this phenomenon suggests that the CeO₂ and ZIF-8/Cu-CDs relationship is not independent, but rather synergistic.

Because of its excellent solubility throughout the reaction, phenol was previously used as a substrate to investigate the catalytic activity of laccase. Different volumes (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mL) of a 10 mM phenol solution were reacted with 500 µL of PBS (pH 7.7) and 200 µL of 4-AP (2%), respectively, to determine the initial rate of the reaction. The catalytic reaction was initiated by introducing 300 µL of CeO₂@ZIF-8/Cu-CDs (50 mg/mL) into the system. The values of K_m and V_{max} , two kinetic parameters, were determined using

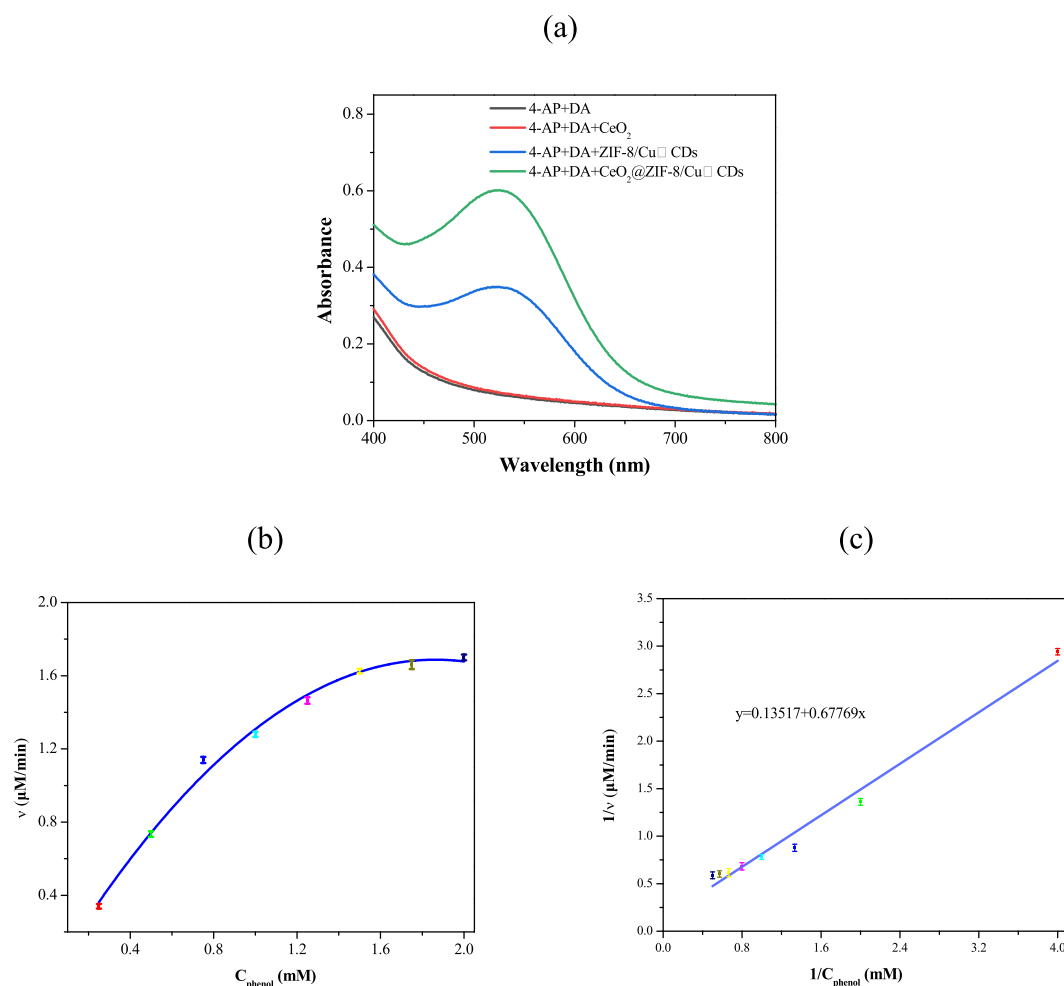


FIGURE 5 (a) The UV spectra of 4-AP + DA, 4-AP + DA + CeO₂, 4-AP + DA + ZIF-8/Cu-CDs, and 4-AP + DA + CeO₂@ZIF-8/Cu-CDs. (b) Michaelis–Menten curves between reaction rate and C_{phenol} catalyzed by CeO₂@ZIF-8/Cu-CDs. (c) Corresponding Lineweaver–Burk plot of CeO₂@ZIF-8/Cu-CDs.

