
Supplementary Materials

Bimetallic Fe₃O₄@Co₃O₄/CN as a Nanozyme with Dual Enzyme-Mimic Activities for the Colorimetric Determination of Mercury(II)

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Text S1 Synthesis of Fe₃O₄ – PVP@ZIF – 67

Synthesis of Fe₃O₄ Particles

Fe₃O₄ was prepared by means of a solvothermal reaction as reported previously. Briefly, 2.70 g of FeCl₃ • 6H₂O and 7.20 g of sodium acetate were dissolved in 100 mL of ethylene glycol under vigorous stirring. The resulting homogeneous yellow solution was transferred to a Teflon – lined stainless – steel autoclave, sealed, and heated at 200°C. After the reaction was allowed to proceed for 8 h, the autoclave was cooled to room temperature. The resulting black magnetite particles were washed several times with ethanol and dried in vacuum at 60°C for 12 h.

Synthesis of Fe₃O₄ – PVP

The PVP – modified Fe₃O₄ nanoparticles were prepared via a previously reported method with slight modification. Fe₃O₄ nanoparticles (1 g) were dispersed into a PVP solution (2.5 g of PVP, 300 mL of ethanol). After the above solution was stirred for 24 h, the PVP – modified Fe₃O₄ nanoparticles were washed using ethanol and distilled water for several times and dried in vacuum at 60°C for 12 h. It was marked as Fe₃O₄ – PVP.

Synthesis of Fe₃O₄ – PVP@ZIF – 67

Fe₃O₄ – PVP@ZIF – 67 was synthesized by microwave – assisted solvothermal method. The microwave instrument (MDS – 8G, China) frequency was 2450 MHz. Sample reaction containers were made of chemical resistant material (strengthened poly tetra fluoroethylene, TFM), which are temperature resistant up to 250°C . The microwave cavity was capable of holding up to 10 × 80 mL of these digestion vessels on a turntable at the same time. The microwave instrument had a high – precision temperature measurement control system, temperature range: 0 – 300°C, accuracy ± 1°C. Fe₃O₄ – PVP microspheres (0.291 g), Co(NO₃)₂ • 6H₂O (0.291 g), 2 – MIM (0.656 g), and 30 mL methanol were reacted under the microwave (300 W) at 150°C for 30 min. The heating ramp and dwelling time were 7 and 23 min, respectively. Purple black solid was produced and washed with methanol several times. The obtained Fe₃O₄ – PVP@ZIF – 67 was dried in vacuum at 60°C for 12 h.

Text S2 Optimization of $\text{Fe}_3\text{O}_4@\text{Co}_3\text{O}_4/\text{CN}$ preparation conditions

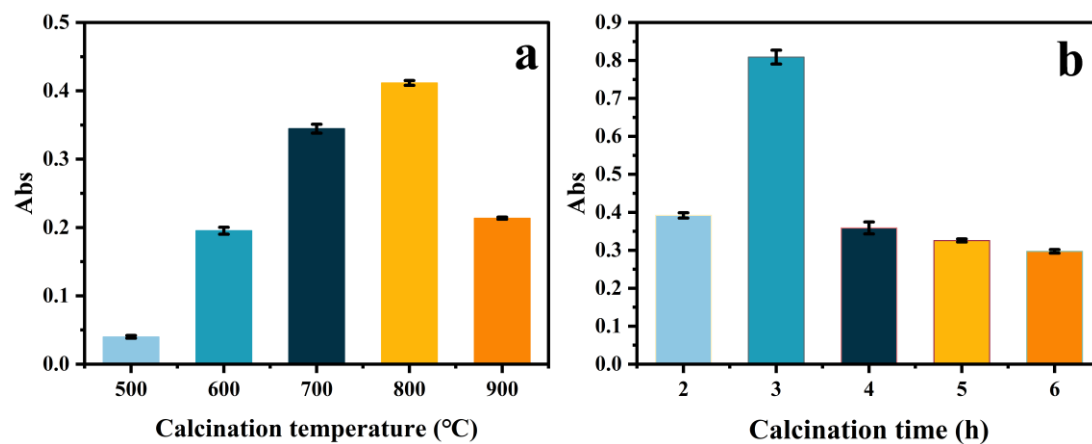


Figure S1. The oxidase - like activity of prepared $\text{Fe}_3\text{O}_4@\text{Co}_3\text{O}_4/\text{CN}$ with different calcination temperature (a) and calcination time(b).

