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Cryst. Res. Technol., 1–6 (2014) / DOI 10.1002/crat.201300440

$Ag@Fe_3O_4$ nanowire: fabrication, characterization and peroxidase-like activity

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Received 26 December 2013, revised 5 March 2014, accepted 12 March 2014
Published online 2 April 2014

With a facile solvothermal method, $Ag@Fe_3O_4$ nanowire was successfully prepared and characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The obtained $Ag@Fe_3O_4$ nanowire posses enhanced peroxidase-like activity with good stability and high absorbance. The optimization of pH, H_2O_2 concentration and loading capacity were carried out. The result of kinetic analysis indicates that the catalyzed reaction followed a Michaelis-Menten behavior. The good peroxidase-like activity makes $Ag@Fe_3O_4$ nanowire be promising for real application in biomedicine.

1 Introduction

Enzymes are biological catalysts involved in almost all reactions in vivo [1, 2]. Enzyme-catalyzed reactions are also of wide spread interest in chemical field due to their high specificity, high efficiency and mild reaction conditions. In addition, enzyme based analysis has recently been applied in clinical diagnosis, environmental science, chemistry, biotechnology and other fields [3–5]. However, limited natural sources, inherent instability, and difficult purification processes with high cost restrict enzyme application to some extent. Therefore, more and more attention has been paid to constructing enzyme mimetic with similar functions to natural enzymes in recent years [6–9].

Combing two nanomaterials into one nanostructure will integrate both of their functions and properties. Some improved properties are also found in nanocomposite. Noble metal nanoparticles (NPs) have attracted great interest owing to their easy bio-functionalization, tunable optical properties and bio-stability [10–12]. In particular, gold and silver NPs has drawn considerable research attention due to their unique optical properties, which make them be ideal color indicating probes for various analytes [13–15]. There are also some reports about the hydroxide enzyme mimic properties of Ag nanostructures. In addition, magnetic iron oxide nanoparticles have been found to possess intrinsic enzyme mimetic activity similar to that of natural peroxidases [16, 17]. Since this report, increasing attention has been paid to the nanoscale peroxidase mimetics and their potential applications [18–20]. This induces us to fabricate $Ag@Fe_3O_4$ nanocomposite to study their enzyme mimetic activity.

In fact, there are some reports on the synthesis of $Ag@Fe_3O_4$ nanocomposite and the investigation of their properties, such as the antifungal activity [21, 22], electro-catalytic properties [23, 24], magnetic properties [25], electrical conductivity [26], optical limiting [27, 28], and so on. To the best of our knowledge, no attempt has been made to investigate the enzyme-mimic activities of the $Ag@Fe_3O_4$ nanocomposite. Herein, with a facile polyol synthesis route, Ag nanowire was prepared. After that a solvothermal method is involved for the loading of Fe_3O_4 on the silver nanowire, forming $Ag@Fe_3O_4$ nanocomposite. The obtained $Ag@Fe_3O_4$ nanowire can catalyze the oxidation of the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) with high efficiency and good stability in the presence of H_2O_2. The reaction parameters of pH, H_2O_2 concentration and loading capacity were optimized. The catalyzed reaction followed a Michaelis-Menten behavior.

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2 Experimental

Chemicals: 3, 3′, 5, 5′-Tetramethylbenzidine (TMB), o-phenylenediamine (OPD), 3,3′-diaminobenzidine (DAB) and polyvinylpyrrolidone (PVP) were purchased from Sigma-Aldrich. hydrogen peroxide (30%), $AgNO_3$, $Fe(NO_3)_3 \cdot 9H_2O$, ethylene glycol (EG), diglycol and other chemical reagents were of analytical grade and used without further purification. The deionized water used throughout the experiment was made by our laboratory.

Preparation of Ag nanowire: A polyol route was used for the synthesis of Ag nanowire. In brief, EG (20ml) was heated in oil bath at 150 °C for 1 h before adding silver nitrate and PVP. Then 5 ml of silver nitrate EG solution (containing 0.24 mol of $AgNO_3$ in EG) and 5 ml of PVP EG solution (containing 0.24 mol of PVP calculated on the monomer) were added drop wise simultaneously. When the first drop of silver nitrate and PVP/EG solutions was added, the mixture turned yellow immediately. Then the solution became opaque gradually with the injection. The solution turned turbid with a gray color in about 15 min, indicating the appearance of Ag nanowire. The reaction was continued at 150 °C for another 3 h. After finishing the reaction a gray precipitate remained. Finally the products were washed nine times with ethanol, dried at 30 °C for 6 h.