TABLE 1 Comparison of the kinetic parameters [Michaelis–Menten constant (K_m) and the maximal velocity (V_{max})] of laccase-like activity of CeO₂@ZIF-8/Cu-CDs and other materials.

Catalyst	K_m (mM)	V_{max} (μ M/min)	Reference
Cu-CH	0.42	7.3	[40]
Fe1@CN20	0.07	2.25	[41]
AMP-Cu	0.09	1.30	[42]
Cu-Cys NLs	0.14	1.44	[43]
Bpy-Cu	0.21	4.11	[44]
Laccase	0.36	0.28	This work
ZIF-8/Cu-DACDs	0.27	0.95	This work
CeO ₂ @ZIF-8/Cu-CDs	0.20	1.48	This work

the Michaelis–Menten equation (Figure 5b,c). The CeO₂@ZIF-8/Cu-CDs demonstrated a K_m of 0.20 mM and V_{max} of 1.48 μ M/min. In comparison to other laccase mimic enzymes listed in Table 1, the CeO₂@ZIF-8/Cu-CDs exhibited favorable reaction rates and substrate affinity. At the same time, the V_{max} value of CeO₂@ZIF-8/Cu-CDs for phenol is five times higher than that of laccase and the K_m value indicates that the affinity of CeO₂@ZIF-8/Cu-CDs for phenol is lower than that of laccase and ZIF-8/Cu-CDs. This suggests that the developed nanozyme demonstrated superior kinetic performance [40–44].

3.3 | Mechanism of catalysis by CeO₂@ZIF-8/Cu-CDs

To confirm the catalytic mechanism of the CeO₂@ZIF-8/Cu-CDs, the formation of reactive free radicals and positive ions ($\cdot$ OH, $\cdot$ O₂, H⁺, or Cu²⁺/Cu⁺) that are normally thought to be formed in nanozyme catalyzed reactions was studied. Methanol, ascorbic acid (5 mM), sodium oxalate (Na₂C₂O₄, 5 mM) and ethylenediaminetetraacetic acid (EDTA, 5 mM) are widely recognized as effective capture agents for $\cdot$ OH, $\cdot$ O₂, H⁺, and Cu²⁺/Cu⁺ [45, 46]. The absorbance of the system was found to be essentially unaffected by the addition of Na₂C₂O₄ and EDTA. This finding demonstrates the lack of electron–hole generation during the catalytic process and points to the lack of free copper or cuprous ions on the surface of CeO₂@ZIF-8/Cu-CDs (Figure 6a). The addition of methanol resulted in a decrease in the absorbance of the system, providing evidence for the generation of hydroxyl radicals ($\cdot$ OH) during the catalytic process. Upon the introduction of ascorbic acid, the absorbance of the system decreased considerably, providing strong evidence for the significant contribution of the superoxide radical ($\cdot$ O₂) to the catalytic process.

To investigate the effect of oxygen on the catalytic activity of CeO₂@ZIF-8/Cu-CDs, laccase activity was measured in both air and nitrogen environments under identical experimental conditions. The laccase activity of CeO₂@ZIF-8/Cu-CDs in nitrogen, as depicted in Figure 6b, was found to be significantly low, hence limiting its ability

to efficiently catalyze the oxidation of DA. Therefore, the participation of atmospheric oxygen is crucial in this chemical reaction.