Text S3 Procedure of the catalytic performance for Fe₃O₄@Co₃O₄/CN

The peroxidase – like (POD) catalytic performance was completely investigated as follows: 200 μL of Fe₃O₄@Co₃O₄/CN suspension (2.0 mg/mL, water suspension), 40 μL of TMB solution (10 mM, ethanol solution), and 50 μL of H₂O₂ (40 mM, diluted with water) were added in 3710 μL of HAc – NaAc buffer solution (pH 3.5) in turn. The mixed solution (with a total volume of 4.0 mL) was incubated at 45°C for 15 min and then immediately separated by an external magnet. The color and ultraviolet – visible absorption spectroscopy ranged from 200 nm to 800 nm of the isolated supernatant were recorded.

The oxidase – like activity of Fe₃O₄@Co₃O₄/CN was estimated by catalytic oxidation of TMB in the absence of H₂O₂. In brief, 100 μL of Fe₃O₄@Co₃O₄/CN water suspension (2.0 mg/mL) and 40 μL of TMB ethanol solution (10 mM) were added in 3860 μL of HAc – NaAc buffer solution (pH 3.5) in turn. The suspension was incubated at 40°C for 15 min and then immediately detached by an external magnet. The supernatant was then measured by ultraviolet–visible absorption spectroscopy.

Text S4 Optimization of experimental conditions of oxidase - like activity of Fe₃O₄@Co₃O₄/CN.

Similar to other artificial enzymes, the oxidase - like activity of the Fe₃O₄@Co₃O₄/CN would be also dependent on the solution pH, reaction temperature, reaction time and concentration of the Fe₃O₄@Co₃O₄/CN. The general operation of the oxidase - like activity investigation experiment was as follows: 100 µg· mL⁻¹ Fe₃O₄@Co₃O₄/CN catalyzed the reaction containing TMB (0.1 mM) in acetate buffer (0.1 M, pH 3.5) at 35°C for 10 min. The following experiments were optimized based on the above initial conditions by the single - factor experiment. As shown in Fig. S2(a), in the pH range of 2.0 - 3.5, the catalytic activity increases with increasing pH of the solution. However, the catalytic activity decreases again in the pH range of 3.5 - 5.0. Therefore, 3.5 was chosen as the optimum pH value for the Fe₃O₄@Co₃O₄/CN nanozymes system and used for the subsequent work. Secondly, the effect of temperature at 20°C - 70°C on the catalytic activity of oxidase was examined (**Error! Reference source not found.**(b)). The catalytic activity increased more gently from 20°C to 40°C, reached a peak at 40°C, and decreased linearly above 40°C. Thirdly, the change of oxidase - like catalytic activity was examined for incubation times ranging from 5 min to 45 min (**Error! Reference source not found.**(c)), with the peak of oxidase - like activity at 15 min. At last, as shown in the **Error! Reference source not found.**(d), the oxidase - like activity of the Fe₃O₄@Co₃O₄/CN increase with increasing the concentration of the Fe₃O₄@Co₃O₄/CN from 5 to 50 µg/mL whereas the activity decrease from 50 µg/mL to 100 µg/mL. This can be explained that as the higher the concentration of catalyst, the more the number of catalytic sites. However, excess material dose can lead to excess active sites competing with each other, resulting in the effective active sites cannot be fully utilized. Here, we used a concentration of 50 µg/mL Fe₃O₄@Co₃O₄/CN in further detection for mercury ion. All in all, the optimal oxidase - like activity of the Fe₃O₄@Co₃O₄/CN was obtained under the following conditions: reaction temperature and time 40°C for 15 min, pH 3.5, and 50 µg/mL Fe₃O₄@Co₃O₄/CN, which was listed in Table S1.

