Polymeric Nanocapsule Enhances the Peroxidase-like Activity of Fe₃O₄ Nanozyme for Removing Organic Dyes

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Abstract: Peroxidase-like nanozymes are nanoscale materials that can closely mimic the activity of natural peroxidase for a range of oxidation reactions. Surface coating with polymer nanogels has been considered to prevent the aggregation of nanozymes. For a long time, the understanding of polymer coating has been largely limited to its stabilization effect on the nanozyme in aqueous media, while little is known about how polymer coating plays a role in interaction with substrates and primary oxidants to dictate the catalytic process. This work reported a facile sequential modification of Fe₃O₄ nanoparticles to polyacrylamide coated nanozymes, and as low as 112 mg/L samples with only 5 mg/L Fe₃O₄ could nearly quantitatively (99%) remove a library of organic dyes with either H₂O₂ or Na₂S₂O₅ as primary oxidants. The catalytic results and molecular simulation provide both experimental and computational evidence that the hydrogen bonding interaction between the reactant and nanozyme is key for the high local concentration hence catalytic efficiency. We envision that this work, for the first time, provides some insights into the role of polymer coating in enhancing the catalytic activity of nanozyme apart from the well-known water dispersity effect.

Keywords: polymeric nanocapsule; Fe₃O₄ nanoparticle; nanozyme; peroxidase-like activity; advanced oxidation processes; organic pollutant

1. Introduction

Advanced oxidation processes (AOPs), producing highly oxidative hydroxyl radicals that react with persistent organic pollutants, have become a popular technique for wastewater remediation [1]. Among various AOPs, the Fenton process, in which Fe²⁺ triggers a decomposition of H₂O₂ into hydroxyl radicals, has attracted significant attention due to its simplicity, diverse applications, and easy operation of the reaction system [2]. Fe²⁺ could be replaced by Fe³⁺ or other transition metal ions and result in a similar process. Fenton or Fenton-like reactions produce a high concentration of hydroxyl radicals when using hydrogen peroxide as the primary oxidant [3]. These highly reactive hydroxyl radicals are responsible for further reactions involving a radical process, such as radical polymerization and pollutant degradation. However, the traditional Fenton process shows a low efficiency in generating hydroxyl radicals from hydrogen peroxide (<30%), therefore requiring the addition of a large amount of H₂O₂ (30–6000 mM) and Fe²⁺ (18–410 mM) [4]. While a high concentration of Fe²⁺ can easily form an iron slurry, leading to the toxicity of the catalyst, the system also incurs a high cost.

Nanozymes are nanomaterials that display enzyme-like reactivity but can overcome the limitations of natural enzymes, including high cost and poor stability upon changing the reaction and storage environment [5]. Recently, using metallic compounds as...
nanozymes represents a viable strategy to remove organic pollutants [6]. Firstly reported by Yan and coworkers in 2007, the ferromagnetic Fe₃O₄ nanoparticles have shown intrinsic peroxidase-like catalytic activity that could activate hydrogen peroxide and produce hydroxyl radicals, leading to the oxidation of multiple substrates [7]. Their high specific surface area and excellent stability make nanoparticles expose much more active sides during the reaction and thus can simultaneously transfer more electrons in the catalysis process. Other than Fe₃O₄ nanoparticles, many other metal nanoparticles, such as FeS [8], Cu₂Fe₂O₄ [9], CeO₂ [10], MnO₂ [11], CdS [12], and CuO-Fe₃O₄ [13] have demonstrated a similar peroxidase-like activity as Fe₃O₄. These nanozymes can efficiently oxidize and remove organic pollutants when mixed with hydrogen peroxide [14], peroxymonosulfate [9,15], or persulfate [8,16,17]. Interestingly, the Fe₃O₄ nanocatalyst can activate both hydrogen peroxide and peroxymonosulfate and persulfate [18]. Among the available non-noble metal oxide or sulfide nanoparticles, Fe₃O₄ nanoparticles stand out as an ideal catalyst due to their minimum environmental footprint, cost-effectiveness, easy access and simple separation and recycling by an external magnetic field. Unfortunately, the organic pollutants and oxidized products can also bind to the surface of nanozymes, and block the active sites, leading to the aggregation of nanoparticles and the drastic attenuation of its enzyme-like activity.