The UV-visible absorption spectra of CeO₂, ZIF-8/Cu-CDs, and CeO₂@ZIF-8/Cu-CDs are displayed in Figure 6c. Because of the surface plasmon resonance (SPR) effect, CeO₂@ZIF-8/Cu-CDs exhibited a stronger absorbance in the visible range than CeO₂ and ZIF-8/Cu-CDs. The results showed that the enhanced absorbance of CeO₂@ZIF-8/Cu-CDs might be attributed to its greater ability to absorb visible light [47]. Furthermore, the electron transfer from formic acid to riboflavin sodium phosphate (FMN) is illustrated in Figure 6d. The inclusion of CeO₂@ZIF-8/Cu-CDs resulted in a notable drop in the absorbance of FMN at wavelengths of 370 and 450 nm, indicating the reduction of FMN. This result confirmed that CeO₂@ZIF-8/Cu-CDs are capable of producing a reduced form of FMN via electron transfer from formic acid to FMN [48]. Due to the greater work function of ZIF-8/Cu-CDs, the band gap of CeO₂ was greater than that of ZIF-8/Cu-CDs, as illustrated in Figure 6e. As a consequence, electrons are transferred from CeO₂ to ZIF-8/Cu-CDs until equilibrium is reached and a new energy level is formed. Photo-generated electrons can react with oxygen molecules adsorbed on the surface of CeO₂@ZIF-8/Cu-CDs upon exposure to visible light to produce $\cdot$ O₂ and $\cdot$ OH (Figure 6f) [49]. The superoxide radical and hydroxyl radicals were also verified by ESR (Figure 6g). The synergistic effect of these two radicals enhanced the efficiency of the catalytic oxidation of DA. The resultant oxidation product was then subjected to a chromogenic reaction with 4-AP, resulting in the formation of a red compound (Figure 1) [50].