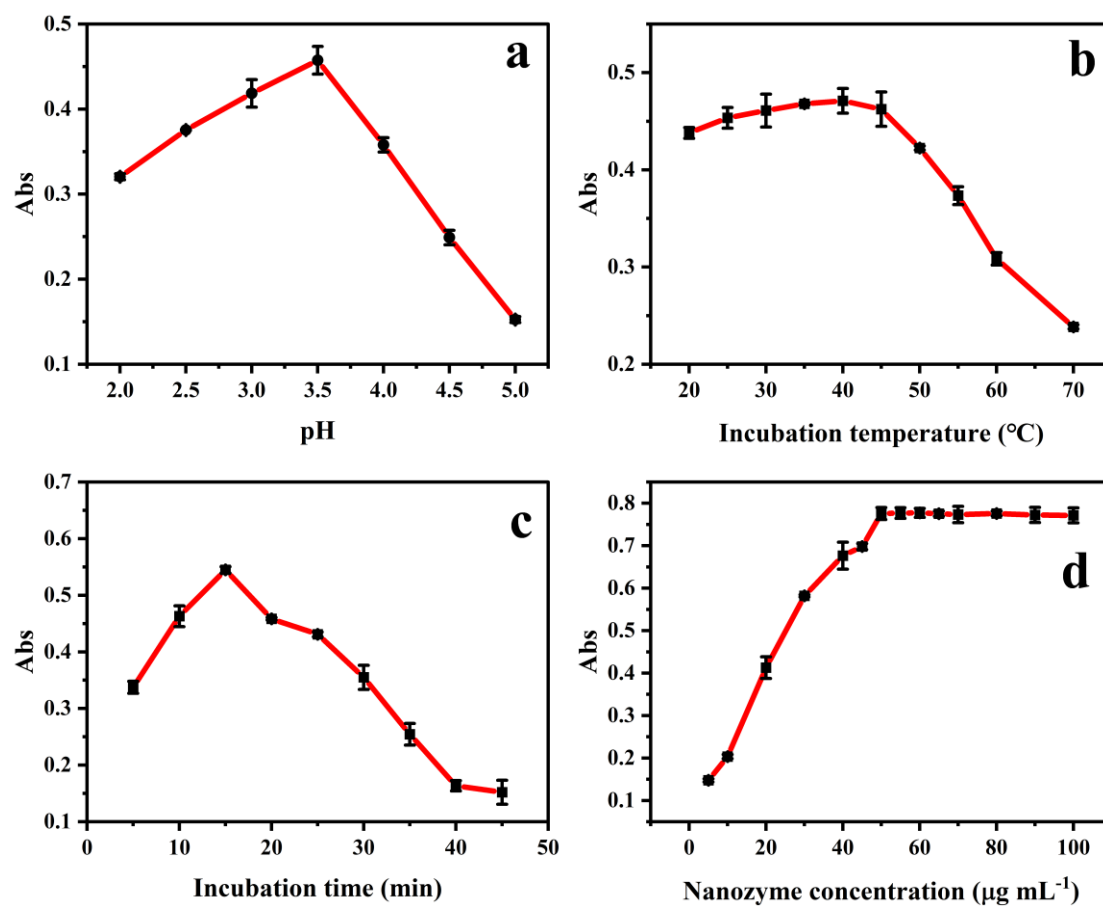


Figure S2. Examining the effect of pH (a), incubation temperature (b), incubation time (c) and nanozyme concentration (d) on $\text{Fe}_3\text{O}_4@\text{Co}_3\text{O}_4/\text{CN}$ oxidase activity.

Text S5 Optimization of experimental conditions of peroxidase – like activity of Fe₃O₄ – PVP@ZIF – 67.

The POD – like activity of Fe₃O₄ – PVP@ZIF – 67 was strongly influenced by the buffer solution concentration, pH, reaction temperature, reaction time, nanozymes concentration and H₂O₂ concentration. In this experiment, the above six influencing factors were investigated under the initial reaction conditions through a single – factor experiment with POD – like activity as an indicator. The general operation of the POD – like activity investigation experiment was as follows: 50 µg·mL⁻¹ Fe₃O₄ – PVP@ZIF – 67 catalyzed the reaction containing TMB (0.1 mM) in acetate buffer (0.1 M, pH 3.5) at 45°C for 15 min. The following experiments were optimized based on the above initial conditions by the single – factor experiment. (i) The amount of nanozyme was tested from 5 to 50 µg·mL⁻¹; (ii) the incubation time was set from 5 to 60 min; (iii) the incubation temperature was ranged from 20 to 70° C; (iv) the pH was adjusted from 2.5 to 6.5; (v) the buffer concentration was investigated from 0.05 to 0.5 M; (vi) the H₂O₂ concentration was in the range from 0.05 to 1.0 mM.

