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Enhanced Peroxidase-Like Activity of MoS$_2$ Quantum Dots Functionalized g-C$_3$N$_4$ Nanosheets towards Colorimetric Detection of H$_2$O$_2$

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Abstract: MoS$_2$ quantum dots (QDs) functionalized g-C$_3$N$_4$ nanosheets (MoS$_2$@CNNS) were prepared through a protonation-assisted ion exchange method, which were developed as a highly efficient biomimetic catalyst. Structural analysis revealed that uniformly-dispersed MoS$_2$ QDs with controllable size and different loading amount grew in-situ on the surface of CNNS, forming close-contact MoS$_2$@CNNS nanostructures and exhibiting distinct surface properties. Compared to MoS$_2$ QDs and CNNS, the MoS$_2$@CNNS nanocomposites exhibited a more than four times stronger peroxidase-like catalytic activity, which could catalyze the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of H$_2$O$_2$ to generate a blue oxide. Among the MoS$_2$@CNNS nanocomposites, MoS$_2$@CNNS(30) was verified to present the best intrinsic peroxidase-like performance, which could be attributed to the more negative potential and larger specific surface area. A simple, rapid and ultrasensitive system for colorimetric detection of H$_2$O$_2$ was thus successfully established based on MoS$_2$@CNNS, displaying nice selectivity, reusability, and stability. The detection limit of H$_2$O$_2$ could reach as low as 0.02 µM. Furthermore, the kinetic and active species trapping experiments indicated the peroxidase-like catalytic mechanism of MoS$_2$@CNNS. This work develops a novel, rapid, and ultrasensitive approach for visual assay of H$_2$O$_2$, which has a potential application prospect on clinical diagnosis and biomedical analysis.

Keywords: MoS$_2$; g-C$_3$N$_4$; peroxidase-like; colorimetric detection; H$_2$O$_2$

1. Introduction

Over past decades, enzyme mimetics have caused extensive concern due to their favorable superiorities against harsh conditions compared to natural enzymes, such as low cost, easy preparation...
and storage, better stability and reusability, and nice practicability [1–3]. Hence, it is interesting and challenging to develop novel and effective enzyme mimetics. Recently, various enzyme mimetics have been reported and widely used in clinical diagnosis and biomedical analysis, including nanomaterials [4–7], Schiff-base complexes [8], cyclodextrin [9], hemin [10], and DNA complexes [11]. Among them, nanomaterials are becoming a novel efficient mimic peroxidase in catalyzing \( \text{H}_2\text{O}_2 \)-mediated reaction due to their intrinsic properties like natural enzymes in size, shape and surface charge [4–6]. Moreover, nanomaterials exhibit nice surface properties with larger specific surface areas, more surface activation centers, and controlled catalytic potentials, which can highly promote their peroxidase-like catalytic performances [5]. Thus, the field for seeking novel nanomaterials such as peroxidase mimetics has been rapidly developed since \( \text{Fe}_3\text{O}_4 \) magnetic nanoparticles were first found to present the intrinsic peroxidase-like activity like that of horseradish peroxidase (HRP) in 2007 [4]. Thereafter, many kinds of nanomaterials have been exploited as peroxidase mimetics, and have exhibited good peroxidase-like properties, such as magnetic nanomaterials (CoFe\(_2\)O\(_4\) [12] and FeVO\(_4\) [13]), carbon nanomaterials (carbon nanotubes [14], carbon dots [15], graphene oxides [16], and C\(_3\)N\(_4\) [17]), noble metal nanomaterials (gold, silver and platinum) [18] and their alloys (AgVO\(_3\) nanobelts [19], FeSe-Pt@SiO\(_2\) nanospheres [20], Fe\(_3\)O\(_4\)-Pt nanocomposites [21], and Fe\(_2\)O\(_4\)-Au nanohybrids [22]), and other nanomaterials (BiOI nanoflowers [23], CeVO\(_4\) nanorods [24] and MoS\(_2\) nanoflakes [25]). Despite this progress, there is still an urgent demand to pursue novel nanomaterials with highly-efficient and stable peroxidase-like activities to overcome their inherent disadvantages, including the loss of noble metals, environmental pollution, difficulty in separation, and recyclability.

Recently, special attention has been focused on graphene-like, two-dimensional (2D) nanomaterials owing to their 2D layer structure with high energy surfaces analogous to graphene [25–27]. As typical 2D nanomaterials, graphitic carbon nitride (g-C\(_3\)N\(_4\)) possesses a stacked 2D structure and appropriate band gap (2.7 eV) owing to the \( sp^2 \) hybridization of carbon and nitrogen, resulting in the formation of a stable and extended \( \pi \)-conjugated system [25–27]. And the unique graphite-like structure and tunable electronic structure of g-C\(_3\)N\(_4\) lead to its large specific surface area, high thermal and chemical stability, and rapid electron transfer, accompanied by the advantage of being metal-free, abundant in natural resources, and economical, endowing g-C\(_3\)N\(_4\) with extensive potential for use in new energy, sensor, and catalysis applications [25–27]. Currently, g-C\(_3\)N\(_4\) materials have been reported as peroxidase mimetics [17], showing nice peroxidase-like activities and further extending their application areas in biotechnology. However, in view of the high recombination rate of photoinduced electron-hole pairs, the catalytic efficiency of pure g-C\(_3\)N\(_4\) is greatly restricted [27]. Hence, various of methods have been performed to further improve the catalytic activity. Among them, constructing a functionalized hybrid structure using g-C\(_3\)N\(_4\) as the supporter by doping with other efficient catalysts is an especially effective way, which can apparently adjust the electronic structure and accelerate electron transport [28,29]. Up to now, lots of g-C\(_3\)N\(_4\)-based composites have been designed and exploited to explore the synergistic enhancement effect, such as Cu/g-C\(_3\)N\(_4\) [28], MnSe-g-C\(_3\)N\(_4\) nanosheets [29], Fe-g-C\(_3\)N\(_4\) [30], Co-g-C\(_3\)N\(_4\) [31], g-C\(_3\)N\(_4\)/BiFeO\(_3\) nanocomposites [32], and so on, all of which exhibited an improved peroxidase-like activity. Therefore, in-depth investigations are indeed of great demand for designing and fabricating novel g-C\(_3\)N\(_4\)-based nanocomposites to further facilitate the peroxidase-like activity.

