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COMMUNICATION

Green synthesis of Fe₃O₄ nanoparticles with controlled morphology using urease and their application in dye adsorption

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This paper describes a simple and environmentally friendly method for preparing highly stable dispersions of Fe₃O₄ nanoparticles with controlled morphologies in the same synthesis system for the first time. During the process, the Fe₃O₄ nanoparticles are formed using urease as a multifunctional reagent, including catalyst, template and dispersant, due to its enzymatic activity and special enzymatic steric structure. A possible formation mechanism of such Fe₃O₄ nanoparticles with various morphologies, including nanospheres, nanosheets and nanorods, is proposed. The as-prepared nanoparticles show larger specific surface area and stronger magnetism, which make them perform better dye adsorption capacity and have a great potential in application of wastewater treatment.

Fe₃O₄ magnetic nanoparticles have attracted much interest due to their property of magnetic segregative and potential applications in different areas,^[1] such as supercapacitor electrode materials,^[1c] heavy metal adsorption,^[2] recyclable catalysts for catalytic oxidation of alcohols,^[3] magnetic carriers for protein separation^[4] and wastewater treatment.^[5] Moreover, Fe₃O₄ nanoparticles with various controlled morphologies may own some unique physical and chemical properties, such as specific surface area and unique lattice plane. To date, a variety of Fe₃O₄ nanoparticles with various morphologies, including sphere, cube, wire, tube, polyhedron, core-shell structure and ring, have been synthesized.^[6] For example, Baoyou Geng *et al.* have synthesized single-crystalline Fe₃O₄ nanotubes by using egg albumin as a nanoreactor.^[7] Kok Chung Chin *et al.* have synthesised Fe₃O₄ Nanosheets by oxidizing Fe substrates in acidic solution on a hotplate.^[8] Xiyun Li *et al.* have synthesised single-crystalline triangular Fe₃O₄ nanoprisms by hydrothermal route.^[9] Xiyun Li *et al.* have prepared 3D porous flowerlike hierarchical nanostructures by a precursor-templated conversion method.^[6h]

Nevertheless, most of these literatures only focus on a kind of morphology, of which are unsuitable for the synthesis of different morphologies nanoparticles. To our best knowledge, there have not been any literatures reporting a synthesis system that can prepare Fe₃O₄ nanoparticles with various morphologies via simply adjusting

of reaction variants. What's more, these reported strategies have the disadvantages of high reaction temperature, high pressure and strong basic conditions, expensive and complicated apparatus, time-consuming and tedious synthetic processes, or the use of organic solvents.^[6f-h,10] To overcome these shortcomings, many attempts have been tried by researchers.^[11] Yet, Fe₃O₄ nanoparticles with different morphologies fabricated by a general green chemistry method are still highly required.

In this paper, we developed a simple and environmentally friendly method for preparing highly stable dispersions of Fe₃O₄ nanoparticles with controlled morphologies, including nanospheres, nanorods and nanosheets, in the same synthesis system for the first time. Urease, a hydrolytic enzyme, was used as a catalyst and template reagent to catalyze the hydrolysis of urea and make the pH increase slowly, which intrigues the nucleation of Fe₃O₄ nanoparticles and provides the appropriate mild reaction condition for the formation of Fe₃O₄ nanoparticles with different morphologies. The morphologies of these Fe₃O₄ nanoparticles are controlled through simply adjusting the reaction temperature. No other organic reagent and polymer dispersant is added to avoid any adverse effect on environment in our method. Due to the large specific surface area and high saturation magnetization, the Fe₃O₄ nanoparticles with various morphologies have great potential in dye adsorption, which is of great importance for wastewater treatment^[12]. In this work, we take these Fe₃O₄ nanoparticles as typical examples to study their dye adsorption ability and Congo red is chosen as the absorbed dye, which is harmful to the environment and public health.