From **Error! Reference source not found.**, it can be seen that the maximum absorbance value was achieved when the concentration of HAc – NaAc was 0.1 M; the maximum absorbance was achieved at 40 µg · mL⁻¹ of Fe₃O₄ – PVP@ZIF – 67; the best absorbance value was achieved at pH 3.5; the maximum absorbance was achieved at an incubation temperature of 50°C; the absorbance reached its peak at 35 min of reaction; the maximum absorbance was achieved at a concentration of H₂O₂ of 0.6 mM. The optimum conditions for catalysis were determined as follows: 0.1 mM TMB, 0.1 M HAc – NaAc (pH 3.5), 40 µg · mL⁻¹ Fe₃O₄ – PVP@ZIF – 67, 0.6 mM H₂O₂ and a water bath at 50°C for 35 min, which was listed in Table S1.

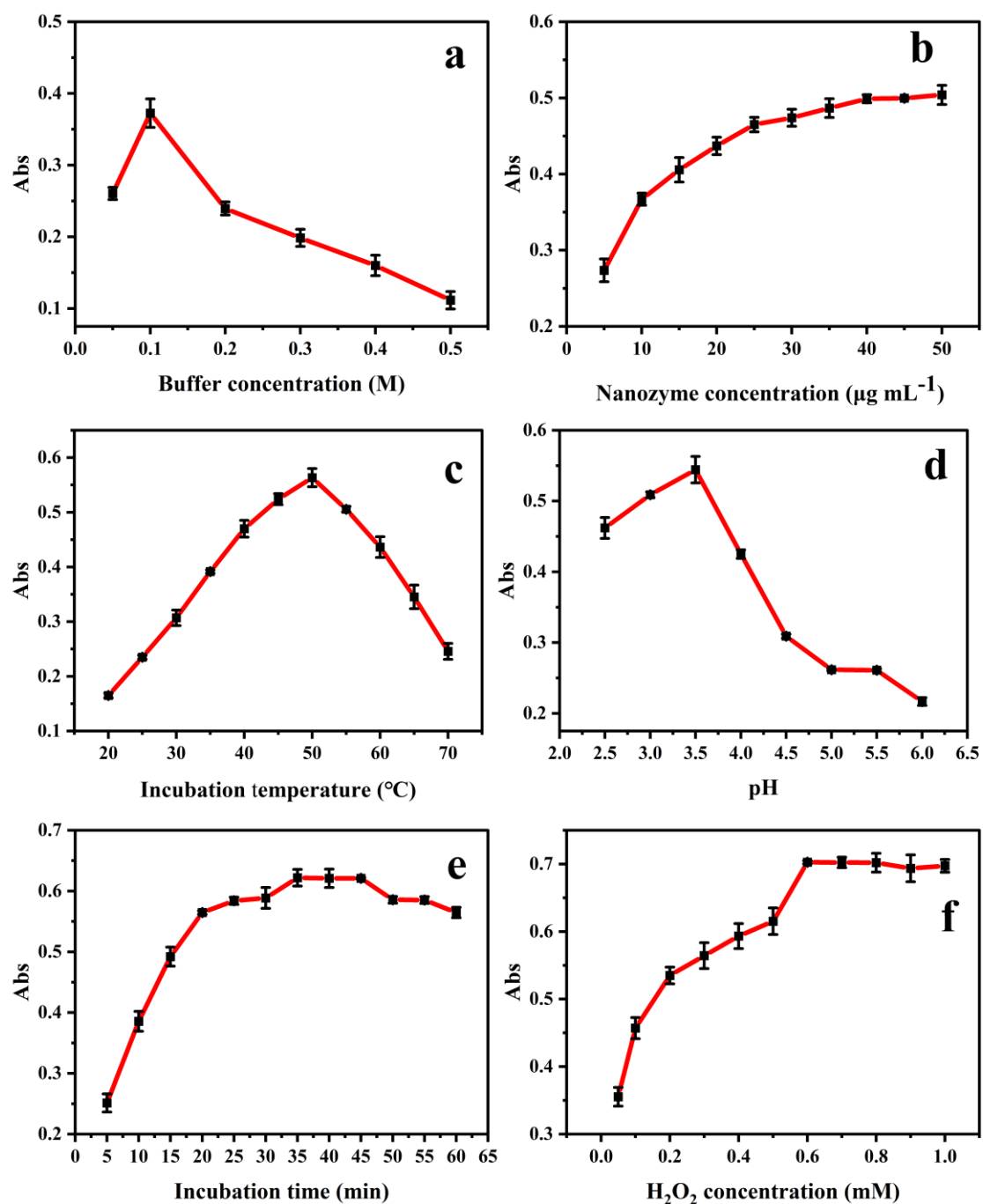


Figure S3. Investigation of the effects of the buffer concentration (a), nanozyme concentration (b), incubation temperature (c), pH (d), incubation time (e) and H_2O_2 concentration (f) on the peroxidase - like catalytic activity of Fe_3O_4 - PVP@ZIF - 67.