Surface modification is a potential method to inhibit aggregation and maintain the stability of Fe₃O₄ nanoparticles [19]. For example, Fe₃O₄ coated with dopamine could afford nanoparticles with better water dispersibility than the naked Fe₃O₄ nanoparticles [20]. Polydopamine has also been coated on the surface of Fe₃O₄ microspheres to assist the immobilization of Ag nanoparticles on the surface of Fe₃O₄ microspheres, resulting in fast adsorption of a model dye methylene blue (MB) [21]. Glutathione-coated Fe₃O₄ nanoparticles exhibited enhanced peroxidase-like activity for oxidizing 2,4-dichlorophenol [14]. Moreover, with the help of molecularly imprinted polymers, Liu and collaborators recently created substrate-binding pockets on the surface of Fe₃O₄ nanozymes, showing a nearly 100-fold enhancement in the substrate selectivity compared to the bare Fe₃O₄ [22]. While these studies provide evidence that surface modification of nanozymes with polymers could prevent aggregation and improve substrate selectivity, little is known about how the polymer layers interact with organic substrates, hence promoting the catalytic process.

In this work, we proposed a facile method for preparing monodisperse Fe₃O₄ nanozymes with excellent water dispersity via the classic dopamine coating and in situ polymer encapsulation. The prepared samples showed enhanced peroxidase-like activity and achieved the purpose of removing organic dyes. Importantly, we successfully employed molecular simulation methods to study the interaction and diffusion of substrates into the polymer coating, demonstrating a coordination effect of organic dyes, persulfates, and amino groups from polymers. This provided some important insights into the selection of appropriate polymers for nanozyme modification toward disparate application scenarios.

2. Results
2.1. Design and Synthesis of Fe₃O₄@Gel

Here, the oleic acid-coated nanoparticles were referred to as OA-Fe₃O₄. The alkane chains of OA are helpful for Fe₃O₄ nanoparticles to be dispersed in non-polar solvents, such as n-hexane. On the other hand, dopamine (DA) could assist Fe₃O₄ NPs dispersion in polar solvents, such as methanol or water. In this work, we coated Fe₃O₄ nanoparticles with dopamine through a phase-transfer process from the n-hexane phase to the methanol phase (Figure 1B). The obtained nanoparticle in methanol is referred to as DA-Fe₃O₄. DA has also been used to directly coat the Fe₃O₄ nanoparticles in an alkaline solution [21,23]. The driving force for such a phase-transfer process was attributed to the strong interaction with Fe₃O₄ exerted by DA. The hydroxyl groups and amide groups of dopamine endow hydrophilic to the surface of Fe₃O₄ nanoparticles and ensure their aqueous dispersibility. The averaged hydrodynamic diameter of DA-Fe₃O₄ was about 24.4 nm, as determined by DLS in Figure 2A. However, serious aggregation and precipitation of DA-Fe₃O₄ still
appeared after three-day storage in an aqueous solution (pH = 7) as the naked Fe₃O₄ (see Figure 1C).

Figure 1. (A) Schematic illustration for preparing the water-dispersible Fe₃O₄ nanoparticles via polymeric encapsulation (Fe₃O₄@Gel). (B) Nanoparticle samples dispersed in n-hexane and methanol, respectively (OA-Fe₃O₄ & DA-Fe₃O₄); (C) Nanoparticle samples freshly prepared and after three days of storage in an aqueous solution.

Figure 2. Physical characterization of the naked Fe₃O₄, DA–Fe₃O₄, and Fe₃O₄@Gel. (A) Size distribution measured by DLS; (B) XRD patterns; (C) Magnetization curves; (D) FT–IR spectra.

To tackle this problem, we further performed a chemical modification to encapsulate DA-Fe₃O₄ nanoparticles into a thin polymer layer. The amino groups of dopamine coating on DA-Fe₃O₄ NPs were chemically modified with acryloyl groups and then copolymerized with AAM monomer. The polymerization formed a porous hydrogel layer that covered DA-Fe₃O₄ NPs. Hereafter, the Fe₃O₄ embedded nanogel/nanocapsule is referred to as
Fe₃O₄@Gel. As shown in Figure 1C, Fe₃O₄@Gel was stable during three-day storage in an aqueous solution, and its hydrodynamic diameter was about 78.8 nm (see Figure 2A). We can imagine that the aggregation of Fe₃O₄ nanoparticles should be effectively inhibited after polymeric encapsulation. Notably, the corresponding peaks for DA-Fe₃O₄ totally disappeared in the diameter distribution of Fe₃O₄@Gel, indicating that all DA-Fe₃O₄ nanoparticles should be encapsulated into polymeric nanogel.

2.2. Physical Characterization of Samples

Except for the hydrodynamic diameter in an aqueous solution, more characterizations were carried out for the three Fe₃O₄ samples. In the X-ray diffraction (XRD) pattern of naked Fe₃O₄ and DA-Fe₃O₄ (see Figure 2B), Miller indices with strong response peaks were 220, 311, 400, 440, and 511, respectively. Such patterns were well-matched with the standard Fe₃O₄ (JCPDS no. 19-0629) [14], indicating high purity and good crystalline morphology for the surface-modified DA-Fe₃O₄.