Preparation of Ag@Fe_3O_4 nanowire: Ag nanowire (0.01 mmol) and $Fe(NO_3)_3$ (0.04 mmol) were dissolved in 11 ml of diglycol, then sodium acetate (0.12 mol) and 3 ml of EG were added in turn, a orange-yellow solution was then obtained. The resultant mixture was transferred to 20 ml of teflon-lined stainless steel autoclave, which was sealed and maintained at 200 °C for 10 h. The autoclave was cooled to room temperature naturally. The precipitate, Ag@Fe_3O_4 (sample 1) was washed with distilled water and ethanol for nine times, and dried at 30 °C for 6 h. Changing the quality of $Fe(NO_3)_3$ to 0.12 mmol and 0.2 mmol, Ag@Fe_3O_4 nanocomposite with different content of iron (sample 2, sample 3) were obtained, respectively.

Catalytic oxidation of TMB by Ag@Fe_3O_4 nanowire: To investigate the catalytic activity of the as-prepared nanocomposite, the oxidation reaction of TMB was tested in the presence of H_2O_2. In a standard procedure, 1 mL of TMB (0.1 mmol/L), 0.5mg of Ag@Fe_3O_4 nanowire, 2 mL H_2O_2 (0.1mmol/L) were orderly added into 5 mL acetic acid/sodium acetate buffer solution with pH of 4 at 25 °C (keep constant). The reaction system will change to be blue, suggesting the oxidation of TMB. The reaction was carried out in a small cuvette and monitored by observing the absorbance evolutions at 652 nm. For investigating the influence of various reaction parameters on the oxidation reaction, different reaction condition such as pH, concentration of TMB and H_2O_2 were used for catalytic reaction.

Characterizations: The phases of the as-prepared products were characterized by powder X-ray diffraction analysis (XRD-6000) using Cu Kα radiation (λ = 1.5406 Å). The morphologies, sizes of the products were examined using scanning electron microscopy (SEM, JSM-6480) and transmission electron microscopy (TEM, JEOL 2100). UV-vis spectra were collected on Hitachi UV-3000 spectrophotometer.

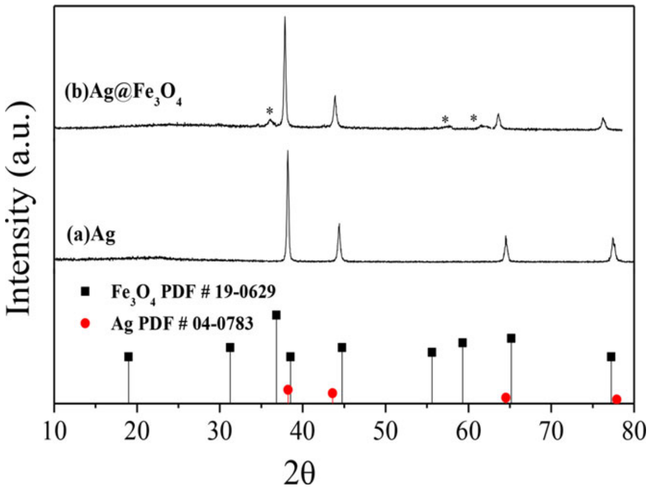

Fig. 1 XRD of Ag nanowire (a) and Ag@Fe_3O_4 nanowire (sample 1) (b).

3 Results and discussion

XRD analysis was firstly used to determine the crystal structure and phase purity of the obtained products. The XRD pattern of the as-prepared Ag nanowire (figure 1a) shows diffraction peaks at 38.2, 43.6, 64.2 and 77.1 corresponding to the (111), (200), (220), (311) crystal planes of face-centered cubic Ag (JCPDS, No. 04-0783). The strong diffraction peaks suggest that the Ag nanowire has a good crystalline. No peaks from impurity are observed in the pattern. The XRD spectrum of Ag@Fe_3O_4 nanowire (figure 1b) shows the diffraction peaks at 38.2, 43.6, 64.2 and 77.1 corresponding to face-centered cubic Ag. Some weak diffraction peaks such as that positioned at 37.0 is attributed to Fe_3O_4 (JCPDS No. 19-0629). The diffraction peaks from Fe_3O_4 are marked with an asterisk sign (*) in the spectrum.