As one of the typical 2D transition metal dichalcogenides, MoS\(_2\) materials show excellent catalytic activity and long-term stability, which has been widely used in the fields of electronic devices, battery materials, and catalysts [33,34]. In addition, when the size decreased to less than 10 nm, MoS\(_2\) QDs will exhibit unique extra electrical/optical properties owing to the stronger quantum confinement and edge effects [35,36], further improving its catalytic performance. Moreover, MoS\(_2\) with diverse nanostructures (nanoflakes [25] and nanoparticles [37]) and several MoS\(_2\)-based hybrids [38–40] have been reported as enzyme mimetics recently. Thus, on account of the matching energy band structure and nice catalytic performance, MoS\(_2\) QDs is becoming an ideal candidate for coupling with g-C\(_3\)N\(_4\) to
facilitate the peroxidase-like ability by promoting the separation and transport of electron-hole pairs. However, to the best of our knowledge, the peroxidase-like activity of MoS$_2$ QDs-g-C$_3$N$_4$ hybrids has not been investigated until now, which deserves further and deeper exploration.

Herein, in view of the superiority of g-C$_3$N$_4$ and MoS$_2$ QDs, the MoS$_2$@CNNS nanocomposites with different morphologies and surface properties were successfully designed and prepared via a protonation-assisted ion exchange method, which were used as efficient artificial enzymes. With the assistance of H$_2$O$_2$, the MoS$_2$@CNNS nanocomposites could catalyze the oxidation of the peroxidase substrate TMB to generate a blue colored reaction. Thus, a simple, rapid, ultrasensitive, selective, and stable system for the colorimetric detection of H$_2$O$_2$ was developed (Scheme 1). In addition, the morphology, crystal structure, surface properties, catalytic kinetics and mechanism, reusability and selectivity of the MoS$_2$@CNNS nanocomposites were studied. The peroxidase-like catalytic activities of MoS$_2$@CNNS nanocomposites had been greatly enhanced after the incorporation between CNNS and MoS$_2$ QDs, which could be mainly attributed to the synergistic interaction, accompanied by the more negative charge and larger specific surface area. The MoS$_2$@CNNS nanocomposites could have promising and broad applications in catalysis, biotechnology, and clinical diagnostics.

![Scheme 1. Schematic illustration of peroxidase-like catalytic reaction of MoS$_2$@CNNS nanocomposites.](image)

2. Materials and Methods

2.1. Materials and Reagents

MoS$_2$ powder and 3,3′,5,5′-Tetramethylbenzidine (TMB) were purchased from Sigma-Aldrich (Shanghai, China). Melamine (C$_{3}$H$_{6}$N$_{6}$), Na$_2$MoO$_4$.2H$_2$O, Tioacetamida (TAA, CH$_3$CSNH$_2$), 1-methyl-2-pyrroldione (NMP, C$_5$H$_6$NO), HCl, H$_2$O$_2$ (3 wt %), ethanol, isopropanol alcohol (IPA), p-benzoquinone (BQ), FeCl$_3$.6H$_2$O, CuSO$_4$.5H$_2$O, KNO$_3$, NaClO, glucose (Glu), lactose (Lac), L-valine (L-Val), glycine (Gly), and other chemicals were all of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Lircon antiseptic liquid was obtained from Shandong Lierkang Disinfection Technology Co., Ltd. (Dezhou, China). All aqueous solutions were prepared with Milli-Q water (Millipore, Boston, MA, USA).

2.2. Preparation of the Catalysts

The MoS$_2$@CNNS nanocomposites were prepared according to our previous report by an in-situ ion exchange method in a protonation process [36]. The bulk g-C$_3$N$_4$ was firstly prepared via a calcination process. Then g-C$_3$N$_4$ nanosheets (CNNS) were obtained by the ultrasonic exfoliation method using bulk g-C$_3$N$_4$ powder. After a protonation process in HCl (37%) and an ion exchange
process under a hydrothermal condition, the MoS$_2$@CNNS nanocomposites were finally obtained and denoted as MoS$_2$@CNNS(30) (‘30’ stands for the molar ratio of Na$_2$MoO$_4$·2H$_2$O/CNNS was 30:1).

In addition, other MoS$_2$@CNNS nanocomposites with different molar ratio were prepared as controls under the same conditions as those mentioned above, which were denoted as MoS$_2$@CNNS(15) and MoS$_2$@CNNS(45), respectively. Moreover, pure MoS$_2$ QDs were prepared as control via an ultrasonic exfoliation method [41]. In brief, 100 mg of MoS$_2$ powder was added into 10 mL of NMP and sonicated for 3.5 h. Then, the dispersion was kept stable overnight and centrifuged at 5500 r/min for 90 min. Finally, the MoS$_2$ QDs were obtained by collecting the top part of the dispersion.