The vibration sample magnetometer behavior of naked Fe₃O₄ and Fe₃O₄@Gel were measured. As shown in Figure 2C, the saturation magnetic induction of naked Fe₃O₄ was 72.8 emu/g, and slowly decreased to 64.9 emu/g after encapsulation in polymer nanogel but was still greater than 16.3 emu/g [24]. Such larger values verified that dopamine modification and polymeric encapsulation exerted little influence on the magnetic property of Fe₃O₄ nanoparticles.

The functional groups of the surface-modified Fe₃O₄ samples have also been characterized by FT-IR spectroscopy (Figure 2D). The Fe-O stretching vibration was near 588 cm⁻¹, a typical absorption band for Fe₃O₄. Compared with naked Fe₃O₄, some new absorption bands appeared in the infrared spectrum of DA-Fe₃O₄. The narrow peak located at 1488 cm⁻¹ was attributed to the C=O stretching of the phenolic hydroxyl group. The peak located at 1297 cm⁻¹ can be attributed to the C=O stretching of the phenolic hydroxyl group validating the successful preparation of DA-Fe₃O₄ [20]. For the sample Fe₃O₄@Gel, the broad peak occurring between 3500 and 3200 cm⁻¹ corresponds to hydrogen-bonded O-H stretching. The increased intensity of the peak at 1629 cm⁻¹ indicated the collective signals from the aromatic ring of DA, N-H bends, and C=O stretches of amide bonds of the polymer layer.

The dried sample of Fe₃O₄@Gel was further characterized by TEM. As shown in Figure 3, the diameter of Fe₃O₄@Gel was estimated between 10 nm and 20 nm. The high-resolution TEM image in Figure 3C displays a crystal lattice fringe with about 0.25 nm, fitting well with the cubic structure Fe₃O₄ (311) planes [14,25]. As the dried polymer nanocapsule formed a thin film around the Fe₃O₄ surface and resulted in a slight aggregation of the samples. Careful observation reveals that the Fe₃O₄@Gel samples presented a core-shell morphology with the inner Fe₃O₄ core (dark grey) and the outer polymer layer (light grey). Moreover, the elemental mapping (Figure S1 in Supplementary Materials) demonstrates that the Fe elements were in the core area while the N elements were in the shell area, unambiguously indicating the core-shell structure of Fe₃O₄@Gel nanoparticles.

2.3. Peroxidase-Mimicking Activity of Fe₃O₄@Gel

The Fe₃O₄ nanoparticles could catalytically oxidize peroxidase substrates in company with H₂O₂, exhibiting a peroxidase-like activity as firstly reported by the Yan group [7]. In this work, the peroxidase-like activities of modified Fe₃O₄ samples were evaluated according to the catalytic oxidation of TMB, a peroxidase chromogenic substrate. As shown in Figure 4A, DA-Fe₃O₄ exhibited improved peroxidase-mimicking activity, ca. 2.7-times of naked Fe₃O₄ nanoparticles. Since the naked Fe₃O₄ nanoparticles were prepared without oleic acid or dopamine, their lower catalytic degradation ability suggested that dopamine was conducive to the preparation of water-dispersed Fe₃O₄ nanoparticles, which could effectively enhance its catalytic performance. After polymer encapsulation, the enzymatic performance was further increased to ca. 3.1 times of naked Fe₃O₄ nanoparticles. As a comparison, the polymeric nanogel exhibited no significant enzymatic activity. Intuitively, the polymer nanogel seems to prevent the diffusion of organic substrates to the nanozyme.
centers. However, the results suggested that the permeability of the porous polymeric layer is good enough to allow the free diffusion of substrates and hydrogen peroxide molecules. In our previous work on encapsulating horseradish peroxidase (HRP), the low Michaelis-Menten constant and high catalytic efficiency validated the good permeability for the free diffusion of substrate molecules into the enzyme active site [26,27].

![Figure 3](image-url)  
Figure 3. TEM images of Fe₃O₄@Gel nanoparticles (A, B) and the enlarged nanoparticles showing the polymer layer (C) with an average diameter of 12.0 ± 2.8 nm. The accelerated voltage was 80 kV.

![Figure 4](image-url)  
Figure 4. Peroxidase mimic activities of Fe₃O₄ samples based on the oxidation of TMB (A) and IC (B). Reaction conditions: [H₂O₂]₀ = 20 mM, [Fe₃O₄] = 5 mg/L, [TMB]₀ = 200 mg/L, [IC]₀ = 200 mg/L, pH = 7, and T = 30 °C.

