Microwave assisted polyl process for time-saving synthesis of superparamagnetic nanoparticles and application in artificial mimic enzyme

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Abstract
A microwave assisted polyl process accomplished within 10 min was developed for synthesis of superparamagnetic Fe3O4 nanoparticles (MNPs) with well controlled size between 2 and 6 nm. Effects of reaction time and temperature on the size of the MNPs were investigated through transmission electronic microscope, x-ray diffraction pattern, thermogravimetric and magnetic analysis. The results indicate that longer reaction time or higher temperature lead to formation of MNPs with larger size. As a proof-of-concept, the MNPs were utilized as peroxidase and their activity was also investigated. Oxidation of typical substrate, 3, 3’, 5, 5’-tetramethylbenzidine, can be proceeded by using the MNPs as artificial mimic enzyme. The MNPs display the maximal catalyzed activity under the optimum condition as pH = 3.5, 40 °C and concentration of TMB and H2O2 with 120 and 110 mmol L−1, respectively. This work provides a new way for fast synthesis of MNPs, which are of potential application in artificial mimic enzyme.

1. Introduction

Enzymes have been widely utilized in many fields such as biomedicine, pesticides and food science for their high substrate specificity, activities and yields [1, 2]. However, the disadvantages of natural enzymes like low stability, high cost and easy to lose catalysis activity limit their further application [3–6]. Therefore, it is of great significance to develop artificial mimic enzymes which can overcome the disadvantages of natural ones [7–12]. Several types of artificial mimic enzymes such as nobol metal or metal oxide based nanoparticles were developed and found peroxidase-like activities [13–19].

Peroxidases can catalyze oxidation reactions with hydrogen peroxide (H2O2) or alkyl peroxide. Many peroxidase enzymes (including the haem-containing enzyme HRP) and enzyme mimetics also contain Fe3+ or Fe2+ in their reaction centers. Based on the above aspiration, Yan et al [20] found that MNPs have an intrinsic enzyme mimic activity similar to natural peroxidases. The MNPs with given sizes as 30, 150 and 300 nm can be used for catalyzing the reaction of the substrate 3,3,5,5-tetramethylbenzidine (TMB) in the presence of H2O2 to produce a blue colour reaction. The other reports also testified that MNPs can be an ideal substitute for peroxidase enzymes and have greatly potential applications in artificial mimic enzymes [11, 18, 21–26]. Glucose provides human life activities with energy and plays an important role in maintaining the normal operation of various organs in the human body. Nowadays, more and more people suffer from diabetes. Development of fast and precise detection of blood sugar content is highly desired for the prevention and treatment of diabetes. It is found that H2O2 is produced by glucose reacting with glucosidase. The content of glucose can be determined by detecting the concentration of H2O2 in the reaction of glucose and glucosidase [17, 27–29]. Hui Wei and Erkang Wang firstly utilized MNPs as peroxidase mimetics to detect H2O2 and glucose [30]. Several other efforts have
been made to design and synthesize MNPs based artificial enzyme for effectively detection of glucose content [18, 23, 29].

Monodisperse and uniform size are desired when they are used as artificial enzymes. There are several methods such as coprecipitation, high temperature decomposition and non-aqueous solvent which have been well developed for the synthesis of magnetite nanoparticles [31]. Coprecipitation method is facile to prepare large scale of nanoparticles with high water stability but the size of MNPs can not be precise controlled. High temperature decomposition of iron precursors such as Fe(acac)₃ and Fe(CO)₅ leads to MNPs with well-controlled size [32, 33] but hydrophobic surface make them disperse in organic solvents and further modification is needed to transform them into hydrophilic phase. Polyols are ideal non-aqueous solvents which can dissolve most of inorganic metal compounds and provide high reaction temperature for the synthesis of nanomaterials. Moreover, polyols possess not only excellent reducibility at high temperature, capable of reducing most metal ions to metal elements, but also act as stabilizer to prevent nanoparticles from aggregation. A lot of metal and metal oxide nanomaterials [34], especially MNPs, have been successfully synthesized by polyol method [35–38]. Generally, anhydrous ferric chloride is used as precursors in the the polyol syntheses of MNPs [36, 39, 40]. The alkalinity atmosphere is required to hydrolyze Fe³⁺ to Fe(OH)₂ which is partially reduced to Fe(OH)₃ by polyol at high temperature conditions, finally leading to formation of Fe₃O₄ particles through dehydration [40]. The amount of added alkaline substance and water produced during the reaction process play very important roles to control the speed of nucleation and growth, then leading to precise tuning of particle size [38, 40]. Therefore, the syntheses of magnetite nano particles or spheres should be carried out in the sealed Teflon-lined autoclave to prevent water escaping [41, 42]. Most polyol processes are time consuming from several to dozens of hours because the up-limit temperature is 200 °C in the sealed Teflon-lined autoclave and the efficiency of heat transfer is low by traditional heater. Therefore, the development of modified polyol process with higher reaction temperature (>200 °C) and time saving is of significance in the one-step synthesis of MNPs with uniform size and high water stability.