2.3. Characterization

The crystal phase and structure of the as-prepared samples were analyzed by powder X-ray diffraction (XRD) measurements on a Germany Bruker D8 Advanced powder diffractometer using Cu K$_\alpha$ radiation ($\lambda = 0.15406$ nm). The morphology and microstructure of the as-prepared samples were observed by transmission electron microscopy (TEM), high resolution transmission electron microscopy (HRTEM), and selected area electron diffraction (SAED) (JEOL JEM-2100, Tokyo, Japan). Contents of S and Mo in MoS$_2$@CNNS nanocomposites were measured via an inductively coupled plasma emission spectrometer (ICP-AES, Varian725-ES, Palo Alto, CA, USA). The specific surface areas were determined by an automatic nitrogen adsorption specific surface and pore size distribution analyzer (NOVA 4000e, Quantachrome Instruments, Boynton Beach, FL, USA) at 77 K after a pretreatment at 473 K for 2 h. The zeta potential tests were determined on a zeta potential measuring instrument (Zetasizer Nano-ZS, Malvern Instruments Ltd., Malvern, UK), which was examined six times (each time being the average of 100 runs) at pH 4.0, and the mean values and standard deviations were calculated automatically based on Smoluchowski’s equation.

2.4. Peroxidase-Like Activities and Steady-State Kinetic Assay

The catalytic oxidation experiments (a peroxidase substrate TMB with H$_2$O$_2$) were carried out at room temperature to comparably investigate the peroxidase-like activities of the as-prepared catalysts, including MoS$_2$ QDs, CNNS and MoS$_2$@CNNS nanocomposites. Typically, the tests were performed by adding 200 µL of 600 µg/mL catalysts into the reaction systems containing 500 µL of 50.0 mM phosphate buffer solution (PBS, pH = 4.0), 100 µL of 8.0 mM TMB, and 200 µL of 10.0 mM H$_2$O$_2$. Then, the reaction systems were monitored in a time-scan mode at 652 nm by an UV-visible spectrophotometer (Shimadzu UV-2500, Kyoto, Japan) right after all of the components were added and mixed. The effect of MoS$_2$@CNNS(30) concentration (0–200 µg/mL), H$_2$O$_2$ concentration (0–5.0 mM), pH (2.0–9.0), and temperature (10–50 °C) on the peroxidase-like activity of MoS$_2$@CNNS(30) were also tested by the same procedures mentioned above to probe the optimal reaction conditions (Supplementary Information Figure S2 and Figure S3).

The steady-state kinetic tests were performed in a time course mode at 652 nm by an UV-visible spectrophotometer under the optimal experimental conditions (25.0 mM PBS, pH = 4.0, 25 °C, 120 µg/mL MoS$_2$@CNNS(30)) by varying the concentration of TMB at a fixed concentration of H$_2$O$_2$ or vice versa [4,42]. The Michaelis-Menten constant was measured using the Lineweaver-Burk double reciprocal plot according to the equation $1/v = (K_m/V_{\text{max}}) \times (1/[S]) + 1/V_{\text{max}}$ [4,42], where $v$ is the initial reaction velocity, $K_m$ is the Michaelis-Menten constant, $V_{\text{max}}$ is the maximum reaction velocity, and $[S]$ is the concentration of substrate.

2.5. Analysis of Active Species

To explore the roles of active species played in the catalytic reaction, the active species trapping experiments were carried out. In brief, scavengers (10.0 mM IPA, a scavenger of ·OH; 10.0 mM BQ, a scavenger of ·O$_2$·) were added into the reaction systems respectively to remove the active species under the optimal experimental conditions [23,24,43]. Then, the absorbance and color change of the reaction system was recorded at 652 nm by an UV-visible spectrophotometer.
2.6. H$_2$O$_2$ Detection

A colorimetric detection system of H$_2$O$_2$ was set up based on the nice peroxidase-like performances of MoS$_2$@CNNS(30). The reaction system contained 100 µL of 8.0 mM TMB, 200 µL of 600 µg/mL MoS$_2$@CNNS(30), 200 µL of H$_2$O$_2$ with different concentrations (0–0.1 mM), and 500 µL of 50.0 mM PBS (pH = 4.0). Then, the absorbance change of the mixture was recorded on time-mode at 652 nm with an UV-visible spectrophotometer to obtain a standard curve.

In addition, the specificity and selectivity of MoS$_2$@CNNS(30)-based detection system were evaluated by adding some potential interfering substances into the reaction system under the same experimental conditions mentioned above, such as metal and non-metal ions (20.0 mM of Fe$^{3+}$, Cu$^{2+}$, NO$_3^-$, and ClO$^-$) and common organic compounds (20.0 mM of glucose (Glu), lactose (Lac), L-valine (L-Val), and glycine (Gly)). The practical detection assay of H$_2$O$_2$ based on MoS$_2$@CNNS(30) was performed by adding commercial antiseptic solution (containing about 0.79 M H$_2$O$_2$) into the reaction system instead of standard H$_2$O$_2$ with the other operations fixed. The antiseptic liquid was diluted 1000 times before the test.