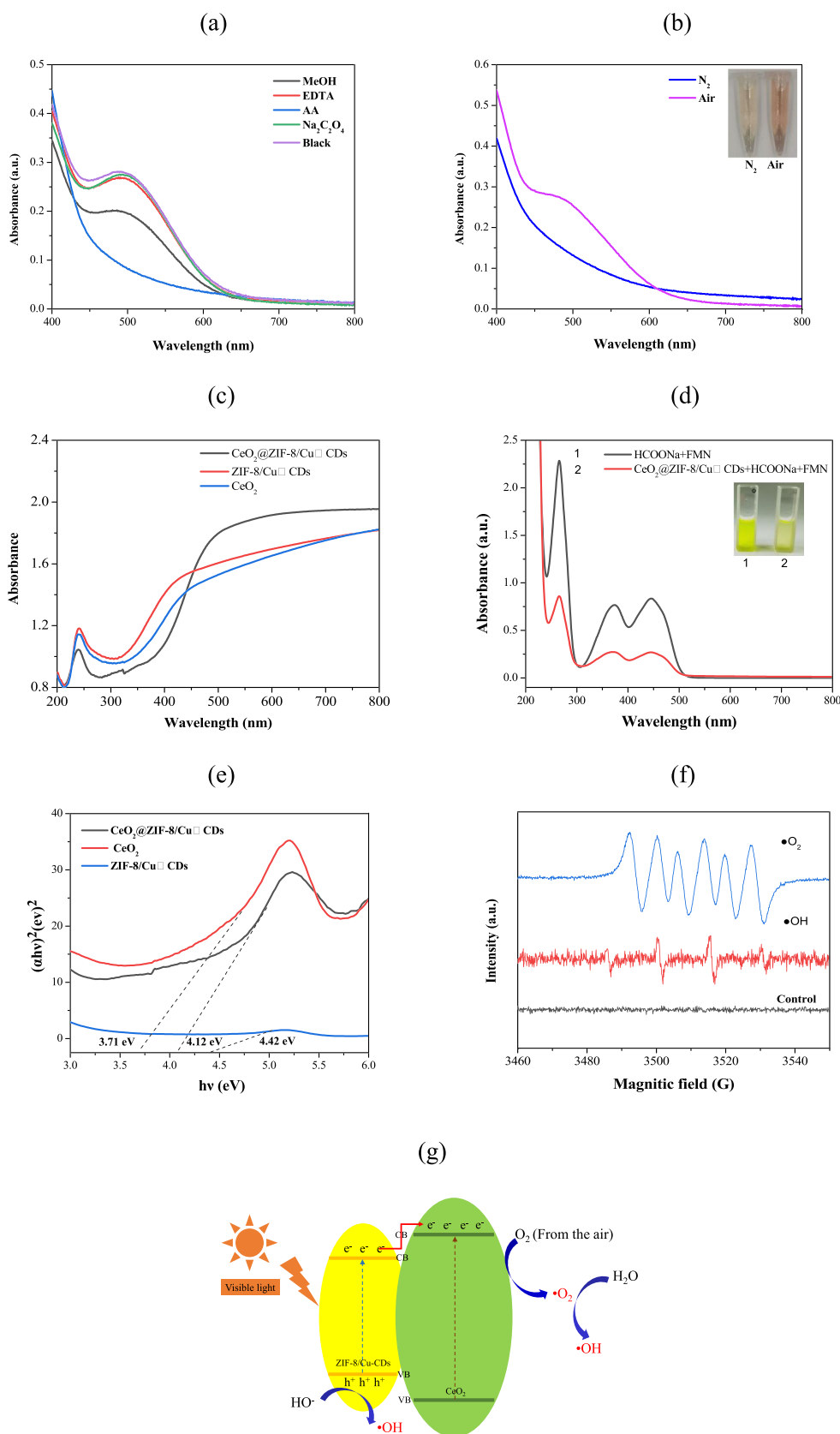
3.4 | Reusability and repeatability

To evaluate the reusability of the CeO₂@ZIF-8/Cu-CDs, a washing step with pure water was performed, allowing the catalyst to be utilized once more for subsequent catalytic cycles. Figure 7a shows that the absorbance appears to be 88.6% after five cycles of recycling. Consequently, the nanozymes can be reused for up to five cycles without appreciably altering their catalytic efficiency. To assess the repeatability of the CeO₂@ZIF-8/Cu-CDs synthesis procedure, different laboratory technicians prepared CeO₂@ZIF-8/Cu-CDs at various time points following the synthesis process. These samples were then utilized for DA analysis. The findings depicted in Figure 7b demonstrate that the relative standard deviation (RSD) of absorbance in the testing system is below 5.0%. The results revealed that CeO₂@ZIF-8/Cu-CDs exhibit not only excellent catalytic efficiency but also notable reusability and remarkable repeatability.

3.5 | Parameters optimization for DA detection

To assess the amount of CeO₂@ZIF-8/Cu-CDs used, different concentrations of the material were systematically studied. As the CeO₂@ZIF-8/Cu-CDs concentration was increased from 0.125 to 1 mg/mL, the data indicated an improvement in the system's

FIGURE 6 (a) The UV spectra of free radical trapping experiment. (b) The UV spectra of oxygen influence experiment. (c) The UV spectra of CeO_2 , ZIF-8/Cu-CDs, and $\text{CeO}_2@ZIF-8/\text{Cu-CDs}$. (d) The UV spectra of HCOOK + FMN and $\text{CeO}_2@ZIF-8/\text{Cu-CDs}$ + HCOOK + FMN. (e) Kubelka–Munk function versus energy plot. (f) The corresponding $\cdot\text{O}_2$ and $\cdot\text{OH}$ detected by ESR. (g) Mechanism schematic for $\text{CeO}_2@ZIF-8/\text{Cu-CDs}$ nanozymes.



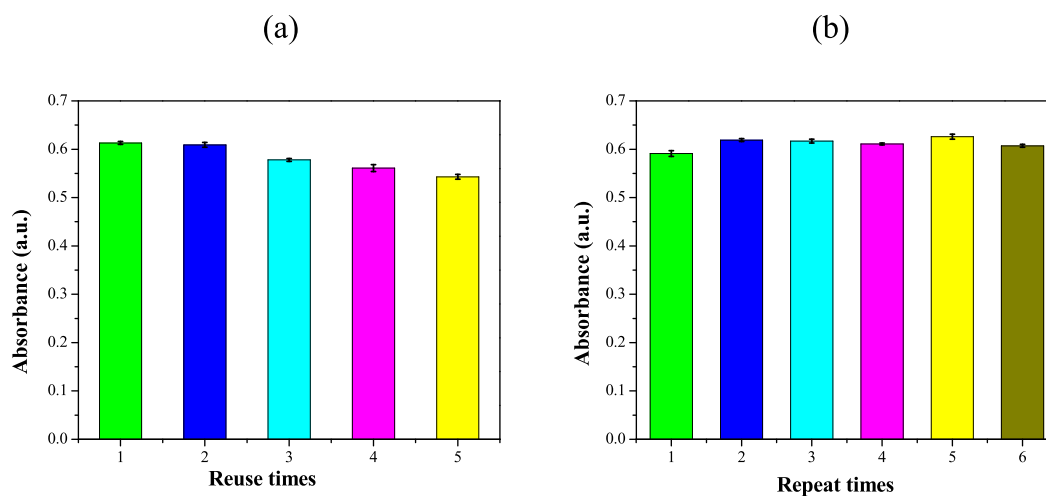


FIGURE 7 Reusability and repeatability of CeO₂@ZIF-8/Cu-CDs.

absorbance. The absorbance of the system tended to remain constant when the concentration of the material exceeded 1 mg/mL (Figure 8a). Consequently, 1 mg/mL was determined to be the optimal concentration of CeO₂@ZIF-8/Cu-CDs.