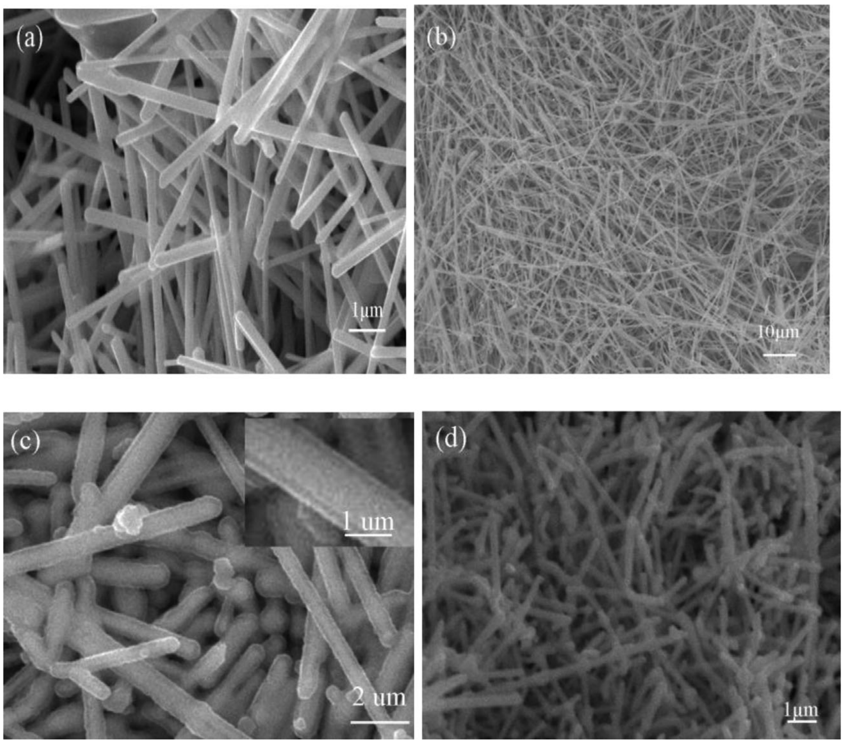

Fig. 2 SEM images of Ag nanowire (a, b) and Ag@Fe_3O_4 nanowire (sample 1) (c, d).

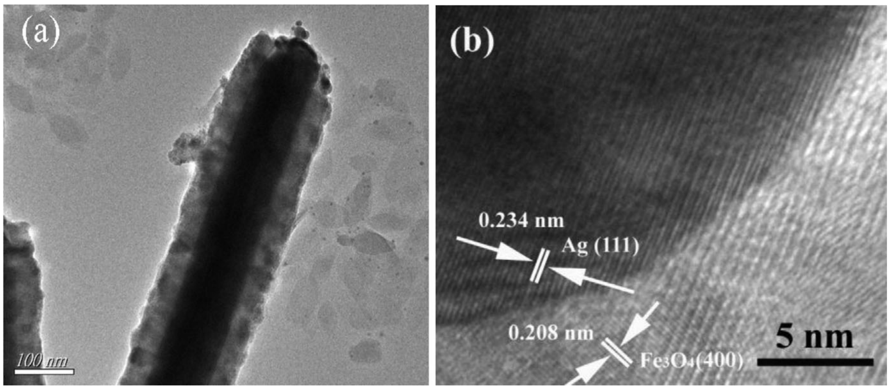

Fig. 3 TEM (a) and HRTEM (b) images of Ag@Fe_3O_4 nanowire (sample 1).