Microwave heating is of extremely high energy efficiency as well as no temperature gradient and hysteresis effect in the high temperature reactions [43]. To set power as 300 W, most polar solvents can be heated up to reach or exceed their boiling points even in a few seconds of microwave irradiation. This ensures that all particles have the same nucleation and growth history, leading to uniform particle size. In this work, we combined the advantages of polyols and microwave heating together to develop a modified polyol process. The syntheses of MNPs can be accomplished within 10 min. As a proof-of-concept, the nanoparticles were preliminary utilized as peroxidase and the activity were also investigated. Oxidation of TMB can be proceeded by using the nanoparticles as artificial mimic enzyme. The nanoparticles display the optimum catalyzed activity under the conditions: pH = 3.5, temperature 40 °C, concentration of H₂O₂ with 120 mmol l⁻¹. The study provides a new way for fast synthesis of superparamagnetic Fe₃O₄ nanoparticles, which are of potential application in artificial mimic enzyme.

2. Experiments

2.1. Materials and equipment
Anhydrous Ferric Chloride (FeCl₃, 99%), Diethylene Glycol (99%), Anhydrous Sodium Acetate (99%), Diammonium-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS), 1,2-diaminobenzene (OPD) and 3,3',5,5'-tetramethylbenzidine (TMB) were purchased from Aladdin.

Single mode microwave synthesizer (Discover S-Class, CEM), Ultrafiltration device (AMICON 8050, MILLIPORE).

2.2. Synthesis of MNPs
In a typical synthesis, 4.0 g (50 mmol) of anhydrous sodium acetate and 2.6 g (16 mmol) of anhydrous ferric chloride were ultrasonic-assisted dissolution in 40 ml of diethylene glycol (DEG) to form clear brown solution. The synthesis was accomplished by adding 5 ml of the above solution into a 10 ml-microwave-heating glass tube and setting the reaction parameters as 240 °C, 10 min and 200 W of up-limit power. After reaction, the solution was quickly cooled down room to temperature by high-pressure air flow provided by air compressor and black colloidal dispersion was obtained. The MNPs were deposited by adding 20 ml of 5:1/v/v ethyl acetate-ethanol solution and separated by centrifuge at a speed of 5000 rpm/min. The deposit was dispersed in 40 ml of water and further purified with millipore ultrafiltration device. The final dispersion was concentrated to a volume of 5 ml. Black powder was prepared from freeze drying. The other samples were prepared by the similar procedure but setting the different reaction parameters. The samples were marked as MNP-temperature-time. For example, MNP-240-10 means that the MNPs were prepared under the setting parameters as 240 °C and 10 min.
2.3. MNPs catalyse the oxidation of peroxidase substrates

In a typical procedure, 50 μl of dispersion, containing 0.6 mg of MNPs, was added into 5 ml of acetic acid-sodium acetate buffer solution. Then, 350 μl of TMB (0.05 mol l⁻¹) and 40 μl of H₂O₂ (0.2 mol l⁻¹) were added into the above solution to make uniform solution. The solution was determined by ultraviolet spectrophotometer (PerkinElmer LAMBDA750). Iron concentration was determined by ICP.

The kinetic parameters were calculated based on the function:

\[ v = \frac{V_{\text{max}} \times [S]}{K_m + [S]} \]  \hspace{1cm} (1)

\[ K_{\text{cat}} = \frac{V_{\text{max}}}{[E]} \]  \hspace{1cm} (2)

Where \( v \) is the initial velocity, \( E \) is the MNP concentration, \( V_{\text{max}} \) is the maximal reaction velocity, \([S]\) is the concentration of substrate and \( K_m \) is the Michaelis constant.