2.4. Efficient Removal of Organic Dyes with H₂O₂

Next, we explored the effect of polymeric encapsulation on the catalytic performance of Fe₃O₄ on organic dyes. Indigo carmine (IC) was chosen as another target for catalytic oxidation. As shown in Figure 4B, in the IC/H₂O₂ mixed solution, IC can be rapidly degraded with the addition of Fe₃O₄@Gel; the removal efficiency was as high as 57.6% after 15 min and 99% after 180 min. In comparison, there is almost no IC degradation after the addition of naked Fe₃O₄, DA-Fe₃O₄, or nanogel without Fe₃O₄. As aforementioned, the peroxidase-mimicking activity of DA-Fe₃O₄ and Fe₃O₄@Gel were very similar when oxidating TMB. In our opinion, this can be rationalized by the adsorption effect between the polymeric nanogel and dyes. The nanogel is composed of polyacrylamide, which is rich in amide groups. Each IC molecule contains two sulfonic acid groups and thus could be adsorbed into the nanogel through hydrogen bonding. As shown in Figure 5A, the increased local concentration of IC inside the gel increased the oxidation reaction rate.
Next, the catalytic performance was evaluated with various concentrations of $\text{H}_2\text{O}_2$ and $\text{Fe}_3\text{O}_4@\text{Gel}$. Increasing $\text{H}_2\text{O}_2$ could enhance the removal efficiency of IC, and the enhancement became less intense when $\text{H}_2\text{O}_2$ was above 20 mM, indicating that the local concentration of $\text{H}_2\text{O}_2$ inside the nanocapsules approached saturation (see Figure S2A). Similarly, the saturation concentration of $\text{Fe}_3\text{O}_4@\text{Gel}$ was $[\text{Fe}_3\text{O}_4] = 5 \text{ mg/L}$, and double loading of $\text{Fe}_3\text{O}_4@\text{Gel}$ only increased the removal efficiency from 79.7% to 93.8% (see Figure S2B). In the following studies, the dosage of $\text{Fe}_3\text{O}_4@\text{Gel}$ was fixed at 112 mg/L, which is equivalent to $[\text{Fe}_3\text{O}_4] = 5.0 \text{ mg/L}$.

The magnetic property of $\text{Fe}_3\text{O}_4@\text{Gel}$ could also be used in the effective collection of nanozymes under external magnetic fields (Figure 5). Reuse experiments were performed to evaluate the catalytic sustainability of $\text{Fe}_3\text{O}_4@\text{Gel}$. It was revealed that more than 85% of IC could still be removed in the third run. Considering the mass loss in recirculation, we can speculate that $\text{Fe}_3\text{O}_4@\text{Gel}$ nanoparticles could be a good choice in the continuous treatment of wastewater. In contrast, the catalytic performance of the $\text{Fe}_3\text{O}_4$ nanoparticles recycled three times is only 40% of the fresh sample when oxidizing acetaminophen [28].

2.5. Efficient Removal of Organic Dyes with $\text{Na}_2\text{S}_2\text{O}_8$

Other than $\text{H}_2\text{O}_2$, $\text{Na}_2\text{S}_2\text{O}_8$ can also supply free radicals in the advanced oxidation process. Here, another catalytic oxidation system was constructed based on $\text{Na}_2\text{S}_2\text{O}_8$ in company with $\text{Fe}_3\text{O}_4@\text{Gel}$. As shown in Figure 6, IC can be fully removed by both $\text{Fe}_3\text{O}_4@\text{Gel}/\text{H}_2\text{O}_2$ and $\text{Fe}_3\text{O}_4@\text{Gel}/\text{Na}_2\text{S}_2\text{O}_8$, while a remarkable difference is displayed in the oxidation of other dyes, i.e., AO7, DR81, and AZO. The oxidation performance of $\text{Fe}_3\text{O}_4/\text{Na}_2\text{S}_2\text{O}_8$ was significantly higher than that of $\text{Fe}_3\text{O}_4/\text{H}_2\text{O}_2$. This may be attributed to the longer half-life of sulfate radicals ($^3\text{SO}_4^-$), supplying a higher probability of encountering and oxidizing dye molecules before quenching. Nevertheless, the polymer encapsulation significantly enhanced the catalytic oxidation performance of the $\text{Fe}_3\text{O}_4$ nanozyme, regardless of the primary oxidants $\text{H}_2\text{O}_2$ or $\text{Na}_2\text{S}_2\text{O}_8$. Especially when oxidizing AZO, the removal efficiency was improved by 77%. Considering that all dyes in this work (i.e., IC, AO7, DR81, and AZO) contain sulfonic acid groups, the polymeric nanocapsules should be able to adsorb these dyes, thereby improving their catalytic oxidation efficiency.
were scavenged when the concentration of TBA exceeds 5 mM [29].

quickly, and the removal efficiency was as high as 73% after 150 min. As the amount of TBA
hydroxyl radicals are direct oxidants to the substrates, and almost all the hydroxyl radicals
and the removal efficiency decreased from 65% to 11%. These data agreed with the fact that
radical (•OH) scavenger. As depicted in Figure 7A, the concentration of

DMPO-OH (1:2:2:1) and DMPO-SO₄⁻ adduct () in the Fe₃O₄@Gel/H₂O₂ and
Fe₃O₄@Gel/Na₂S₂O₈ systems, respectively.