Table S1 Comparison of optimal catalytic conditions for Fe₃O₄@Co₃O₄/CN and Fe₃O₄-PVP@ZIF-67.

| | Fe ₃ O ₄ @Co ₃ O ₄ /CN | Fe ₃ O ₄ - PVP@ZIF - 67 |
|--|--|---|
| TMB concentration (mM) | 0.1 | 0.1 |
| Buffer concentration (M) | 0.1 | 0.1 |
| Buffer solution pH | 3.5 | 3.5 |
| Nanozyme concentration (μg • mL ⁻¹) | 50 | 40 |
| Incubation temperature (°C) | 40 | 50 |
| Incubation time (min) | 15 | 35 |
| H ₂ O ₂ concentration (mM) | – | 0.6 |

Text S6 Steady - state kinetics of Fe₃O₄@Co₃O₄/CN and Fe₃O₄ - PVP@ZIF - 67

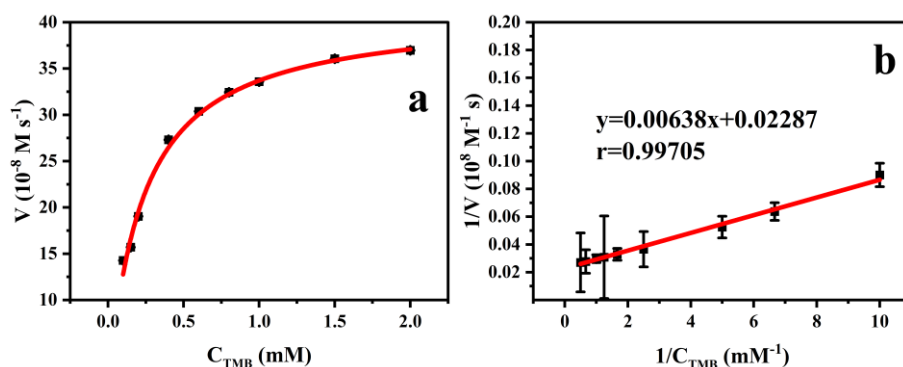


Figure S4. Steady - state kinetic assay of Fe₃O₄@Co₃O₄/CN microspheres(a) and double - reciprocal plots derived from Michaelis - Menten curves(b).

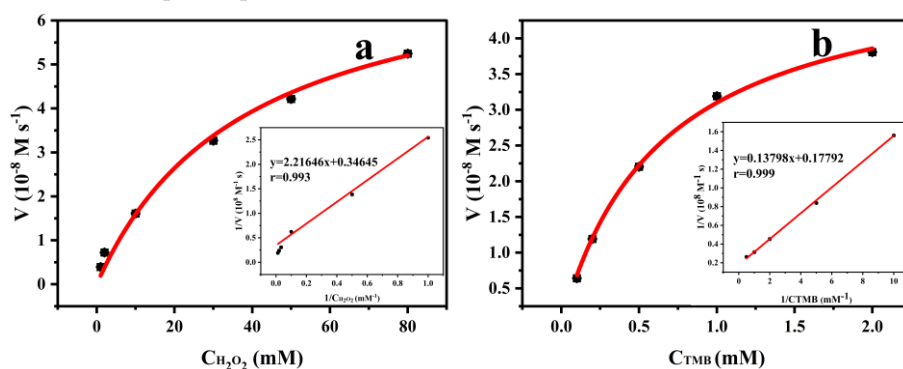


Figure S5. Steady - state kinetic assay of Fe₃O₄ - PVP@ZIF - 67 microspheres(a) and double - reciprocal plots derived from Michaelis - Menten curves(b).