Figure 1 shows the SEM images of the Fe₃O₄ nanoparticles with various morphologies. We can find that the Fe₃O₄ nanospheres, Fe₃O₄ nanosheets and Fe₃O₄ nanorods all perform approximately uniform. Fe₃O₄ nanospheres are obtained at 60 °C with diameter of about 19±5 nm (Figure 1a, 1b). When the temperature is kept at 40 °C for 2 hours, we can get thin Fe₃O₄ nanosheets in large numbers (Figure 1c, 1d) and their thickness is less to several nanometers, which can be seen more clearly in the TEM images in Figure 2. When the reaction mixture is kept at 40 °C for an hour and then stilled for another hour with the temperature rising to 60 °C, Fe₃O₄ nanorods are formed uniformly with the averagely length of longer than 100 nm and diameter of section of 10±4 nm (Figure 1e, 1f). Through analysing the EDX pattern in Figure S2, we can demonstrate that these three kinds of nanoparticles with various

morphologies mainly consist of oxygen and iron element as well as little impurity element, including silicon, carbon etc., which may come from scanning substrate.

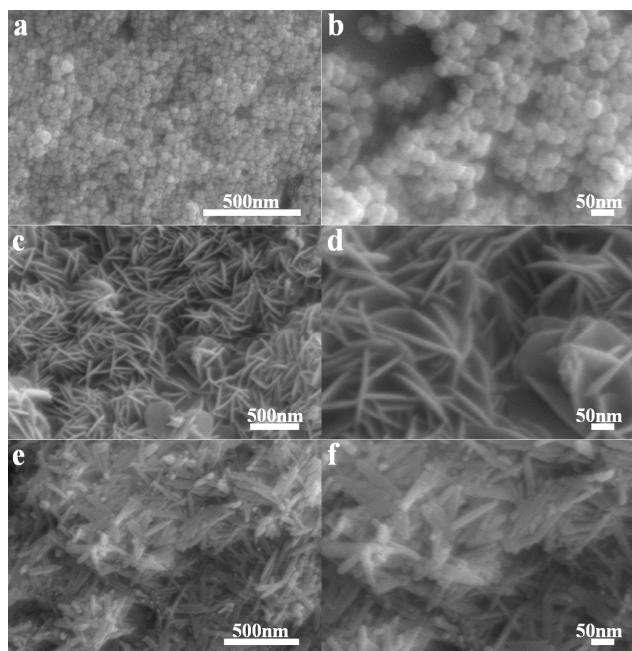


Fig. 1 SEM images of Fe₃O₄ nanospheres (a-b), Fe₃O₄ nanosheets (c-d) and Fe₃O₄ nanorods (e-f).

The size and morphologies of Fe₃O₄ nanoparticles are further examined with transmission electron microscope (TEM). As shown in Figure 2a and 2b, the nanospheres are well-distributed and its diameter is about 19±5 nm. Figure 2c and 2d show the TEM image of Fe₃O₄ nanosheets and they can indicate more clearly that the nanosheets perform quite thin to several nanometers. Figure 2e shows that the length of the Fe₃O₄ nanorods is longer than 100 nm with a diameter of section of 10±4 nm, which can be seen more clearly in Figure 2f. All these analysis results are well identical with those in Figure 1, and all these morphology characteristics make them perform better adsorptive property of dye and be applied to wastewater treatment.

In the reaction solution, urease catalyzes the hydrolysis of urea and releases of hydroxyl ion (Equation 1). The pH of the reaction mixture tends to increase due to the production of hydroxyl ion. When it is higher than 7.6, precipitation reaction of ferrous chloride will proceed and ferrous hydroxide is produced (Equation 2). Owing to that some atmosphere influxes into the reactor, oxidation process of a part of ferrous hydroxide proceeds and ferroferric oxide materials are obtained (Equation 3).^[6a,10,13] With the consecutive producing of hydroxyl ion, the catalytic activity of urease decreases gradually due to moving away from the optimal pH value. Thus the rate of decomposition of urea is gradually hampered and the reaction condition is kept nearly constant, which is appropriate for the aggregation of nanoparticles. The symmetrical size and morphology of these materials are mainly due to the regulation of urease.

Ammonia yielded from the hydrolysis of urea using urease can catalyze the mineralization of crystalline Fe₃O₄.^[14] As shown in Scheme 1, due to the thermostability of urease, when the reaction temperature reaches 60 °C, the catalytic activity of urease is higher. Because the hydrolysis reaction of urea is very rapid, the concentration of hydroxyl ion increases significantly. There are no surfactants or templates involved in this process and the surfaces of Fe₃O₄ crystal nucleus are smooth. Thus the Fe₃O₄ nanospheres are

formed by free growing up and aggregation of the freshly Fe₃O₄ crystal nucleus, driven by the minimization of interfacial energy.^[6a,15]