2.7. Stability and Reusability of the Catalysts

The stability and reusability of MoS$_2$@CNNS(30) were studied by recycling the H$_2$O$_2$ detection assays 10 times under optimal experimental conditions. The reaction system was monitored at 652 nm by a UV-visible spectrophotometer for 10 min. After each cycle, the MoS$_2$@CNNS(30) samples were collected by centrifugation, washed with Milli-Q water and alcohol, dried at 60 °C for 30 min, and then reused in the next cycle. The crystal structure and morphology of MoS$_2$@CNNS(30) samples after 10 cycles were characterized by XRD and TEM as described above.

3. Results

3.1. Characterization of the Catalysts

The crystal phase, structure, and crystallinity of the as-prepared samples were determined by XRD. It can be seen in Figure 1a that two tiny diffraction peaks can be barely seen in the XRD pattern of MoS$_2$ QDs, which can be attributed to the (100) and (110) lattice planes of MoS$_2$, respectively. However, the two diffraction peaks were extremely weak, which implied that the MoS$_2$ QDs had poor crystallinity [36]. In addition, the CNNS samples show a strong peak at 27.29°, which can be attributed to the characteristic of the stacking peak of π-conjugated layers and indexed for the interlayer reflection of a graphite-like structure as the (002) peak [29,36,44], consistent with the literature value (JCPDS No. 87-1526). Moreover, the absence of a (100) peak and the presence of a sharp (002) peak further confirmed the layered structures of CNNS samples and the good crystallinity of CNNS. As for the MoS$_2$@CNNS composites (Figure 1b), all the characteristic diffraction peaks can be well indexed to the graphite-like structure of CNNS (JCPDS No. 87-1526) and hexagonal phase MoS$_2$ (JCPDS Card No. 37-1492), indicating that MoS$_2$ QDs were successfully formed on the surface of CNNS through the aid of ion-exchange process. Furthermore, with increasing the loading amount of MoS$_2$ QDs, the relative intensity of corresponding (100) and (110) diffraction peaks of MoS$_2$ strengthened gradually, while the characteristic peaks of MoS$_2$ were still relatively weak and broad in the composites owing to the size effect of quantum dots. There were no significant shifts of the characteristic diffraction peaks occurring in the MoS$_2$@CNNS composites, implying that MoS$_2$ QDs existed as a separate phase rather than being incorporated into the lattice of CNNS [36]. No impurity peaks were observed in the obtained samples, indicating the pure phase of the samples. Hence, these results indicated that the MoS$_2$@CNNS nanocomposites were successfully obtained through the synergistic effect of anion-exchange and hydrothermal process.
structures with the loading amount of MoS2, which could provide a larger surface area and more reactive sites in the MoS2. Thus, the as-prepared 2D CNNS nanosheets exhibited a thinner and exfoliated lamellar structure, which could provide a larger reaction area, which effectively promoted the in-situ reaction process, resulting in the formation of MoS2 nanostructures. Moreover, the HRTEM image was examined to give further insight into the crystal structure of the MoS2@CNNS nanostructure. It can be seen in Figure 2e that a lattice spacing of 0.27 nm was obviously observed in the CNNS, which is in accordance with the (002) lattice plane of tetragonal C3N4. In addition, the QDs displayed well-defined lattice fringes parallel to each other with the same interplanar spacing of 0.27 nm, which can be indexed to the (100) lattice plane of hexagonal-phase MoS2. Meanwhile, several apparent diffraction rings were observed in the selected area electron diffraction (SAED) pattern of MoS2@CNNS(30) (Figure 2h), which can be well indexed to the lattice planes of MoS2 and g-C3N4, respectively, coinciding well with the XRD results. Therefore, these results indicated that a close-contact and well-defined MoS2@CNNS nanostructure was formed via the planar in-situ growth of uniformly-dispersed MoS2 QDs on the surface of CNNS during the protonation-assisted ion exchange process, which is a facile and controllable approach to obtain uniform and dispersive MoS2 QDs on the surface of substrate.

In order to further probe the contents of S and Mo in MoS2@CNNS nanocomposites, ICP-AES was examined. Quantitative analysis results showed the loading amount of MoS2 QDs on the surface of CNNS is about 2.0, 5.7 and 5.7 wt % for MoS2@CNNS(15), MoS2@CNNS(30) and MoS2@CNNS(45), respectively (Table S1). These results indicated that with increasing the amount of Na2MoO4·2H2O in the ion exchange process, the loading capacity and protonated reactive site of CNNS was reaching saturation, which greatly restricted the formation of more MoS2 QDs.

Figure 1. XRD patterns of MoS2 QDs and g-C3N4 (a) and MoS2@CNNS nanocomposites (b).
As is well known, a larger specific surface area will lead to more active sites, better adsorption performance, and faster electron transfer for catalysts, and will further improve the catalytic activities. Therefore, based on the XRD, TEM, ICP-AES, BET, and zeta potential results mentioned above, it can be deduced that the MoS\(_2@\)CNNS nanocomposites prepared via the protonation-assisted ion exchange process showed different morphologies and structures due to the molar ratio.

In addition, the Brunauer Emmett Teller (BET) specific surface area is a significant affecting factor for the catalytic abilities of catalysts, which was determined by the nitrogen adsorption method [23,24,45]. And the specific surface areas of MoS\(_2@\)CNNS(15), MoS\(_2@\)CNNS(30), MoS\(_2@\)CNNS(45), CNNS, and MoS\(_2\) QDs were measured as 45.58, 75.93, 69.37, 29.85 and 16.13 m\(^2\)/g, respectively. It can thus be seen that the MoS\(_2@\)CNNS(30) nanocomposites presented a larger specific surface area among the as-prepared catalysts (Figure S1a), though MoS\(_2@\)CNNS(45) loaded a little bit more MoS\(_2\) QDs, while the agglomeration of the QDs could decrease the specific surface area. As is well known, a larger specific surface area will lead to more active sites, better adsorption performance, and faster electron transfer for catalysts, and will further improve the catalytic activities. Hence, MoS\(_2@\)CNNS(30) nanocomposites are expected to display enhanced peroxidase-like activity.