Figure 2(a, b) shows a typical SEM image of the prepared Ag nanowire, illustrating the presence of a high concentration of Ag nanowire with random arrangements. The length and diameter of the Ag nanowire were about 20-50 μm and 250 nm, respectively. While the typical diameter of Ag@Fe_3O_4 nanowire was about 400 nm and the length was similar to Ag nanowire (figure 2c, 2d). From the inset image in figure 2c we could see that the surface of Ag nanowire was coated with Fe_3O_4, there is a clear layer boundary between them.The TEM images (figure 3) were also confirmed the morphology of Ag@Fe_3O_4 nanowire. The typical lattice fringe spacings of Fe_3O_4 layer were determined to be 0.208 nm, corresponding to the (400) planes of cubic magnetite (figure 3b).

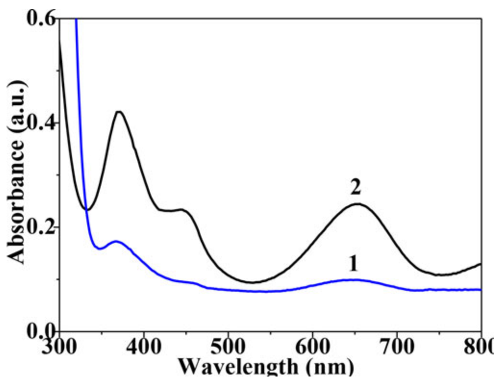

Fig. 4 Typical absorption spectra of the TMB-H_2O_2 mixed solutions in the absence (1) and in the presence (2) of Ag@Fe_3O_4 nanowire (sample 1) in acetate buffer with pH of 4.0.

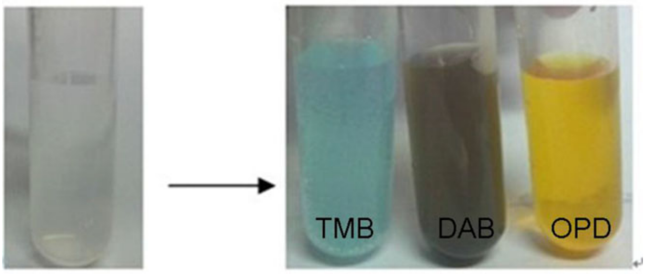

Fig. 5 Images of oxidation color reaction of TMB, DAB and OPD by H_2O_2 with Ag@Fe_3O_4 nanowire (from left to right, the substrate is TMB, DAB and OPD).

It is known that with Horseradish Peroxidase (HRP) as catalyst, TMB can be oxidized into SQI^+ or $TMQD^{2+}$ by H_2O_2 [29], both of them are blue compounds with stable resonant structure. The reaction system usually shows three absorbance peaks at 370 (SQI^+), 450 ($TMQD^{2+}$) and 652 nm (the association of both objects). Without catalyst, TMB can also be oxidized by H_2O_2 but with lower speed, as shown in figure 4, curve 1. When Ag@Fe_3O_4 was involved as the enzyme-like catalyst, the reaction tuned to be blue quickly. As shown in figure 4, curve 2, the absorbance curve shows three absorbance peaks at 370, 450 and 652 nm, respectively.

Besides TMB, the substrates DAB and OPD were also used to investigate the peroxidase-like properties of Ag@Fe_3O_4 (sample 1) nanowire. It was found that the obtained Ag@Fe_3O_4 nanocomposite can catalyze various peroxidase substrates in the presence of H_2O_2 producing different color, producing a brown color for DAB and an orange color for OPD (figure 5).

The peroxidase-like activities of the Ag@Fe_3O_4 nanowire depend on the pH, H_2O_2 concentration, TMB concentration, loading capacity and so on. The effect of pH on the peroxidase-like activities of the Ag@Fe_3O_4 nanowire was measured firstly by varying the pH from 1 to 12, whereas keeping the concentrations of TMB (0.1mM) and H_2O_2 (0.1mM) constant. The acidic buffer solutions were prepared from acetic acid and sodium acetate, while the alkali solutions were prepared from sodium carbonate and sodium bicarbonate. The catalytic activity of the Ag@Fe_3O_4 nanowire shows a peak at pH 5.0 (figure 6a). This situation is similar to natural enzymes, the best catalytic activity is demonstrated at a special pH value. When pH > 5.0, the catalytic activity decreased quickly with the increasing of pH. We also found that the Ag@Fe_3O_4 nanowire show the best catalytic activity with TMB concentration of 0.3 mM and H_2O_2 concentration of 0.1 mM (figure 6b, 6c). Meanwhile, the