Due to the inherent instability and vulnerability of nanozymes to degradation in acidic environments, the investigation focused on the analysis of the alkaline buffer system. Figure 8b illustrates that the maximum absorbance was attained in PBS at a pH of 7.7. This is consistent with previous studies that have highlighted the importance of the buffer system.

To determine the effect of reaction conditions on catalytic activity, five distinct reaction conditions were evaluated for 30 min: indoor light, light-emitting diode (LED) light, infrared light, ultrasound, and a combination of infrared light and ultrasound. The reaction conducted under indoor light conditions exhibited the highest absorbance, as illustrated in Figure 8c. Therefore, the subsequent determination used indoor light as the reaction condition.

To gain further insights into the prospective applications of the CeO₂@ZIF-8/Cu-CDs nanozyme, it is critical to analyze the impact of hydrogen peroxide on its catalytic activity. A DA solution (6.25, 12.5 and 25 µg/mL) was mixed with 100 µL of PBS (pH 7.4) and 25 µL of 4-AP (2%). In one group, 50 µL of hydrogen peroxide (50 mM) was added, while the other group did not receive any hydrogen peroxide. After that, 50 µL of CeO₂@ZIF-8/Cu-CDs at a concentration of 50 mg/mL was added. The findings illustrated in Figure 8d demonstrate that CeO₂@ZIF-8/Cu-CDs had improved effectiveness in the oxidation of DA. However, the absorbance of the system was observed to decrease upon the introduction of hydrogen peroxide. This indicates that the ·OH produced by hydrogen peroxide decomposition tends to adsorb on the surface of the nanozyme, resulting in the reduction of free ·OH in the solution. Therefore, the DA assay did not necessitate the application of hydrogen peroxide to facilitate oxidation.

3.6 | Influence of interfering ions and substances

The evaluation of the detection method focused on assessing its ability to resist interference. Various analogues and disruptors of DA were employed for this purpose, including different ions such as K⁺, Na⁺, Mg²⁺, Ca²⁺, Cl⁻, and SO₄²⁻. Furthermore, additional substances that have the potential to disrupt the detection process were incorporated into the assessment, including uric acid, glucose, urea, ascorbic acid and cholesterol. The intensity of the absorbance was significantly increased only by DA, as illustrated in Figure 9a,b. Under the experimental conditions, the presence of any of the biomolecules or inorganic ions tested had no discernible effect on the activity of the CeO₂@ZIF-8/Cu-CDs. It is noteworthy to notice that chemicals such as glucose, uric acid, urea, and cholesterol, which are strongly correlated with urine and serum, exhibited minimal impact on the CeO₂@ZIF-8/Cu-CDs nanozymes, demonstrating their resistance to interference. Using CeO₂@ZIF-8/Cu-CDs nanozymes for DA detection was found to have high selectivity and excellent anti-interference properties.

3.7 | Method validation

As the concentration of DA was raised, the color of the solution containing CeO₂@ZIF-8/Cu-CDs changed from colorless to red. The colorimetric detection limit was experimentally determined to be 8.5 ng/mL using the formula 3s/K. The limits of detection (LODs) for DA were much lower than the corresponding values for urine (87 ng/mL) and blood serum (2.45 µg/mL) [51]. Different concentration of DA was dissolved with 400 µL PBS (pH 7.7) and detected in the presence of 4-AP and CeO₂@ZIF-8/Cu-CDs under the optimum conditions. Quantitative analysis of DA yielded a good linear relationship ($A = 0.09503C + 0.1328$, $R^2 = 0.9966$) between 0.05 and