The catalytic performance was also evaluated with different loading of Na₂S₂O₈ and Fe₃O₄@Gel, and various pH. As shown in Figure S3, Fe₃O₄@Gel showed the best degradation efficiency of AO7 when pH = 7; the degradation was slightly weakened in the alkaline environment, and was only 20% in the acidic environment, especially at pH = 4. Furthermore, Figure S4 shows that increasing Na₂S₂O₈ could also enhance the removal efficiency of AO7, indicating that the local saturation concentration of Na₂S₂O₈ inside the nanocapsules should be about 10 mM. Similarly, the plateau concentration of S₂O₈⁵⁻ was close to that of H₂O₂ when using Fe₃O₄@Gel. Notably, Fenton or Fenton-like systems often used a different design of the catalysts and reaction conditions, which could be difficult for a direct comparison. As such, we have listed the catalysis performance of previous work, from which one could have a better understanding of the pros and cons of each system (Table S1).

2.6. Identification of Primary Reactive Oxidants

The catalytic degradation is usually through a radical mechanism. To identify the types of radicals in our systems, we performed EPR experiments by adding the spin-trapping agent DMPO. It is known that the Fenton reaction generates hydroxyl radicals (•OH) from H₂O₂, whereas the •SO₄⁻ is produced from the decomposition of Na₂S₂O₈. As illustrated in Figure 7B, the characteristic quartet peak with an intensity ratio of 1:2:2:1 indicated large quantities of DMPO-OH adducts in the Fe₃O₄@Gel/H₂O₂ system. In contrast, the obvious signals of both DMPO-OH (1:2:2:1) and DMPO-SO₄⁻ (1:1:1:1:1:1) appeared for the Fe₃O₄@Gel/Na₂S₂O₈ system. The highly reactive hydroxyl radicals are supposed to initiate the degradation reaction of the substrates. Deactivation of quenching these radicals with more radical-reactive species should then inhibit the degradation reaction. To prove this hypothesis, we took the Fe₃O₄@Gel/H₂O₂ system as an example by adding tert-butanol (TBA) as a typical hydroxyl radical (•OH) scavenger. As depicted in Figure 7A, the concentration of IC decreased quickly, and the removal efficiency was as high as 73% after 150 min. As the amount of TBA increased from 1 mM to 8 mM, the degradation process of IC was significantly inhibited, and the removal efficiency decreased from 65% to 11%. These data agreed with the fact that hydroxyl radicals are direct oxidants to the substrates, and almost all the hydroxyl radicals were scavenged when the concentration of TBA exceeds 5 mM [29].
was used to track the aggregating process. The large number of atomic contacts formed between IC and H$_2$O during their short lifetime (ca. 1 s) significantly inhibited, and the removal efficiency decreased from 65% to 11%. These data agreed with the fact that hydroxyl radicals are direct oxidants to the sub-

hydroxyl radicals cannot capture the organic pollutants low (<1000 ppm) in wastewater, hydroxyl radicals are direct oxidants to the sub-

Our previous work [30] revealed that the molecular interactions between the PAM nanogel and the dye (AZO) enable the dye molecules to be efficiently absorbed into the nanogel interior. In this work, IC was chosen as the mode dye, and acrylamide tetramers were used to represent the PAM nanocapsule. Using molecular simulations, we compared the intermolecular interactions existing in two typical systems, i.e., the PAM/H$_2$O$_2$/IC system and the PAM/Na$_2$S$_2$O$_8$/IC system. The three components in the simulated system were first randomly placed inside the simulation box and then aggregated, driven by molecular interactions. The number of atomic contacts between every two components was used to track the aggregating process.

As presented in Figure 8, the number of atomic contacts between dye and H$_2$O$_2$/Na$_2$S$_2$O$_8$ was the most among all the formed contacts, which increased and then approached a plateau after about 20 ns. This means that the simulated system reached the thermodynamic equilibrium after 20 ns. Several insights could be obtained from the simulation results. In the PAM/H$_2$O$_2$/IC system, the number of atomic contacts between PAM and IC increased rapidly and reached its equilibrium value of 5960 after 15 ns, indicating a strong interaction between PAM and IC. In other words, the interaction between H$_2$O$_2$ and PAM was much weaker, and the number of associated atomic contacts was only 2041. Given that the large number of atomic contacts formed between IC and H$_2$O$_2$ was ca. 15,023, H$_2$O$_2$ should also be largely absorbed into the nanocapsule. As illustrated in Figure 9, IC was the key in the adsorption process, which can interact with PAM and H$_2$O$_2$ to promote aggregation of the three components, increasing the local concentration of IC and H$_2$O$_2$ inside the nanocapsule. Considering that the concentrations of organic pollutants are very low (<1000 ppm) in wastewater, hydroxyl radicals cannot capture the organic pollutants during their short lifetime (ca. 1 µs) [31] via free diffusion. In the PAM nanocapsule, the locally concentrated dye and H$_2$O$_2$ were endowed with increased possibilities to be oxidized by hydroxyl radicals.
Figure 8. Time evolution of the simulated system as revealed by two order parameters. They are the number of atomic contacts (A) and hydrogen bonds (B) between different components, i.e., polymeric gel (PAM), dye (IC), and H$_2$O$_2$.