Table S2 Comparison of steady - state kinetic parameters of different nanozymes

| nanozyme | K_m | V_m (10^{-8} M s^{-1}) | ref |
|--|--------|--------------------------------------|-----------|
| Fe ₃ O ₄ @Co ₃ O ₄ /CN | 0.28 | 43.70 | This work |
| Fe ₃ O ₄ - PVP @ZIF - 67 | 0.78 | 5.62 | This work |
| Fe ₃ O ₄ | 0.37 | 2.60 | [1] |
| Co ₃ O ₄ HNCs | 0.13 | 31.96 | [2] |
| Co ₃ O ₄ /CuO HNCs | 0.22 | — | [3] |
| Fe SAEs | 0.13 | 2.25 | [4] |
| NiCo ₂ O ₄ MS | 0.13 | 1.00 | [5] |
| CoSe ₂ | 0.47 | 17.00 | [6] |
| CeO ₂ NPs | 0.42 | 10.04 | [7] |
| Au@Hg ⁰ /WO ₃ HNFs | 145.58 | 2.21 | [8] |

Text S7 Catalytic mechanism of Fe₃O₄@Co₃O₄/CN

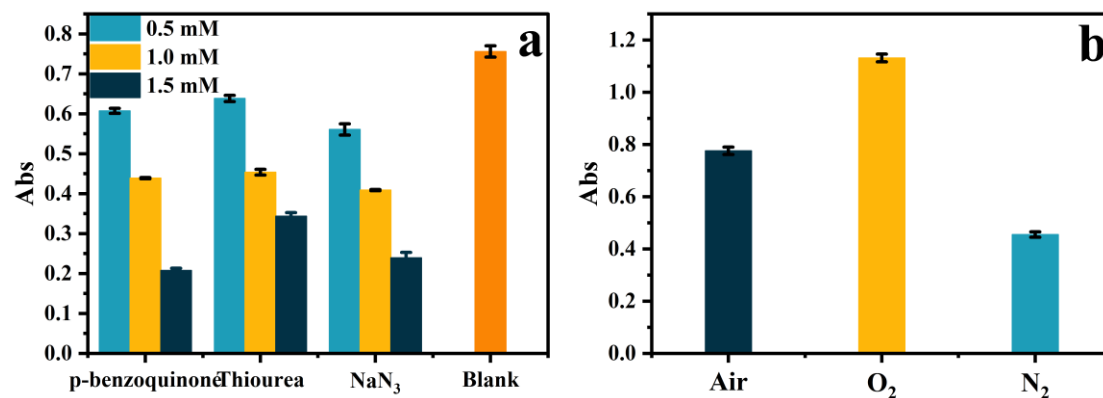


Figure S6. The catalytic oxidation of TMB in the presence of various radical scavengers (a).

Effect of N₂/O₂ purging on the catalytic oxidation of TMB (b).

Text S8 Analytical performance

First, the accompanying standard curves were established by adding different concentrations of Hg^{2+} standard solutions under optimal reaction conditions. Next, 5.5 μM , 7 μM and 8.5 μM of Hg^{2+} standard solutions were added to the actual water samples of known concentrations, and the absorbance values at 652 nm were measured in three parallel operations for each level of concentration.

Table S3 Recoveries and precisions for Hg^{2+} spiked at three levels.

| Spiked level | Original amount (μM) | Spiked amount (μM) | Measured amount (μM) | Recovery (%) | Average recovery(%) | RSD (%) |
|--------------|-----------------------------------|---------------------------------|-----------------------------------|--------------|---------------------|---------|
| Low-1 | 0 | 5.5 | 5.17 | 94.0 | | |
| Low-2 | 0 | 5.5 | 5.01 | 91.2 | | |
| Low-3 | 0 | 5.5 | 5.40 | 98.1 | | |
| Middle-1 | 0 | 7.0 | 6.69 | 95.6 | | |
| Middle-2 | 0 | 7.0 | 6.54 | 93.5 | 96.3 | 3.1 |
| Middle-3 | 0 | 7.0 | 6.92 | 98.9 | | |
| High-1 | 0 | 8.5 | 8.37 | 98.5 | | |
| High-2 | 0 | 8.5 | 8.15 | 95.8 | | |
| High-3 | 0 | 8.5 | 8.60 | 101.2 | | |