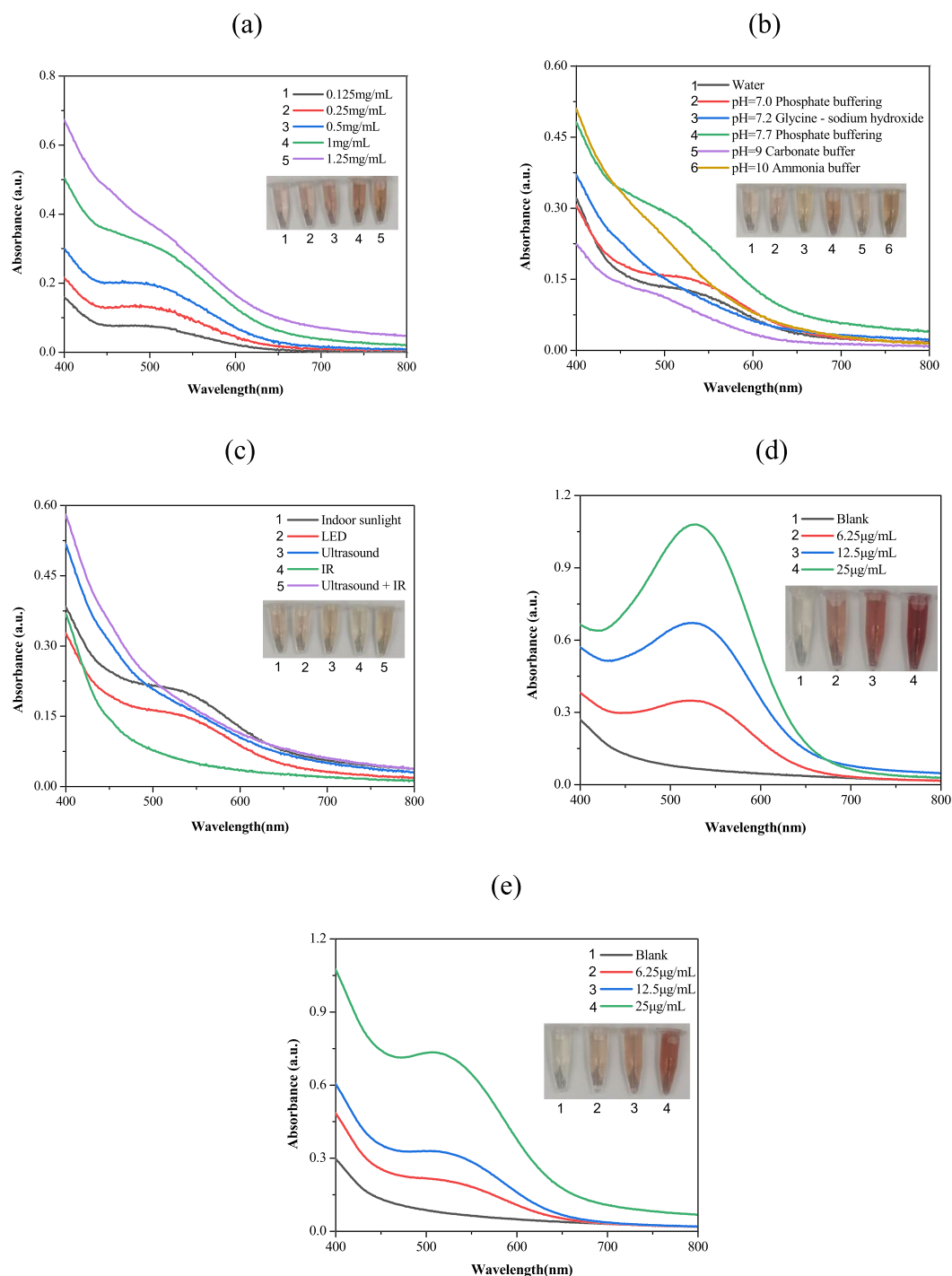


FIGURE 8 Effect of (a) $\text{CeO}_2\text{@ZIF-8/Cu-CDs}$ concentration, (b) buffer system, (c) reaction condition, (d) no hydrogen peroxide, and (e) added hydrogen peroxide.