Furthermore, the zeta potentials of the MoS\(_2@\)CNNS nanocomposites were tested to explore their adsorption properties to molecules with different charges, which is also a significant indicator of the catalytic activities for catalysts. Thus, the zeta potentials of MoS\(_2@\)CNNS nanocomposites were determined as \(-30.48, -63.38, -53.66, -16.35\) and \(-3.76\) mV for MoS\(_2@\)CNNS(15), MoS\(_2@\)CNNS(30), MoS\(_2@\)CNNS(45), CNNS, and MoS\(_2\) QDs, respectively (Figure S1b). The results indicated that different quantities of MoS\(_2\) QDs loaded onto the surface of CNNS contributed to different surface charges of MoS\(_2@\)CNNS nanocomposites, which would further lead to different peroxidase-like activities. Therefore, based on the XRD, TEM, ICP-AES, BET, and zeta potential results mentioned above, it can be deduced that the MoS\(_2@\)CNNS nanocomposites prepared via the protonation-assisted ion exchange process showed different morphologies and structures due to the molar ratio.
of Na$_2$MoO$_4$·2H$_2$O/CNNS, leading to their different surface/interface properties and further contributing to the distinct peroxidase-like performance.

3.2. Peroxidase-Like Activities of MoS$_2$@CNNS(30) Nanocomposites

To investigate the peroxidase-like activity of MoS$_2$@CNNS(30) nanocomposites, the TMB catalytic oxidation experiments were conducted with or without H$_2$O$_2$ by the UV-visible absorption spectra in the range of 400–800 nm. It can be seen in Figure 3a that low absorption presented in the TMB + MoS$_2$@CNNS(30), the H$_2$O$_2$ + TMB system, and the H$_2$O$_2$ + MoS$_2$@CNNS(30) system, while the H$_2$O$_2$ + TMB + MoS$_2$@CNNS(30) system exhibited an evident absorption peak at 652 nm, indicating that the MoS$_2$@CNNS(30) nanocomposites could play a key role in catalyzing the oxidation of TMB in the presence of H$_2$O$_2$. In addition, the significant color changes of different reaction systems were observed in Figure 3b, which coincided with the absorption spectra. It can be seen that the TMB + MoS$_2$@CNNS(30) system, the H$_2$O$_2$ + TMB system, and the H$_2$O$_2$ + MoS$_2$@CNNS(30) system were almost colorless. However, the H$_2$O$_2$ + TMB + MoS$_2$@CNNS(30) system showed an apparent color variation, presenting a deep blue color, further demonstrating the excellent peroxidase-like properties of the MoS$_2$@CNNS(30) nanocomposites.

![Figure 3](image_url)

Figure 3. UV-visible absorption spectra (a) and color changes (b) of different reaction systems (a. H$_2$O$_2$ + MoS$_2$@CNNS(30), b. TMB + MoS$_2$@CNNS(30), c. H$_2$O$_2$ + TMB, and d. H$_2$O$_2$ + TMB + MoS$_2$@CNNS(30)); UV-visible absorption spectra (c) and color changes (d) in the presence of different MoS$_2$@CNNS nanocomposites.

What’s more, the peroxidase-like activities of other MoS$_2$@CNNS, pure MoS$_2$ QDs, and CNNS samples were studied and compared via the catalytic oxidation of TMB in the presence of H$_2$O$_2$. It can be seen in Figure 3c that the MoS$_2$@CNNS(30)-based assay system revealed the strongest absorption peak at 652 nm, followed by MoS$_2$@CNNS(45), MoS$_2$@CNNS(15), CNNS, and MoS$_2$ QDs, confirming the best peroxidase-like catalytic activity of MoS$_2$@CNNS(30). The color variations of
different reaction systems also presented a similar result with the absorption spectra. As shown in Figure 3d, the MoS2@CNNS(30)-based assay system showed the deepest blue color compared to that of other materials. Therefore, these results further indicated the enhanced peroxidase-like activities of the MoS2@CNNS(30) nanocomposites compared to other MoS2@CNNS nanocomposites, pure CNNS, and MoS2 QDs. The more negative charge, larger specific surface area, and stable nanostructure of MoS2@CNNS(30) mainly facilitated its superior and enhanced peroxidase-like performance, which could increase the binding affinity and absorption to TMB molecules, improve the electron transfer, accelerate the reaction rate, and further improve the peroxidase-like catalytic performance [29]. On the other hand, one can find that both CNNS and MoS2 QDs showed a certain intrinsic peroxidase-like catalysis behavior toward TMB-H2O2 reaction, which could be attributed to the quantum effect and catalysis-active segments of MoS2 QDs and CNNS. Thus, when MoS2 QDs were loaded on CNNS by the in-situ growth process, the resulting MoS2@CNNS(30) nanocomposites could display much higher peroxidase-like activities than pure CNNS and MoS2 QDs on account of the synergistic effect of high conductivity and electron transfer capability for CNNS and highly-efficient intrinsic catalytic activity for MoS2 QDs [29,36]. The bonding carbon network in CNNS could donate electrons to reduce H2O2 by accelerating the electron transfer from microcosmic point of view [29]. Furthermore, as a good supporter, CNNS could apparently improve the aqueous dispersion and stability of the nanocomposites. Thus, all of the favorable factors effectively improved the peroxidase-like performances of MoS2@CNNS(30) nanocomposites. Hence, these results confirmed the enhanced peroxidase-like activities of the MoS2@CNNS(30) nanocomposites, making it a potential highly-efficient colorimetric sensor for H2O2 detection.