Figure 9. A proposed mechanism for the enhanced removal of dye pollutants via a combination of adsorption and catalytic oxidation. The yellow dashed line denoted the hydrogen bonds formed between PAM, dye, and H$_2$O$_2$.

In the PAM/Na$_2$S$_2$O$_8$/IC system, the number of atomic contacts between Na$_2$S$_2$O$_8$ and IC increased rapidly and reached its plateau value (ca. 22,257) after 20 ns, indicating that the stronger intermolecular interactions were formed between the secondary ammonia of IC and Na$_2$S$_2$O$_8$, such as hydrogen bonding. In contrast, the number of atomic contacts between PAM and IC was only 3154, which is about one-half of that in the PAM/H$_2$O$_2$/IC system. Considering that the number of atomic contacts between PAM and Na$_2$S$_2$O$_8$ was 4025, significantly larger than that between PAM and H$_2$O$_2$ in the PAM/H$_2$O$_2$/IC system, we suppose that Na$_2$S$_2$O$_8$ formed stronger hydrogen bonding with the primary ammonia of PAM, thus decreased the adsorption between PAM and IC. The three components in the PAM/Na$_2$S$_2$O$_8$/IC system can interact with each other, which is conducive to aggregation or adsorption. In contrast, H$_2$O$_2$ in the PAM/H$_2$O$_2$/IC system requires the assistance of IC to enter the nanocapsules. This also rationalizes the stronger oxidizing and removing efficiency of Na$_2$S$_2$O$_8$ on organic dyes in Figure 6.

3. Materials and Methods

3.1. Materials

Ferrous chloride tetrahydrate (FeCl$_2$·4H$_2$O, 99.95%), ferric chloride (FeCl$_3$·6H$_2$O, 99%), Oleic acid (OA, 99%) acrylamide monomer (AAM), N-acryloxy succinimide (NAS), ammonium persulfate (APS), tetramethyl ethylenediamine (TEMED), 3,3',5,5'-Tetramethyl benzidine dihydrochloride (TMB), 5,5-dimethyl-1-pyrroline N-oxide (DMPO), hydrogen peroxide

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\[ \text{FeCl}_2 \cdot 4\text{H}_2\text{O}, 99.95\% \], ferric chloride (\( \text{FeCl}_3 \cdot 6\text{H}_2\text{O}, 99\% \)), Oleic acid (OA, 99%) acrylamide monomer (AAM), N-acryloxy succinimide (NAS), ammonium persulfate (APS), tetramethyl ethylenediamine (TEMED), 3,3',5,5'-Tetramethyl benzidine dihydrochloride (TMB), 5,5-dimethyl-1-pyrroline N-oxide (DMPO), hydrogen peroxide
(30%) and sodium persulfate (Na$_2$S$_2$O$_8$) were obtained from Sigma-Aldrich Co. (Shanghai, China) Ammonia water (NH$_3$·H$_2$O, 25–28%), dopamine hydrochloride and all dyes (Indigo carmine, IC; Acid orange 7, AO7; Azophloxine, AZO; Direct Red 81, DR81) were supplied by Aladdin Co. (Shanghai, China) Other chemicals were of analytical grade and were used without further purification.

3.2. Preparation of Fe$_3$O$_4$ Nanoparticles

3.2.1. Naked Fe$_3$O$_4$ NP

Fe$_3$O$_4$ nanoparticles were prepared by a co-precipitation method with minor modifications from previous reports [32,33]. Briefly, FeCl$_3$·6H$_2$O (2.5 mmol), and FeCl$_2$·4H$_2$O (1.25 mmol) were dissolved in deoxygenated water (5 mL) to get a homogenous mixture. The mixture was slowly dropped into ammonia water (2%, 25 mL, 90 °C) under vigorous stirring (1500 rpm). After that, oleic acid (0.5 mL) was slowly added to the solution and stirred for at least 10 min. The blackish precipitates were gathered with a permanent magnet, washed by deionized water and anhydrous ethanol several times, and then homogenously dispersed in n-hexane (10 mL). Hereafter, the Fe$_3$O$_4$ nanoparticles coated with oleic acid were denoted as OA-Fe$_3$O$_4$ NP in this work.