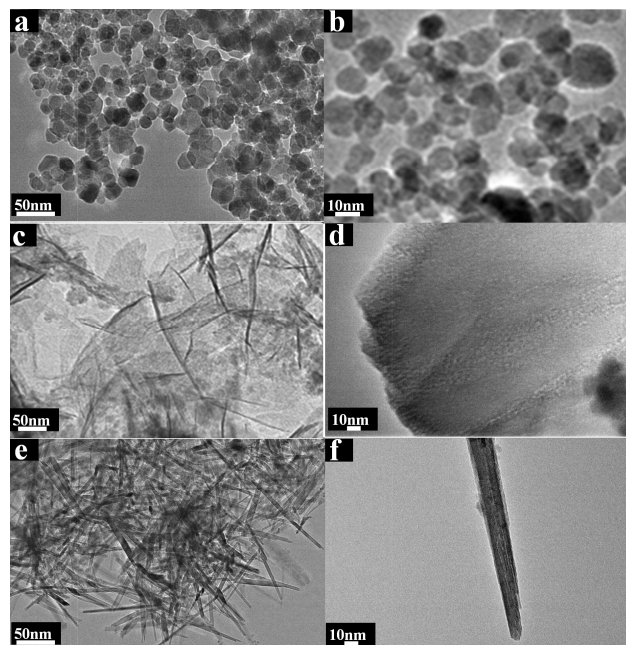
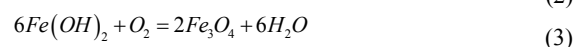
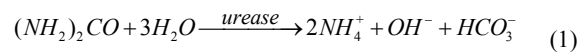
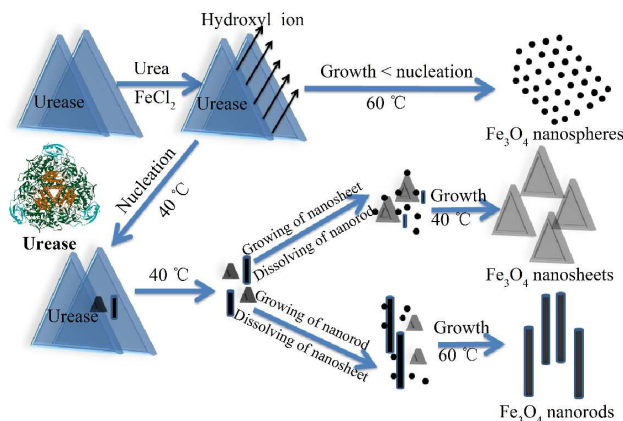


Fig. 2 TEM images of Fe₃O₄ nanospheres (a-b), Fe₃O₄ nanosheets (c-d) and Fe₃O₄ nanorods (e-f).



To explore the formation mechanism of nanosheets and nanorods, the kinetics of the reaction was investigated in detail through SEM analysis, as shown in Figure S1. Because it owns an affinity for ferrous ion in the active sites located between two trimers, urease can act as a template to form Fe₃O₄ nanosheets and nanorods during the process.^[13a] Aqua ammonia produced from the urea-urease reaction also plays a key role in the formation of Fe₃O₄ nanoparticles. The urea-urease reaction acts as a NH₃·H₂O buffer and provides the system with a constant and even supply of OH⁻ anions. As the temperature of the system is decreased to 40 °C, the rate of the urease catalyzed reaction decreases because the enzyme becomes inactive. The slow supply of a low content of OH⁻ anions could be confined in the trimers. Thus, the Fe₃O₄ nanosheets and nanorods nucleus appear between the template simultaneously. The number and size of underdeveloped nanoparticles become different as the reaction temperature changes. As the temperature maintains at 40 °C for another hour, the size of nanosheets becomes larger and their number increases, while the nanorods disappear gradually. These may due to that the nanorods dissolve into small particles, and the nanosheets crystals grow by the adsorption of these particles onto their surfaces (Scheme 1). In contrast, when the reaction temperature rises to 60 °C, the size of nanorods increases while the nanosheets disappear slowly, suggesting that the nanorods crystals grow through adsorbing small particles coming from the slowly dissolving of nanosheets (Scheme 1). In summary, these results are mainly owed to the competitive growth of Fe₃O₄ nanorods and nanosheets at different temperature (High temperature is good for the growth of Fe₃O₄ nanorods and low temperature for Fe₃O₄ nanosheets), containing three stages: preliminary crystallization, selective crystal dissolving, and recrystallization.^[14b] Hence, crystals growth passed

through the dissolution to conduct mass transport and then recrystallization process.^[16] The SAED images in Figure S3 also indicate the growth direction of Fe₃O₄ nanoparticles in various morphologies.