7.5 $\mu\text{g/mL}$ (Figure 10a,b). Six consecutive measurements of DA in PBS at pH 7.7 were performed to determine the repeatability of the $\text{CeO}_2\text{@ZIF-8/Cu-CDs}$ sensor. The concentration of DA in the solution was 0.29 $\mu\text{g/mL}$. The calculated RSD was determined to be less than 5%. The obtained results demonstrate a high level of repeatability and excellent reproducibility for the developed DA sensor.

3.8 | Actual sample assessment

To evaluate the possible applications of the DA sensor, the feasibility of using $\text{CeO}_2\text{@ZIF-8/Cu-CDs}$ for real sample analysis was explored using human urine and serum as representative samples. To confirm the accuracy of the data, recoveries were assessed after the addition of a solution with a known concentration of DA to the real sample.

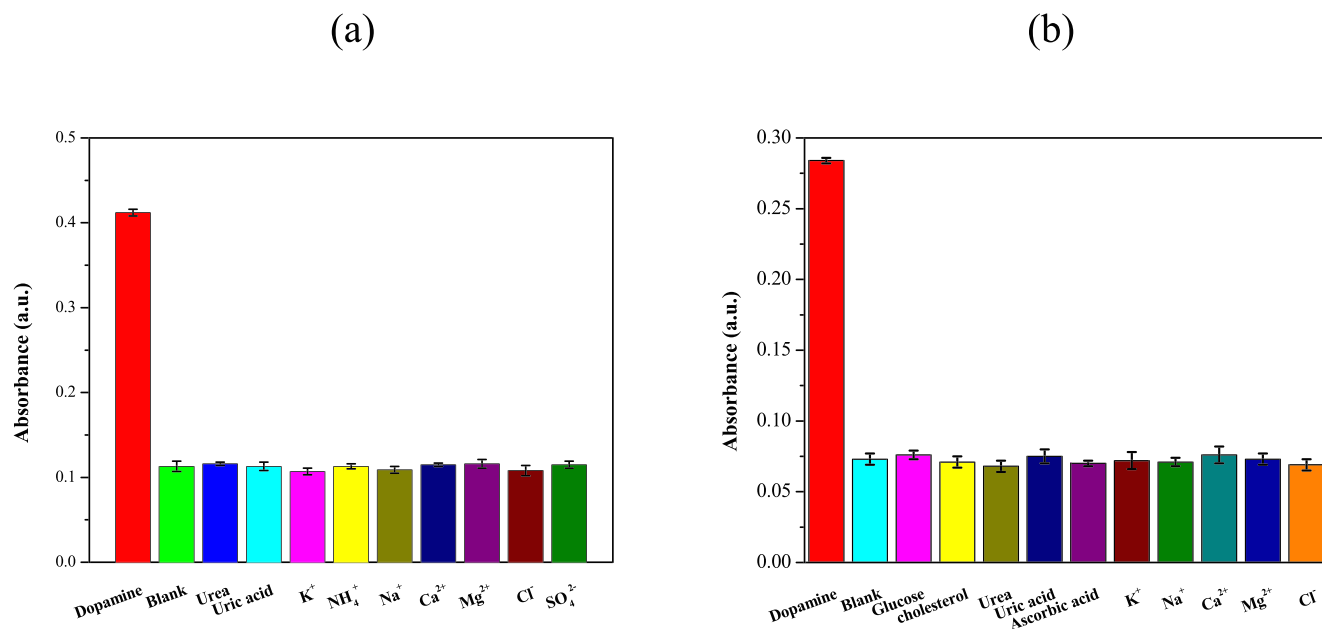


FIGURE 9 Effect of interfering ions and interfering substances in (a) urine and (b) serum.