3.3. Steady-State Kinetics Assay

For further insight into the peroxidase-like catalytic behavior of the MoS2@CNNS(30) nanocomposites, the steady-state kinetic assays were carried out with H2O2 and TMB as substrates. The kinetic data were collected by changing the concentration of one substrate while keeping the other substrate concentration constant [4,42]. Thus, the typical Michaelis-Menten curves were recorded by varying the concentration of TMB or H2O2 while keeping the other one constant, as shown in Figure 4a,b. The steady-state kinetic reaction parameters were calculated on the basis of the Lineweaver-Burk double reciprocal plot (Figure 4c,d) according to the Michaelis-Menten equation: 

\[
\frac{1}{v} = \frac{K_m}{V_{\text{max}}} \times \left( \frac{1}{[S]} \right) + \frac{1}{V_{\text{max}}},
\]

where \( v \) is the initial velocity, \( K_m \) is the Michaelis-Menten constant, \( V_{\text{max}} \) represents the maximal reaction velocity, and \([S]\) signifies the concentration of the substrate [4,29,38,42,46,47], which were listed in Table S2. It is well known that \( K_m \) is an important indicator of the binding affinity of enzyme to the substrates, and that it affects the reaction rate; a smaller value of \( K_m \) generally means a stronger affinity between the enzyme and the substrate [4,29,38,42,46,47]. As listed in Table S2, the \( K_m \) value of MoS2@CNNS(30) with TMB was obviously lower than that of the natural enzyme HRP, implying that MoS2@CNNS(30) had a stronger binding affinity to TMB than HRP, which might be attributed to the CNNS carriers with strong adsorption to TMB [4,29,38,42]. In addition, the \( V_{\text{max}} \) of MoS2@CNNS(30) with TMB was more than two times larger than that of HRP, indicating a favorable tendency of a higher reaction rate, which could be attributed to the presence of tiny MoS2 QDs loaded on the surface of CNNS, as well as the rapid electron transfer capability of CNNS itself [29,36,38]. The \( K_m \) value of MoS2@CNNS(30) with H2O2 was higher than that of HRP, suggesting a lower binding affinity between MoS2@CNNS(30) and H2O2 than that of HRP. Furthermore, the typical Michaelis-Menten behavior towards TMB and H2O2 with various concentrations in the peroxidase-like catalytic reaction were measured, respectively. And the double-reciprocal plots (Figure 4c,d) of initial velocity against one substrate concentration showed the characteristic parallel lines, indicating a typical ping-pong mechanism of the peroxidase-like catalytic reaction [4,23,24,29,38,42,46,47]. Hence, these results inferred that MoS2@CNNS(30) bound and reacted with the first substrate and then released the first product before reacting with the second substrate, which is similar to that of HRP [1–4,42,48].
MoS$_2$@CNNS(30) bound and reacted with the first substrate and then released the first product before @CNNS(30) nanocomposites, actives species trapping experiments were carried out by adding different scavengers (IPA as OH scavengers and BQ as O$_2^-$ scavengers) into the reaction systems under other fixed experimental conditions. It can be seen in Figure 5 that an evident decrease in absorption and color fading of the reaction system was seen with the addition of BQ, while little change in absorption and color fading of the reaction system was seen with the addition of IPA. These results indicated that the MoS$_2$@CNNS(30) nanocomposites could catalytically activate H$_2$O$_2$ to generate O$_2^-$ radicals in the peroxidase-like catalytic reaction, which subsequently play major roles in oxidizing TMB to produce a TMB oxide with a blue color.

On the basis of the above experimental results and some previous literature [1–4,23,24,28–32,37–40], the peroxidase-like catalytic mechanism of MoS$_2$@CNNS(30) nanocomposites was proposed, and is illustrated in Scheme 1. It can be seen that the negatively-charged MoS$_2$@CNNS(30) nanocomposites could act as the peroxidase mimics, facilitating the electron transfer between TMB and H$_2$O$_2$ in the catalytic oxidation reaction. During the reaction process, many positively-charged TMB molecules would easily produce some active radicals such as ·OH and ·O$_2^-$ that could play significant roles in catalytic reaction [23,24,49,50]. Hence, in order to explore the peroxidase-like catalytic mechanism of MoS$_2$@CNNS(30) nanocomposites, actives species trapping experiments were carried out by adding different scavengers (IPA as ·OH scavengers and BQ as ·O$_2^-$ scavengers) into the reaction systems under other fixed experimental conditions. It can be seen in Figure 5 that an evident decrease in absorption and color fading of the reaction system was seen with the addition of BQ, while little change in absorption and color fading of the reaction system was seen with the addition of IPA. These results indicated that the MoS$_2$@CNNS(30) nanocomposites could catalytically activate H$_2$O$_2$ to generate ·O$_2^-$ radicals in the peroxidase-like catalytic reaction, which subsequently play major roles in oxidizing TMB to produce a TMB oxide with a blue color.