Scheme 1 Schematic illustration of assembling Fe₃O₄ nanoparticles.

XRD data was measured to establish the composition and phase purity of the as-prepared Fe₃O₄ nanoparticles as shown in Figure 3a. The positions and relative intensities of the reflection peak of Fe₃O₄ nanoparticles confirm the structure of the magnetite, which agree well with those XRD patterns of Fe₃O₄ PDF card (65-3107). The characteristic peaks can be identified at $2\theta=30.1^\circ$, 35.5° , 43.1° , 53.4° , 57.0° , and 62.6° , which are marked by their indices (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1), and (4 4 0) respectively. Because the crystallinity of the Fe₃O₄ nanoparticles is related to the temperature, the mild low temperature results in the poor crystallinity.

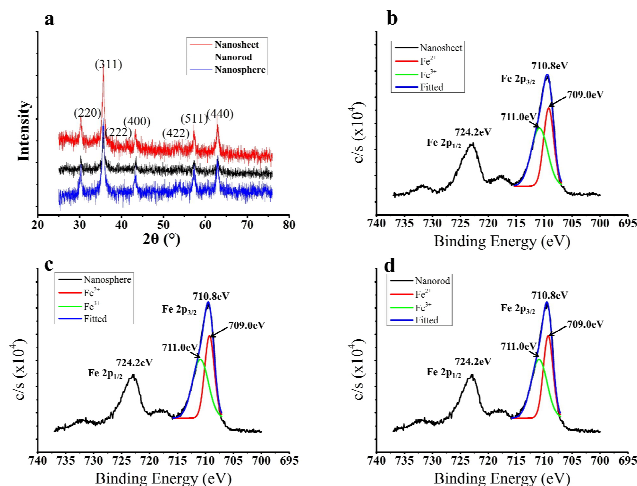


Fig. 3 XRD pattern of Fe₃O₄ nanoparticles (a), XPS spectra of the fitted Fe 2p_{2/3} peak from the Fe₃O₄ nanosheets (b), nanorods (c) and nanospheres (d).

X-ray photoelectron spectroscopy (XPS) was used to study the valence states of the as-prepared Fe₃O₄ nanoparticles, including nanosheets, nanorods and nanospheres. The full XPS spectra in Figure S5 confirm the existence of Fe and O elements in the nanoparticles. Figure 3b–d show XPS spectra of the fitted Fe 2p peak from the products in detail. For Fe₃O₄ nanosheets, nanorods and nanospheres, the XPS spectra of Fe 2p were very similar with two peaks at 710.8 and 724.2 eV, corresponding to the peaks of Fe 2p_{3/2} and Fe 2p_{1/2} in Fe₃O₄, respectively. The Fe 2p_{2/3} peak was

divided into two peaks at 709.0 and 711.0 eV, corresponding to the peak of Fe²⁺ and Fe³⁺, respectively. The relative areas of Fe²⁺ and Fe³⁺ were calculated with the value of 0.33:0.67, corresponding to that of the stoichiometry of Fe₃O₄, which could also be shown as FeO · Fe₂O₃. The results of XPS spectra all confirm the obtaining of Fe₃O₄ nanoparticles.

The composition of the as-prepared nanoparticles can also be confirmed by the FT-IR spectra and TGA curve in Figure S4.

Nitrogen adsorption was measured to confirm the specific surface area of the three kinds of Fe₃O₄ nanoparticles. The consequence shows that the specific surface area is 80.91, 83.06 and 71.19 m² g⁻¹ for Fe₃O₄ nanosheets, Fe₃O₄ nanorods and Fe₃O₄ nanospheres, respectively. The large specific surface area makes Fe₃O₄ nanoparticles own potential application of dye adsorption.