2.4. Characterization

The size and morphology of MNPs were observed on transmission electronic microscopy (TEM, JEM-2010F). X-ray diffraction (XRD) was recorded on X'Pert Pro with Cu target (40 kv, 40 mA). Fourier transformed infrared spectroscopy (FTIR) was measured on a Nicolet6700. ThermoFische. Magnetization curves were measured on a Lakeshore 7400 vibration sample magnetometer (VSM). Hydrodynamic size was measured on Malvern nanosizer A90. The organic fraction of microsphere powder was determined by a TG209, NETZSCH thermogravimetric analyzer (heating from 30 to 1000 °C at 15 °C min⁻¹ under nitrogen flow). Raman spectra were obtained by Renishaw invia Raman microscope using a laser at 532 nm.

Figure 1. High resolution TEM image (a) and enlarged area (b); XRD pattern (c) and Raman spectra (d) of MNP-240-10.
3. Results and discussion

3.1. Phase determination of MNPs

Thanks to high energy efficiency of microwave heating (figure S1(a) available online at stacks.iop.org/NANOX/2/020001/mmedia), the reaction solution, composed of FeCl₃, CH₃COONa and DEG, was heated up to 240 °C (figure S1(c)) with 3 min under 200 W of power and fast cooled down to 60 °C under high pressure air flow (figure S1(d)). The color of reaction solution changed from brown to black after kept 240 °C for 10 min (figure S1(b)), suggesting the formation of MNPs. As shown in figures 1(a) and (b), each particle is single crystal and a value of 0.251 nm between two adjacent lattices corresponds to the lattice spacing of (311) planes of cubic magnetite. Figure 1(c) shows the XRD pattern for MNP-240-10, which prepared from 240 °C and 10 min. The peaks at 2θ = 30.1°, 35.4°, 43.1°, 53.4°, 57.1° and 62.6° can be indexed to (220), (311), (400), (422), (511) and (440) lattice planes of cubic magnetite, respectively (JCPDS 19-629). Raman is required to distinguish crystal phase between Fe₃O₄ and γ-Fe₂O₃ nanoparticles, which have the same inverse spinel structure and similar XRD patterns at nanometer scale. According to the reported results [44], there are three peaks around 350, 500 and 700 cm⁻¹ for γ-Fe₂O₃ while Fe₃O₄ just has one peak near 667 cm⁻¹. As shown in figure 1(d), only one peak is appeared at 680 cm⁻¹, indicating the crystal is Fe₃O₄.

3.2. Analysis of FT-IR

Figure 2 shows FT-IR spectra for MNP-240-10. The characteristic Fe-O vibration band at 580 cm⁻¹ was observed for all samples, suggesting the formation of iron oxide. Two peaks at 3383 and 1064 cm⁻¹ can be assigned to characteristic band absorption of -OH and C-O-C, respectively. Two peaks at 2926 and 2854 cm⁻¹ are corresponded to absorption of -CH₂-. The other two peaks at 1625 and 1430 cm⁻¹ can be attributed to the asymmetric and symmetric C-O stretching modes for the carboxylate (COO⁻) ions. In the synthesis process, there are three components including FeCl₃ as iron precursor, DEG as solvent and CH₃COONa as hydrolysis agents but without stabilizer such as polyacrylic acid or sodium citrate. Sodium acetate should be used up when

Figure 2. FT-IR spectra of MNP-240–10.

Figure 3. Zeta potential versus pH values of MNP-240–10 (a) and stability comparison of magnetite colloidal nanoparticles at different pH value (b).
hydrolysis process proceeds. Therefore, COO$^-$ is resulted from the oxidation of OH-(CH$_2$CH$_2$)$_2$-OH to OH-CH$_3$CH$_2$CH$_2$COO$^-$ at high temperature and then coated on the surface of MNPs, which allows for MNPs with high water stability.

3.3. Zeta potential and water stability

Generally, colloidal dispersion is stabilized by one of steric and charge repulsion or both of them. Larger coating thickness or more quantity of charges on the surface of the nanoparticles result in higher stability. Figure 3(a) shows the zeta potential changing with the pH values. Isoelectric point is about 7.2, indicating that there are carboxyl groups on the surface of MNPs and are stable both in acid and base circumstance. Carboxyl groups on the surface of MNPs were also confirmed by FT-IR (figure 2). To further investigate the stability of MNPs at different pH values, we made three dispersion and adjusted their pH as 5, 7 and 8, respectively (figure 3(b)). As expected, they are stable at pH = 5 and 8 but magnetically deposit at pH = 7, which indicates that the

Figure 4. TEM images of MNPs obtained from different temperature at 10 min (Scale is 20 nm).