3.4. Active Species Analysis and Peroxidase-Like Catalytic Mechanism Study

It is reported that the reaction system contained H$_2$O$_2$, which would easily produce some active radicals such as ·OH and ·O$_2^-$ that could play significant roles in catalytic reaction [23,24,49,50]. Hence, in order to explore the peroxidase-like catalytic mechanism of MoS$_2$@CNNS(30) nanocomposites, actives species trapping experiments were carried out by adding different scavengers (IPA as ·OH scavengers and BQ as ·O$_2^-$ scavengers) into the reaction systems under other fixed experimental conditions. It can be seen in Figure 5 that an evident decrease in absorption and color fading of the reaction system was seen with the addition of BQ, while little change in absorption and color fading of the reaction system was seen with the addition of IPA. These results indicated that the MoS$_2$@CNNS(30) nanocomposites could catalytically activate H$_2$O$_2$ to generate ·O$_2^-$ radicals in the peroxidase-like catalytic reaction, which subsequently play major roles in oxidizing TMB to produce a TMB oxide with a blue color.

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![Figure 4. Steady-state kinetic analysis. The reaction velocity (v) was measured using 120 µg/mL of MoS$_2$@CNNS(30) in 25.0 mM PBS (pH = 4.0) at room temperature. The TMB concentration was varied while the concentration of H$_2$O$_2$ was 2.0 mM (a), the H$_2$O$_2$ concentration was varied while the concentration of TMB was 0.8 mM (b), and the double-reciprocal plots with a fixed concentration of one substrate relative to varying the concentration of the other substrate (c) and (d).]
the electron transfer from MoS\textsubscript{2}@CNNS(30) to H\textsubscript{2}O\textsubscript{2}, and further accelerating the TMB catalytic oxidation reaction rate \cite{4,23,24,28–32,37–40}. Subsequently, the oxidized intermediate -O\textsuperscript{2−} radicals generated in the reaction between MoS\textsubscript{2}@CNNS(30) and H\textsubscript{2}O\textsubscript{2} via one electron transfer would react with TMB molecules to generate a TMB oxide, leading to the color change of the system from colorless to blue \cite{4,23,24,28–32,37–40}. The corresponding chemical equation was: 2H\textsubscript{2}O\textsubscript{2} + TMB \text{catalysts} \rightarrow 2H\textsubscript{2}O + O\textsubscript{2} + oxTMB. Hence, in light of the excellent peroxidase-like catalytic activity, MoS\textsubscript{2}@CNNS(30) nanocomposites exhibited a promising application prospect in medical diagnostics and environmental assay.

![Graph showing absorbance at 652 nm for different scavengers](image)

**Figure 5.** Time-dependent absorbance of reaction solutions at 652 nm in the absence or presence of scavengers (10 mM of IPA and BQ) containing 25.0 mM PBS (pH = 4.0), 2.0 mM H\textsubscript{2}O\textsubscript{2}, 0.8 mM TMB, and 120 \mu{M}/mL MoS\textsubscript{2}@CNNS(30) at room temperature. Inset: related color changes.

### 3.5. Detection of H\textsubscript{2}O\textsubscript{2} by MoS\textsubscript{2}@CNNS(30)-Based Assay System

On the basis of the aforementioned intrinsic and enhanced peroxidase-like catalytic activity of MoS\textsubscript{2}@CNNS(30), a simple, rapid and ultrasensitive colorimetric method for the visual detection of H\textsubscript{2}O\textsubscript{2} was developed. As the absorbance of oxidized TMB was in proportion to the H\textsubscript{2}O\textsubscript{2} concentration, it was a simple approach to determine H\textsubscript{2}O\textsubscript{2} even at low concentrations. Furthermore, compared to some other previously-reported nanomaterials with peroxidase-like activities \cite{17,25,29,30,37,39,42,46,51} (Table S3), MoS\textsubscript{2}@CNNS(30) nanocomposites revealed a reasonable linear range and a lower detection limit for H\textsubscript{2}O\textsubscript{2} detection, further confirming their superior peroxidase-like catalytic activity and sensitivity. Therefore, these results indicated that the visual biosensing platform based on MoS\textsubscript{2}@CNNS(30) was a simple, rapid, and convenient method for H\textsubscript{2}O\textsubscript{2} detection with ultrasensitive response.
3.6. Selectivity and Applicability of MoS$_2$@CNNS(30)-Based Assay System

To estimate the selectivity of MoS$_2$@CNNS(30)-TMB-H$_2$O$_2$ detection system, some other substances, such as Fe$^{3+}$, Cu$^{2+}$, NO$_3^-$, ClO$^-$, Glu, Lac, L-Val, and Gly, with the concentration of 20.0 mM were added into the reaction system respectively as the potential interferents instead of H$_2$O$_2$. In addition, a commercial antiseptic liquid (diluted 1000 times) was used to check the practical applicability and accuracy of the H$_2$O$_2$ detection system in a real sample (RS). It can be seen in Figure 7 that no evident absorbance at 652 nm and color change were observed, though the concentrations of these interferents were 10 times higher than that of standard H$_2$O$_2$ (2.0 mM), indicating a nice selectivity of the detection system. Moreover, the diluted commercial antiseptic liquid presented an obvious absorbance and light blue color, and the concentration of H$_2$O$_2$ in the commercial antiseptic liquid could be calculated to be about 0.77 M based on the calibration curve shown in Figure 6b, which was close to the actual concentration of the commercial antiseptic liquid (0.79 M). Therefore, these results exhibited the favorable applicability in complicated conditions of the MoS$_2$@CNNS(30)-based H$_2$O$_2$ detection system, which favored the practical and rapid determination of H$_2$O$_2$ in various environments.
3.7. Stability and Reusability of MoS\(_2@\)CNNS(30)-Based Assay System