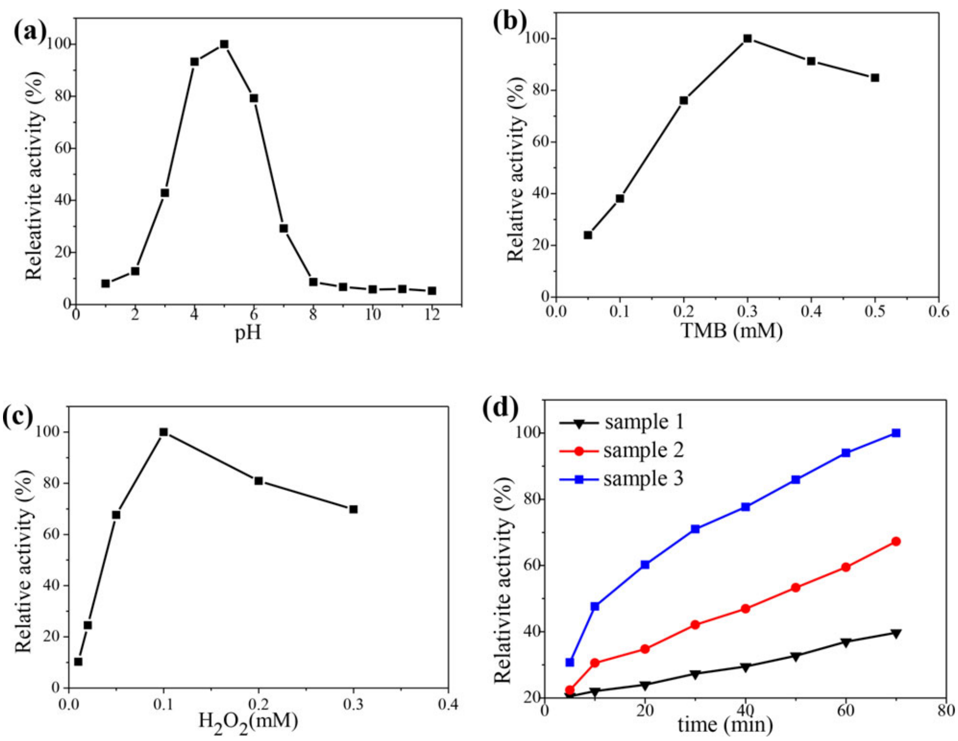

Fig. 6 The peroxidase-like activities of the Ag@Fe_3O_4 nanowire depend on the pH, H_2O_2, TMB concentration and loading capacity. The maximum point in each curve was set as 100%. (a) pH, (b) TMB, (c) H_2O_2, (d) Fe_3O_4 loading capacity.

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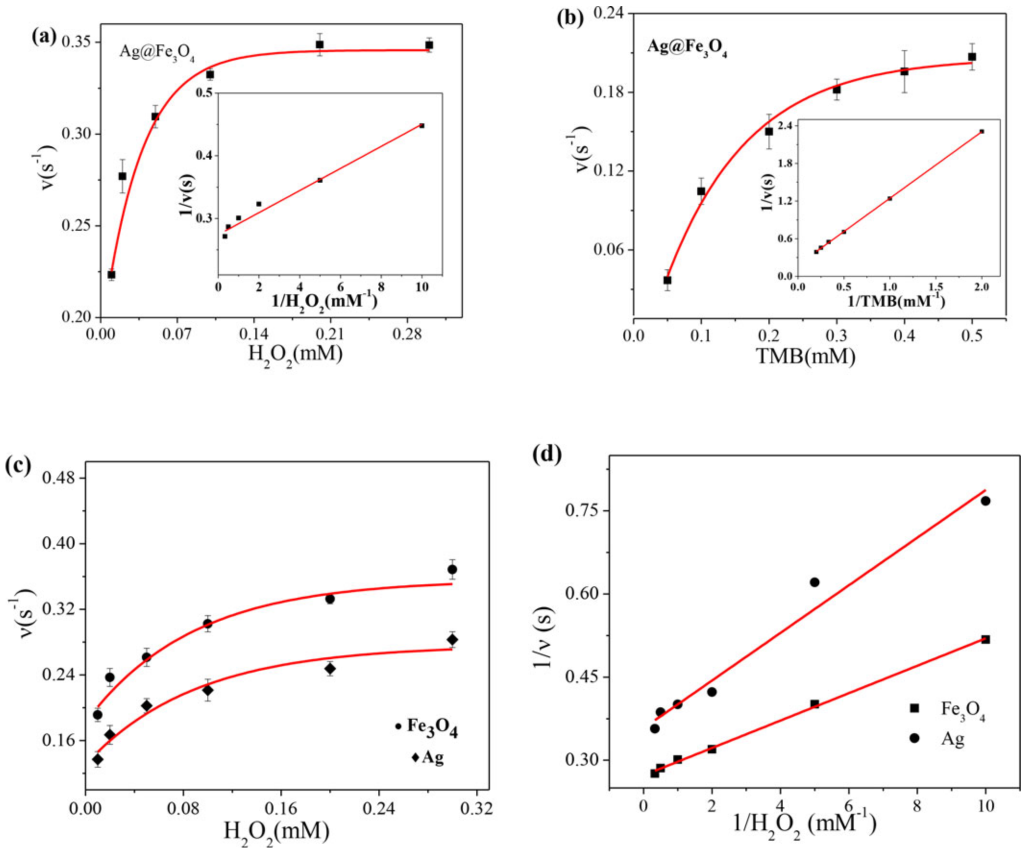