The magnetic properties of the Fe₃O₄ nanoparticles were studied by a vibrating-sample magnetometer and shown in Figure 4. The saturation magnetization (M_S) of Fe₃O₄ nanospheres, nanosheets and nanorods can reach 52.63, 27.62 and 15.76 emu g⁻¹, respectively. The M_S of Fe₃O₄ nanospheres is the strongest among these three kinds of nanoparticles, followed by Fe₃O₄ nanosheets and Fe₃O₄ nanorods. The results are agree well with XRD patterns and better explained with the crystalline degrees at different reaction temperature in Figure 3a. Figure 4b is close-up of origin in a, which shows that their remanent magnetization (M_R) are 7.72, 0.86 and 2.58 emu g⁻¹, respectively. Due to their superparamagnetism properties and large specific surface area, the Fe₃O₄ nanoparticles are possible for application in adsorption of dyes, such as Congo red.

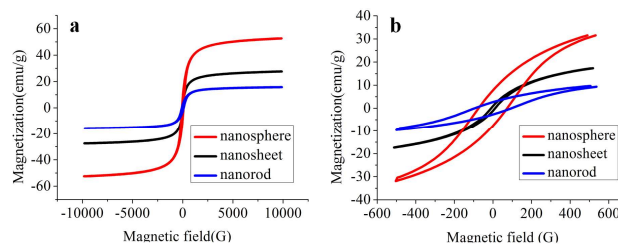


Fig. 4 (a) Magnetic curves of Fe₃O₄ nanoparticles and (b) close-up of origin in (a).

In order to obtain the suitable conditions of the adsorption process, the influences of the initial concentration of Congo red and Fe₃O₄ nanoparticles are discussed in Figure S6, Figure S7 and Figure S8. Through comprehensive analysis, 5 mg ml⁻¹ of Fe₃O₄ nanoparticles and 10 mg L⁻¹ of Congo red were chosen in this paper.

Figure 5a compares the adsorption results of Congo red adsorbed by Fe₃O₄ nanoparticles in various morphologies, which can clearly demonstrate that Fe₃O₄ nanoparticles own better adsorption capacity of Congo red, with the maximum adsorption percent of 87.08%, 78.46% and 68.14% for nanosheets, nanorods and nanospheres respectively. This result of extremely quick adsorption by the Fe₃O₄ nanoparticles owes to the high affinity of the positively charged surface to the negatively charged dye species as well as the contribution of physical absorption.^[17]

Take Fe₃O₄ nanosheets as an example, we studied the adsorption process in detail. Comparing the spectrum at 0 min with that of 10, 20, 30, 40, 50 and 60 minutes in Figure 5b, we can observe that the intensity of the Congo red characteristic peaks decreases gradually. The UV-Vis spectra of Congo red in the presence of Fe₃O₄ nanospheres and nanorods during the adsorption process are shown in Figure S9, the regulation remains similar with Figure 5b. Figure 5c reveals the analytic result of Figure 5b and makes clear the adsorption rate of Congo red. When making a comprehensive analysis, we can find that in 10–15 min, the adsorption rate of Congo red can reach the maximum and when time

is longer than 10 min, the adsorption rate gradually slows down. As adsorption time goes by, the adsorption percent of Congo red gradually reaches the maximum of 85%~90%. Figure 5c also shows the visual adsorption effect of Congo red, which displays that the color of Congo red is bright red before the addition of Fe₃O₄ nanosheets. With the addition of Fe₃O₄ nanosheets, the color of red fades over the time. When the time reaches 50~60 min, the suspension becomes almost colorless. It can make a summary that Fe₃O₄ nanoparticles can be well applied to dye adsorption and wastewater treatment.

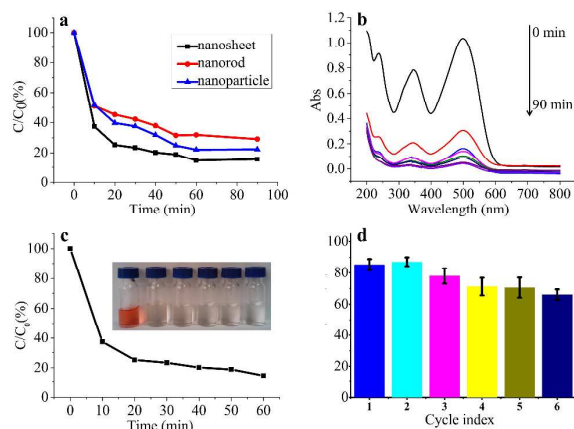


Fig. 5 (a) Adsorption results of Congo red by Fe₃O₄ nanoparticles in various morphologies with the equal concentration of 5 mg ml⁻¹, (b) UV-Vis spectrophotometer spectra of Congo red in the presence of Fe₃O₄ nanosheets (5 mg ml⁻¹) in different adsorption times, the lines from top to bottom represent the spectrum of 0 min to 90 min respectively, (c) adsorption rate of Congo red in the presence of Fe₃O₄ nanosheets (5 mg ml⁻¹), the insert picture represents the visual adsorption effect of Congo red, (d) repeating utilization rate of Fe₃O₄ nanosheets after the adsorption procedure being repeated for 6 times.