Figure 5. TEM images of MNPs obtained from different time at 240 °C (Scale is 20 nm).
dispersion produces precipitate around electric point while are stable at both acid and base circumstance. This results combined with the analysis of zeta potential, we suggest that the water stability of the MNPs is mainly provided by interparticle charge repulsion because the chain length is too short for DEG coated on the surface to give them enough steric repulsion.

3.4. Controlling over particle size

Most polyol processes under traditional heating conditions are time consuming from several to dozens of hours because the up-limit temperature is 200 °C in the sealed Teflon-lined autoclave and the efficiency of heat transfer is low by traditional heater. Several to dozens of hours are required to get the significant changes of particle size. The particle size is mainly controlled by changing the amount of alkaline substance. Herein, microwave assisted polyol process provides a possible way to precise control the size of MNPs through reaction time and temperature. Fixed the reaction time as 10 min and changing the temperature, the particle size can be tuned from 2.1 to 5.5 nm as shown in figure 4. MNPs with the average sizes of 2.1, 2.7, 4.1 and 5.5 nm are corresponded to the reaction temperature of 220, 230, 240 and 250 °C, respectively. Fixed temperature as 240 °C and varying the reaction time from 5 min to 20 min, the sizes were successfully tuned in the range of 3.9 to 5.4 nm as shown in figure 5. MNPs with the average sizes of 3.9, 4.1, 4.7 and 5.4 nm are corresponded to the reaction time of 5, 10, 15 and 20 min, respectively. The results reveal that the sizes of MNPs are more sensitive to the reaction temperature than the reaction time. Generally, reaction rate will rise 3 ~ 4 times when the reaction temperature increase by 10 °C. The nucleation speed greatly enhances with the increase of the temperature that leads to larger size in short time. Thus, particle size is mainly controlled by the nucleation stage when the reaction time is fixed but changes the different temperature. Particle sizes varying with the reaction time mainly result from the well-known Ostwald ripening process in which small nanocrystals dissolve as a result of their high surface energy and the material is subsequently redeposited on the larger nanocrystals. Thanks to higher temperature and energy efficiency provided by microwave assisted polyol method, the MNPs are successfully synthesized within 10 min and their particle sizes are facilely controlled by changing temperature from 220 to 250 °C and the reaction time from 5 to 20 min.

According to reported results, the amount of added alkaline substance and water produced during the reaction process play very important roles to control the speed of nucleation and growth, leading to precise control of particle size. Strong base such as sodium hydroxide leads to fast nucleation and formation of high crystallinity MNPs under high temperature polyol process. Iron precursor such as FeCl3 is hydrolyzed before the reaction reaches to desired high temperature when sodium hydroxide is added in advance. Therefore, sodium hydroxide should be dissolved in polyol separately and then fast injected into the solution heated up to high temperature in advance and possessing iron precursor and stabilizer. The syntheses of MNPs carried out in the sealed Teflon-lined autoclave should mix all reactants together before heating up, in which strong base such as sodium hydroxide is not desired and weak base like urea, sodium acetate and organic amine have been used as hydrolysis agents in high temperature. Weak bases in polyol solution are of low alkalinity and lead to slow nucleation, which make polyol process time consuming from several to dozens of hours. The advantages of microwave-assisted synthesis, such as extremely high energy efficiency and rapid heating and accelerating reaction rate, were testified in this work. The total reaction time was dropped from at least 3 h to 10 min.
3.5. TGA analysis

The organic species and contents were analyzed by TGA. As shown in figure 6, higher reaction temperature and longer reaction time lead to less weight loss. Particle sizes determined by TEM show that higher temperature and longer time result in larger size. Smaller particles are of larger surface area which are capable of loading with more organic species. Organic contents for all of eight samples are listed on table 1, where the contents were calculated by total weight loss from 30 to 1000 °C deducting that caused by evaporation of free or bounded water below 150 °C. The highest organic content is given as 14.6% for MNP-240-5, which is much lower than the reported MNPs with similar size and owing 55% organic proportion prepared in the presence of polyacrylic acid as capping agents. Low organic contents further confirm that the MNPs are not stable around isoelectric point and the stability at both acid and base circumstance is resulted from charge repulsion.