In order to study the stability and reusability of MoS\(_2@\)CNNS(30)-based H\(_2\)O\(_2\) detection system, the peroxidase-like experiments were conducted by repeating the reaction for ten successive cycles. After each cycle, the MoS\(_2@\)CNNS(30) samples were collected, washed several times with Milli-Q water and ethanol, and dried, and then reused in the next cycle. Every cycle lasted for 10 min. It can be seen in Figure 8a,b that there was no significant change of the reaction system absorbance at 652 nm during the recycling tests, accompanied by no color variations of the reaction system observed in the recycling tests, showing the excellent reusability and stable peroxidase-like performance of the MoS\(_2@\)CNNS(30)-based H\(_2\)O\(_2\) detection system. The relative standard deviation (RSD) of the absorbance values was only 1.72%, which further confirmed the nice reproducibility, stability, and reusability of the MoS\(_2@\)CNNS(30)-based assay system even for 10 cycles. Moreover, XRD and TEM were used to further analyze the crystal structure and morphology of MoS\(_2@\)CNNS(30) after ten successive cycles. It can be seen in Figure 8c that the XRD pattern of MoS\(_2@\)CNNS(30) after ten successive cycles displayed no apparent change in both peak intensity and position, implying the stable crystal structure of MoS\(_2@\)CNNS(30) samples kept in the recycling tests. In addition, no significant morphology variation was observed in the TEM image of MoS\(_2@\)CNNS(30) samples after ten successive cycles (Figure 8d), though a little impurity appeared on the surface of CNNS, exhibiting the good stability in crystal structure and morphology. Hence, good stability, reusability, reproducibility and precision of the MoS\(_2@\)CNNS(30)-based assay system suggested that the peroxidase-like colorimetric method might be used to analyze H\(_2\)O\(_2\) in real water samples, which also favored long-term use.

![Figure 8. Stability and reusability experiments of MoS\(_2@\)CNNS(30)-based assay system containing 120 µg/mL MoS\(_2@\)CNNS(30), 25.0 mM PBS (pH = 4.0), 2.0 mM H\(_2\)O\(_2\), and 0.8 mM TMB (a), (b); XRD pattern (c) and TEM image (d) of MoS\(_2@\)CNNS(30) after 10 cycles. Inset: related color changes in 10 cycles.](image-url)
4. Conclusions

In conclusion, the MoS₂@CNNS nanocomposites were successfully synthesized via a protonation-assisted ion exchange method, which were demonstrated to display an enhanced intrinsic peroxidase-like activity. The in-situ growth of MoS₂ QDs on the surface of ultrathin CNNS formed a stable nanostructure, facilitating the synergetic effects of high conductivity and electron-transfer capability for CNNS and intrinsic catalytic activity for MoS₂ QDs, and thus, greatly improving the peroxidase-like performance of the nanocomposites. In addition, the MoS₂@CNNS nanocomposites revealed different morphologies, surface properties, and peroxidase-like activities, owing to the different amount of MoS₂ QDs loading on the surface of CNNS. The MoS₂@CNNS(30) nanocomposites showed the best peroxidase-like ability among the composites, which could be attributed to their more negative potential and larger specific surface area. In the presence of H₂O₂ and the peroxidase substrate TMB, MoS₂@CNNS(30) could induce a typical blue color reaction, thus providing a colorimetric assay for H₂O₂. The catalytic activity was strongly dependent on the catalyst concentration, H₂O₂ concentration, pH, and temperature. Moreover, the kinetic and active species trapping experiments indicated that the catalytic reaction followed a ping-pong mechanism, and the ·O₂⁻ radicals played a pivotal role in the peroxidase-like catalytic reaction. Based on the excellent peroxidase-like catalytic performance of MoS₂@CNNS(30) nanocomposites, a simple, rapid, and ultrasensitive platform for colorimetric detection of H₂O₂ was developed, which exhibited a nice selectivity, reusability, long-term stability, and practicability, making it a potential biosensing material for the practical applications in H₂O₂ detection and biomedical analysis. Furthermore, this work offers some new insights and targeted directions for novel enzymatic mimics with enhanced catalytic activity.

Supplementary Materials: The following are available online at http://www.mdpi.com/2079-4991/8/12/976/s1, Figure S1: Adsorption/desorption isotherms of MoS₂@CNNS(30) (a) and zeta potentials of the MoS₂@CNNS nanocomposites dispersed in ultrapure water (pH = 4.0) (b), Figure S2: Time-dependent absorbance at 652 nm and color changes of 0.8 mM TMB reaction solutions in the absence or presence of different concentrations of MoS₂@CNNS(30) (a) and H₂O₂ (b) in 25.0 mM PBS (pH = 4.0) at room temperature. Inset: related color variations, Figure S3: Dependency of peroxidase-like activity of MoS₂@CNNS(30) on pH (a) and temperature (b) and color changes. Experiments were conducted by using 120 µg/mL of MoS₂@CNNS(30) in 25.0 mM PBS with 2.0 mM H₂O₂ and 0.8 mM TMB as substrates; Inset: related color variations, Table S1: MoS₂ loading amount in MoS₂@CNNS samples determined by ICP-AES, Table S2: Comparison of K_m and V_max between MoS₂@CNNS(30) and HRP for H₂O₂ and TMB, Table S3: Comparison of peroxidase-like activity in the linear range and detection limit of H₂O₂ between MoS₂@CNNS(30) and other peroxidase mimics.

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