Figure 5d shows the correlation between the adsorption efficiency of Congo red and cycle index, which expounds that the adsorption percent can reach 85%~90% at the first time of adsorption and decreases time by time. But when cycle index increases to 6 times, the percent can still reach 65%~70%, with performing better cyclic utilization rate. The samples collected from eluate were analyzed in an UV-Vis spectrophotometer. The positions of peaks of spectrogram agreed well with those of Congo red, which demonstrated the efficient recovery of Congo red.

Conclusions

In summary, Fe₃O₄ nanoparticles with various morphologies were prepared by a simple and environmentally friendly method in the same synthesis system for the first time. The synthetic method had also overcome the disadvantages of high reaction temperature and pressure conditions, expensive and complex equipment, tedious and time-consuming synthetic procedures reported in previous literatures. Urease was used as a multifunctional reagent to intrigue the nucleation and growth of Fe₃O₄ nanoparticles, and to maintain the reaction condition appropriate mild for the even aggregation of Fe₃O₄ nanoparticles, with considering its enzymatic activity, steric structure and thermostability. A possible formation mechanism of such Fe₃O₄ nanoparticles with various morphologies was proposed. These nanoparticles all own well-distributed size, large specific surface area and strong magnetism, which made them perform strong adsorption efficiency and rate as well as well cyclic utilization rate in the adsorption of Congo red. Due to these properties above, the as-prepared Fe₃O₄ nanoparticles in various morphologies obtained

by green chemistry method could be well applied to dye adsorption for wastewater treatment.

Experimental Section

Ferrous chloride (FeCl₂•4H₂O) and urea were purchased from Xilong Chemical Co., LTD. Urease was purchased from J&K Scientific LTD. Aqua ammonia was analytical grade and commercially available products. All the reagents used in this work were products of analytical purity, and they were used without further purification. Deionized water was used in all experiments.

The detail procedure of preparing Fe₃O₄ nanoparticles with various morphologies was as follows, deionized water (50 ml) was heated to 100 °C in a conical flask and then urea (1.2 g) was added into the flask. After cooling down to room temperature rapidly, the pH of the solution was adjusted to 7.0 by using 3 M of aqua ammonia. Then the solution was transferred into a 3-neck flask equipped with ferrous chloride (0.08 g) beforehand. The reaction mixture was kept at constant temperature in water bath with lasting mechanical stirring and urease solution (0.1 ml, 1 mg/ml) was then added into the mixture solution. After maintained at constant temperature for a certain time, the products were separated with a magnet and washed for 3~5 times with deionized water, dried in thermostat drying box at 50 °C. The different reaction temperature gave various morphologies of Fe₃O₄ nanoparticles.

The adsorption experimental process is as below, a certain volume of Fe₃O₄ nanoparticles solution (2 ml, 5 mg ml⁻¹) was added into Congo red solution (5 ml, 10 mg L⁻¹). Subsequently, the mixture solution was placed on the shaking table at 30 °C. Take out 1.5 ml mixture solution every 10 minutes and separate Fe₃O₄ nanoparticles from the solution by a magnet, the UV-Vis adsorption spectrum of the supernatant fluid were measured. In order to analysis the correlation between the adsorption efficiency of Congo red and cycle index, the following process was designed: after adsorption of Congo red, the Fe₃O₄ nanoparticles were separated with a magnet from the solution and washed with alcohol for 3~5 times, subsequently dried in a thermostat drying box at 50 °C. The adsorption experiment was recycled for 6 times.

Acknowledgments

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Notes and references

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† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/c000000x/

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Green synthesis of Fe_3O_4 nanoparticles with controlled morphology using urease and their application in dye adsorption

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This paper describes a green method for preparing Fe_3O_4 nanoparticles employing urease and explores their application in dye adsorption.