![Figure 7](image.png)

**Figure 7.** The measured room-temperature magnetization curves of magnetite nanoparticles from different time (a) and different temperature (b).

| Temperature/°C | Weight loss/% | Organic content/% | Time/min | Weight loss/% | Organic content/% |
|---------------|--------------|-------------------|---------|--------------|------------------|
| <150 °C       | >150 °C      |                   |         | <150 °C      | >150 °C          |
| 250           | 1.5          | 9.1               | 7.6     | 20           | 2.3              | 10.0             | 7.7               |
| 240           | 2.2          | 12.6              | 10.4    | 15           | 2.2              | 11.4             | 9.2               |
| 230           | 2.4          | 14.1              | 11.7    | 10           | 2.2              | 12.6             | 10.4              |
| 220           | 2.4          | 14.5              | 12.1    | 5            | 3.4              | 18.0             | 14.6              |

![Table 1](image.png)

**Table 1.** Organic contents of MNPs obtained from different temperature and different time.

![Figure 8](image.png)

**Figure 8.** (a) Visual color changes of substrates TMB, ABTS and OPD catalyzed by MNP-240-10; (b) UV-visible absorbance spectra of oxidized TMB catalyzed by MNP-240-10 and MNP-220-10, respectively.
3.6. Magnetic analysis
Magnetic properties were measured by vibration sample magnetometer. The measured room-temperature magnetization curves of MNPs were recorded in figure 7. All of eight samples show no remanence and coercivity, indicating that the MNPs are superparamagnetism at room temperature. The magnetization of the MNPs was normalized by deducting the non-magnetic organic coatings according to TGA results. The saturation magnetization are 45.6, 26.8, 18.2 and 9.5 emu·g⁻¹ for MNP-240-20, MNP-240-15, MNP-240-10 and MNP-240-5, respectively (figure 7(a)). The other four samples, MNP-250-10, MNP-240-10, MNP-230-10 and MNP-220-10, have the corresponding values of corresponding saturation magnetization as 38.7, 18.7, 10.2 and 10.1 emu·g⁻¹, respectively. Although the reaction temperature as high as 250 °C, the saturation magnetization is much lower than MNPs with similar size prepared by traditional polyol methods, suggesting low crystallinity of the MNPs. The crystallinity of MNPs in the polyol process mainly dominates by the alkalinity not by the temperature and the time as reported results.

3.7. MNPs catalyse the oxidation of peroxidase substrates
Two MNPs, MNP-240-10 and MNP-220-10, were used for catalyzing the reaction of the substrate TMB in the presence of H₂O₂. As shown in figure 8(a), MNP-240-10 have catalysis activity to all of three different substrates, TMB, ABTS and OPD, which can been directly observed through the solution changing from colorless to blue, green and yellow, respectively (the inset of figure 8(a)). Figure 8(b) shows the UV-visible absorbance spectra of oxidized TMB catalyzed by MNP-240-10 and MNP-220-10. One characteristic absorption peak appears at 652 nm, consistent to the reported results. Therefore, 652 nm is set as excitation wavelength and TMB is used as substrate in the following study.

Both Enzymes and artificial mimic enzymes as catalysis are sensitive to the reaction conditions. Herein, the effects of particle size, pH value of buffer solution, reaction temperature, the concentration of TMB and H₂O₂ on the catalysis activity of MNP-240-10 were investigated. As shown in figures 9(a) and (b), different pH values between 3 and 6 and different temperature from 25 to 60 °C were set for the catalyse reaction of substrate TMB at a given concentration 100 mmol·L⁻¹ in the presence of H₂O₂ with a concentration of 150 mmol·L⁻¹. As shown in figure 9(a), the maximal catalysis activity is exhibited at pH = 3.5 and 40 °C. Subsequently, we measured the
catalysis activity at different concentration and different particle size under pH = 3.5 and 40 °C. As shown in figure 9(c), MNP-220-10 with smaller particle size shows higher catalysis activity than MNP-240-10 with larger particle size. This phenomenon may be attributed to smaller MNPs possessing larger surface area, which have more active sites for reactions. Generally, the active sites are fixed at a certain concentration of MNPs so that there would be of optimum concentration of TMB and H2O2. Herein, the ranges of concentration were 30–180 mmol l⁻¹ for TMB and 30–230 mmol l⁻¹ for H2O2, respectively. The catalysis activity goes up with the increase of TMB concentration and the highest activity appears at 120 mmol l⁻¹ shown as red curve in figure 9(d). Further increasing the concentration of TMB leads to decrease of the catalysis activity, probably due to all of active sites being occupied and incapable of catalyzing the extra TMB. Similar trend was observed and the maximal activity appear at 110 mmol l⁻¹ from different concentration of H2O2 shown as blue curve in figure 9(d). Low level of H2O2 concentration owes low content of hydroxyl radical and there are a large number of residues of exposed active sites. This phenomenon may account for the increase of catalysis activity with raising the concentration of H2O2 in the range of 30–110 mmol l⁻¹. However, the catalysis activity decreases with further increasing the concentration of H2O2 in the range of 110–230 mmol l⁻¹, which is similar to the reaction catalyzed by the haem-containing enzyme. The Kinetic parameters of Km, Vmax and Kcat were measured and the value were listed in the table 2. The Kinetic parameters are comparable with typical reported Fe3O4 results, indicating that the as MNPs are of potential application in artificial mimic enzyme.