3.2.2. DA-Fe$_3$O$_4$ NP

Dopamine was used to modify the surface of Fe$_3$O$_4$ by a phase-transfer method. Typically, 0.64 mL of Fe$_3$O$_4$ (0.08 mmol) in n-hexane was mixed with 2 mL of dopamine (0.8 mmol) in anhydrous methanol and then stirred at 1500 rpm for 1 h. The dopamine coated Fe$_3$O$_4$ NPs were transferred from the non-polar organic phase to the polar methanol phase, and then gathered and washed with deionized water several times. The obtained Fe$_3$O$_4$ NPs coated with dopamine could be well dispersed in water, which was denoted as DA-Fe$_3$O$_4$ NPs hereafter.

3.3. Encapsulation of Fe$_3$O$_4$ Nanoparticles

To synthesize the Fe$_3$O$_4$-embedded nanogel, DA-Fe$_3$O$_4$ NPs were firstly modified to introduce acryloyl groups by reacting surface amide groups with N-acryloylsuccinimide (NAS). The reaction was performed by dissolving DA-Fe$_3$O$_4$ (8 mM, 100 µL) into 1 mL deoxygenated water under magnetic stirring (1500 rpm), followed by a dropwise addition of 20 µL NAS DMSO solution (20 mg/mL). The reaction lasted for two hours at room temperature. Subsequently, the acrylated DA-Fe$_3$O$_4$ was mixed with a 40 mg AAM aqueous solution (2.5 mL). The Fe$_3$O$_4$-embedded nanogel was synthesized via in situ free radical polymerization with the addition of 4 mg APS as initiator and 15 µL TEMED as a stabilizer. After stirring for another two hours, the reaction mixture was concentrated and then washed with deionized water several times.

3.4. Characterization of Fe$_3$O$_4$ Nanoparticles

The morphology of Fe$_3$O$_4$ nanoparticles was characterized with a Talos F200× transmission electron microscope (TEM; Thermo Fischer, Waltham, MA, USA). Fourier transform infrared spectrometer NICOLET IS10 (FT-IR; Thermo Fischer, USA) was used to detect the chemical structures of naked Fe$_3$O$_4$, DA-Fe$_3$O$_4$, and Fe$_3$O$_4$@Gel nanoparticles. X-ray diffraction (XRD) analysis was obtained in an Empyrean X-ray powder diffractometer (PANalytical, Almelo, Holland). Magnetic properties of Bare Fe$_3$O$_4$ and Fe$_3$O$_4$@Gel nanoparticles were examined with a Physical Property Measurement System X-MaxN50 (Quantum Design, San Diego, California, USA). The hydrodynamic diameters of samples were measured using a Zeta Sizer Nano S90 (Malvern, Malvern, UK) after sonicating the aqueous samples for 30 min. Electron paramagnetic resonance (EPR) experiments were performed with DMPO as a spin-trapping agent on an EMX-E spectrometer (Bruker, Karlsruhe, Germany). All the spectroscopic measurements were conducted using a 96-well plate in an Epoch2 microplate reader (BioTek, Winooski, VT, USA).
3.5. Peroxidase-Mimic Activity Assessment

The peroxidase-mimic activity of the samples was measured via a luminescence method [30,34]. Briefly, the peroxidase substrates TMB was used as the subject of the catalytic oxidations, and the oxidated TMB could be detected according to the absorbance at 650 nm. The TMB/H$_2$O$_2$ system was prepared by mixing TMB (10 µL, 10 mg/mL), H$_2$O$_2$ (64 µL, 30%), and acetic acid-sodium acetate buffer solution (800 µL, pH 3.6). The oxidation reaction was started by adding nanozyme sample (200 µL, [Fe$_3$O$_4$] = 74 µg/mL) with stirring at 800 rpm and 30 °C. The color change was recorded for 15 min. The catalytic efficiency can be judged by the rate of forming oxidated TMB.

3.6. Batch Experiments for Dye Degradation

The typical process of using Fe$_3$O$_4$@Gel to treat organic dyes in the reactor was as follows: Dyes (200 µL, 5 mg/mL) and H$_2$O$_2$ (100 µL, 3%) were added sequentially in PBS buffer solutions (pH = 7.0) with stirring at 800 rpm and 30 °C. Then, the reaction was initiated by the addition of the Fe$_3$O$_4$@Gel suspension (200 µL, [Fe$_3$O$_4$] = 74 µg/mL) and the final volume was 5 mL. Each degradation process was performed with three replicates. Organic dyes were also degraded by the Fe$_3$O$_4$/Na$_2$S$_2$O$_8$ system to degrade organic dyes, and the concentration of Na$_2$S$_2$O$_8$ in the reactor was 10 mM or 0.024 mg/mL. Other conditions remained unchanged from the Fe$_3$O$_4$/H$_2$O$_2$ system.