Table S4 Colorimetric method developed for the detection of Hg^{2+} in real water samples.

| | C1 | C2 | C3 | Average | Standard |
|---------|-----------------|-----------------|-----------------|---------|----------|
| Water 1 | ND ^a | ND ^a | ND ^a | — | — |
| Water 2 | ND ^a | ND ^a | ND ^a | — | — |
| Water 3 | ND ^a | ND ^a | ND ^a | — | — |
| Water 4 | ND ^a | ND ^a | ND ^a | — | — |

^a Not Detected.

Text S9

The N₂ adsorption – desorption isotherms of ZIF – 67, Fe₃O₄ – PVP@ZIF – 67, Fe₃O₄ – PVP, and Fe₃O₄@Co₃O₄/CN

The N₂ adsorption – desorption isotherms of ZIF – 67, Fe₃O₄ – PVP@ZIF – 67, Fe₃O₄ – PVP, and Fe₃O₄@Co₃O₄/CN were shown in the following Figure S7(a) and (b). Compared with the pure mesoporous ZIF – 67 (BET surface area: 1328 m²·g⁻¹; pore volume: 0.70 cm³·g⁻¹; average pore diameter: 2.1 nm ~ 5.3 nm), the as – prepared mesoporous Fe₃O₄ – PVP@ZIF – 67 composites (64 m²·g⁻¹; 0.06 cm³·g⁻¹; 2.7 nm ~ 13.2 nm) showed significantly reduced BET surface area and pore volume. This mainly due to the fact that the ZIF – 67 had a low load on the mesoporous Fe₃O₄ – PVP (29 m²·g⁻¹; 0.23 cm³·g⁻¹; average pore diameter 17.5 nm ~ 33.3 nm) and the Fe₃O₄ – PVP plugged the pores of ZIF – 67 to a certain extent, resulting in a dramatically reduction in its specific surface area and pore volume. Moreover, the BET surface area and pore volume of the mesoporous Fe₃O₄@Co₃O₄/CN (27 m²·g⁻¹; 0.01 cm³·g⁻¹; 4.8 nm ~ 9.6 nm) were much smaller than Fe₃O₄ – PVP@ZIF – 67 before calcination. This showed that the collapse of imidazole zeolite skeleton and the formation of cobalt oxide in the calcination process seriously blocked the pore channel of the material, resulting in a serious reduction in the BET surface area and pore volume.

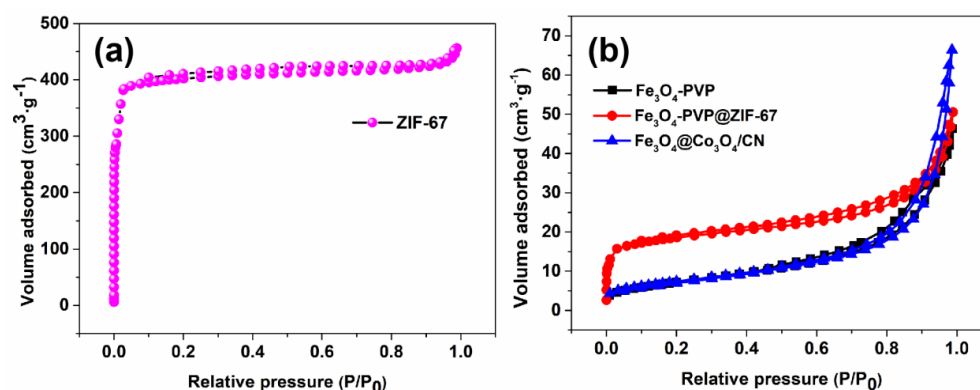


Figure S7. N₂ adsorption – desorption isotherms of ZIF – 67, Fe₃O₄ – PVP@ZIF – 67, Fe₃O₄ – PVP and Fe₃O₄@Co₃O₄/CN.

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