\[ [\text{E}] \text{ is the MNP concentration, } \text{Km is the Michaelis constant, } V_{\text{max}} \text{ is the maximal reaction velocity and } K_{\text{cat}} \text{ is the catalytic constant, where } K_{\text{cat}} = V_{\text{max}}/[\text{E}]. \]

According to reported results [19, 20] and based on our experimental data, we conclude that there are total five reactions:

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^- \quad (1) \]
\[ \text{TMB} + \cdot\text{OH} \rightarrow \text{TMB}^+ + \text{OH}^- \quad (2) \]
\[ \text{H}_2\text{O}_2 + \text{Fe}^{3+} \rightarrow \text{Fe}^{2+} + \text{O}_2 + 2\text{H}^+ \quad (3) \]
\[ \text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{O}_2^- \quad (4) \]
\[ \cdot\text{OH} + \text{TMB} + \text{H}^+ \rightarrow \text{TMB}^+ + \text{H}_2\text{O} \quad (5) \]

The reaction (1) is a typical Fenton reaction. There are Fe²⁺ on the surface of Fe3O4 nanoparticles which react with H2O2 to produce free radical (·OH). The oxidation potential of ·OH is 2.73 V, which has strong oxidation ability. The reaction (2) is typical mechanism for oxidation of 3,3',5,5'-tetramethylbenzidine (TMB). The equations (3) and (4) produce Fe²⁺, Fe³⁺, H^+, which not only make the circulation of Fe²⁺ but also promote the reaction of (1) and (2). H^+ participated in oxidation of TMB to TMB⁺ requires the solution remaining in acidic condition and have enough H^+ ions so that the particles show the maximum catalytic activity at pH = 3.5, which is corresponding to the reported results [20].

4. Conclusions

A series of MNPs with well controlled size between 2 and 6 nm were synthesized within 10 min through microwave assisted polylol process. The advantages of microwave-assisted synthesis, such as extremely high energy efficiency and rapid heating and accelerating reaction rate, were testified in this work. The crystal phase of magnetite was testified by HRTEM, XRD and raman spectra. TF-IR analysis indicates that the surface of MNPs coated with one terminal oxidized DEG, which allows for MNPs with high water stability. Isoelectric point was determined at pH ≈ 7 and the organic content is low according to the measurements of TGA, which accounts for the stability of MNPs resulting from steric repulsion. As a proof-of-concept, the MNPs was utilized as peroxidase and their activity was also studied. Oxidation of typical substrate, 3, 3', 5, 5'-tetramethylbenzidine, can be proceeded by using the MNPs as artificial mimic enzyme. The MNPs display the maximal catalyzed

### Table 2. Comparison of the Kinetic parameters with partial reported results.

| Sample     | Km/mmol l⁻¹ | Vmax/mmol l⁻¹ | Kcat/S⁻¹ |
|------------|-------------|---------------|----------|
| MNP-240-10 | 0.31        | 2.1124⁻⁸      | 4.1124⁻³ |
| MNP-220-10 | 0.35        | 2.5522⁻⁸      | 4.8522⁻³ |
| Fe₃O₄[20]  | 0.998       | 3.448RL⁻⁸     | 3.028RL⁻⁴|
| Fe₃O₄[23]  | 0.24-0.71   | 0.42⁻¹        | 0.2-1.141K⁻⁴|
| Fe₃O₄[22]  | 0.374       | 2.674R⁻¹      |          |
activity under the optimum conditions as pH = 3.5, 40 °C and concentration of TMB and H2O2 with 120 and 110 mmol L−1, respectively. The study provides a new way for fast synthesis of MNPs, which are of potential application in artificial mimic enzyme.

**Data availability statement**

All data that support the findings of this study are included within the article (and any supplementary files).

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