Concentrations of dyes in the degradation process were determined by detecting the absorbance of supernatant. The characteristic absorption peaks of dyes IC, DA81, AO7, and AZO are 610, 510, 484, and 508 nm, respectively. The removal efficiency ($D_\%$) of dye could be determined as $D_\% = C_0 / C_t \times 100\%$, where $C_0$ is the initial concentration of dye, and $C_t$ represents the concentration after elapsed time $t$.

3.7. Molecular Modeling and Computer Simulations

The interaction modes at molecular level among nanocapsule, organic dye, and H$_2$O$_2$/Na$_2$S$_2$O$_8$ were explored based on all-atom molecular dynamics (AAMD) simulations. As verified in our previous work [30], the repeating unit of polyacrylamide could represent the nanocapsule material to form interactions with other components. Gauss view software (version 5.0, Wallingford, CT, USA) was used to construct the structures of acrylamide tetramers, indigo carmine, H$_2$O$_2$, and Na$_2$S$_2$O$_8$, and Automated Topology Builder (ATB) [35] was used to produce the associated topologies. Molecular interactions between each other were described by the GROMOS force field (version 54A8) [36], and water molecules were represented by the classical TIP3P model [37]. The simulated system was composed of 10 acrylamide tetramers, 50 indigo carmine, 200 H$_2$O$_2$, or Na$_2$S$_2$O$_8$, these ratios were set following the experimental feeding ratios. To make the solution electrically neutral, sodium ion (Na$^+$) and chloride ion (Cl$^-$) were added. As the size of the simulation box is 6 nm × 6 nm × 6 nm, periodic boundary conditions were applied in the x-, y-, and z-directions to approximate an infinite environment.

All AAMD simulations were conducted in the isothermal-isobaric (NPT) ensemble using the GROMACS simulation package (version 2018.4) [38] at the National Supercomputing Center in Shenzhen. The simulations were performed with a 2-fs time step at 1 bar and 310 K, and the last 20-ns trajectory was used for data analysis. The PyMOL suite (Schrödinger Co.) [39] was used to visualize the simulated systems. Other details of the simulation can be found in our previous work [30]. The number of atomic contacts between each component of the simulated system was counted via the gmx mindist utility of GROMACS; one atomic contact occurs when the distance between any two atoms in different groups is less than 0.6 nm.

4. Conclusions

We reported a facile method to prepare uniformly dispersed polymer-coated Fe$_3$O$_4$ nanozymes. The surface modification of Fe$_3$O$_4$ NPs with dopamine allows further functionalization with acryloyl groups. Followed by an in situ polymerization, Fe$_3$O$_4$ NPs
were encapsulated in polyacrylamide nanogel. In comparison with the naked Fe$_3$O$_4$ NPs, the polymer-coated nanoparticles displayed an enhanced catalytic removal efficiency to a library of organic dyes when using either H$_2$O$_2$ or Na$_2$S$_2$O$_8$ as primary oxidants. In this study, most dyes were oxidatively removed using as little as 5 mg/L of PAM-coated Fe$_3$O$_4$. The molecular simulation revealed that the coordination interactions of organic dyes, primary oxidants, and the amide groups of polymer nanocapsules could be the key to the enhanced activity of nanozymes. Moreover, the higher removal efficiency using Na$_2$S$_2$O$_8$ as the primary oxidant was attributed to the long life of the $^{*}$SO$_4$ radicals and the stronger interaction between Na$_2$S$_2$O$_8$ and the amide groups of PAM, which allows gradual conversion from sulfate radicals to hydroxyl radicals. As such, the PAM-coated Fe$_3$O$_4$ in the presence of Na$_2$S$_2$O$_8$ demonstrated systematically higher (up to above 90%) catalytic efficiency than those using H$_2$O$_2$ (mostly below 10%). While the small particle size resulted in much higher catalytic efficiency with lower catalyst loading, it resulted in the difficulty of catalyst recycling. Future work will focus on immobilizing these nanogels on the micron size carriers for better recyclability. Overall, this work provided insights into the understanding of why specific polymer coating layers could overall improve the catalytic efficiency and may guide the further design of polymer-based nanozyme in different catalytic applications.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/catal12060614/s1, Figure S1: The corresponding elemental mapping pattern of the dried Fe$_3$O$_4$@Gel; Figure S2: The catalytic degradation of IC using Fe$_3$O$_4$@Gel as a nanozyme with various concentrations of H$_2$O$_2$ (A) and Fe$_3$O$_4$@Gel (B); Figure S3: Influence of various initial parameters on the Fe$_3$O$_4$@Gel-mediated oxidation of dye pollutant: (A) Na$_2$S$_2$O$_8$ dosage, (B) Fe$_3$O$_4$@Gel loading; Table S1: A comparison of catalysis performance between this work and literature that used similar catalysis systems. Refs [40–44] are cited in the Supplementary Materials.

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