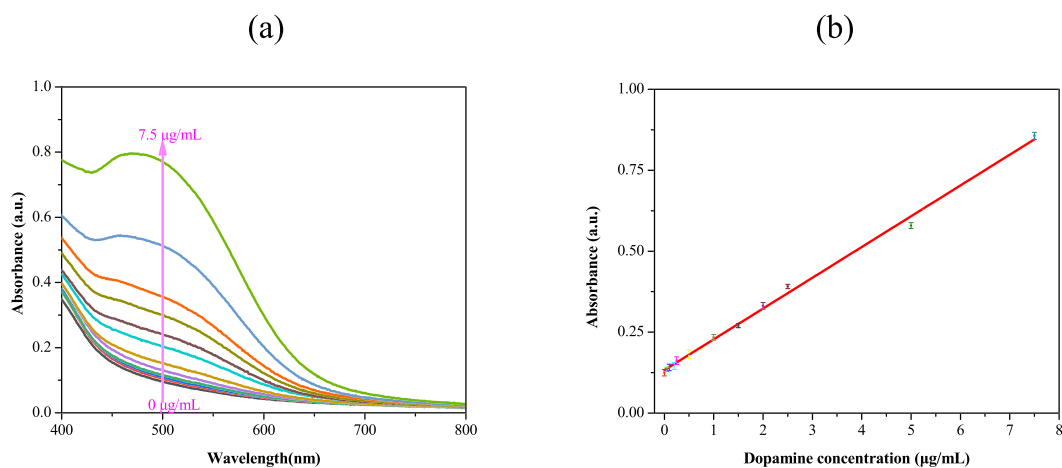


FIGURE 10 (a) The UV spectra of different concentrations of dopamine and (b) the linear relationship between the absorbance and dopamine concentration in the range of 0.05–7.5 µg/mL.

Samples	Added (µg/mL)	Found (µg/mL)	Recovery (%)	RSD (%)
Urine	0	0.263	—	—
	0.05	0.046	92.0	2.1
	0.1	0.112	112.0	1.8
	0.2	0.186	93.0	1.4
Serum	0	0.290	—	—
	0.15	0.430	93.3	3.2
	0.3	0.602	104.0	2.7
	0.6	0.977	114.5	2.1

TABLE 2 Quantification of dopamine (DA) in urine and serum ($n = 3$).

TABLE 3 The overview of analytical data of the reported methods for the analysis of dopamine (DA).

Materials	Detection method	Linearity range (μg/mL)	LOD (ng/mL)	Reference
AuNPs	Colorimetric	0–0.153	14.4	[51]
p-ProH/GCE	Square wave voltammetry	0.153–15.3	45.9	[52]
Mo-W-O/GA/GCE	Electrochemistry	0.15×10^{-3} –68.54	0.122	[53]
Nano-CaCO ₃ /PANI/rGO/GCE	Electrochemistry	0.015–2.14	1.53	[54]
AuNPs	Lateral flow assay	0.5–0.6	50	[55]
CeO ₂ @ZIF-8/Cu-CDs	Colorimetric	0.05–7.5	8.5	This work

The results of this examination are displayed in Table 2. The recovery data exhibited a range of 92.0% to 114.5%, with an RSD of around 4%. This suggests that the composition of the human urine and serum matrix had a minimal impact on the simultaneous measurement of DA. Table 3 compares the outcomes of various DA detection methods. Relatively, colorimetric detection based on nanozymes has demonstrated benefits of simplicity, rapidity, low matrix interference, and high sensitivity. Thus, the developed colorimetric biosensor represents a potential new technique for presumptive testing for DA.

4 | DISCUSSION

In summary, a colorimetric sensor of CeO₂@ZIF-8/Cu-CDs nanozymes with laccase activity was designed for the sensitive and selective detection of DA. The sensor was developed in a three-step synthetic process using a cost-effective and efficient hydrothermal approach. Additionally, the nanozymes displayed remarkable processability and were recyclable. The CeO₂@ZIF-8/Cu-CDs catalyzed the oxidation of DA and demonstrated a colorimetric response with an 8.5 ng/mL detection limit. Human urine and blood serum samples were used to test the colorimetric response of a developed sensor. The observed recovery rates of DA in urine and serum samples, which exceeded 92%, offer evidence for the outstanding efficacy of the developed sensor in detecting DA in physiological settings. This sensor enables the sensitive, rapid and cost-effective detection of DA in human urine and blood serum samples. The lowered detection limit of CeO₂@ZIF-8/Cu-CDs designates them as potential biosensing candidates for pre-clinical applications.

AUTHOR CONTRIBUTIONS

Qinhong Yin: Conceptualization; funding acquisition; software; writing—original draft. **Yutong Wang:** Methodology; validation. **Dezhi Yang:** Resources; supervision; visualization. **Yaling Yang:** Conceptualization; resources; writing—review and editing. **Yanqin Zhu:** Conceptualization; funding acquisition; project administration; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are contained within the article.

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