Fig. 7 The reaction rate of Ag@Fe$_3$O$_4$ nanowire in TMB-H$_2$O$_2$ reaction system with different concentrations of (a) H$_2$O$_2$ and (b) TMB, (the insert image is the double-reciprocal plots of activity of Ag@Fe$_3$O$_4$ nanowire); (c) the reaction rate of Ag nanowire and Fe$_3$O$_4$ nanoparticle in TMB-H$_2$O$_2$ reaction system with different concentrations of H$_2$O$_2$, (d) double-reciprocal polts of activity of Ag nanowire and Fe$_3$O$_4$ nanoparticle. Error bars shown represent the standard error derived from three repeated measurements.

composition of Ag@Fe$_3$O$_4$ nanowire has a big influence on the catalytic activity. The activities change with loading capacity of Ag@Fe$_3$O$_4$ nanocomposite in the order sample 3 > sample 2 > sample 1 (figure 6d), that is, the more loading capacity the higher catalytic activity.

The mechanism of the peroxidase-like catalytic activities of the Ag@Fe$_3$O$_4$ nanowire was further investigated. From previous control experiments, we have known that the reaction was dependent on the substrate concentration (TMB and H$_2$O$_2$). In order to get a better insight into the nanocomposite, several experiments were performed whereby the concentration of either TMB or H$_2$O$_2$ was varied while keeping the other concentration constant. The concentration of the Ag@Fe$_3$O$_4$ nanowire (4.48×10^{-9}M) was kept constant in all these experiments. We observed that the oxidation reaction catalyzed by the Ag@Fe$_3$O$_4$ nanowire follows a Michaelis-Menten behavior towards the components, TMB and H$_2$O$_2$ (figure 7a and 7b). The apparent kinetic parameters were calculated based on the function $V = V_{max} \times [S] / (K_m + [S])$, where [S] is the concentration of substrate, K_m is the Michaelis constant, V_{max} is the maximal reaction velocity. The kinetic parameters for TMB and H$_2$O$_2$ were determined at pH of 5.0. The V_{max} obtained was 2.288×10^{-8} Ms^{-1}, from which it was determined that the Ag@Fe$_3$O$_4$ nanowire mediate the oxidation of TMB in the presence of H$_2$O$_2$ with a turnover frequency (K_{cat}) of 4.72 s^{-1}. The K_{cat} was determined as $K_{cat} = V_{max} / [E]$, where [E] was taken as the enzyme concentration. By the Line weaver-Burk linearization process (inset of figure 7a and 7b) values for K_m (TMB) of 3.46 mM and K_m (H$_2$O$_2$) of 75.2 mM were found. We also did similar experiments to determine the catalytic properties of pure Ag nanowire and pure Fe$_3$O$_4$ nanoparticles for comparison (figure 7c and 7d). The Fe$_3$O$_4$ nanoparticles were prepared according to literature [30] with a litter modification and the diameter was about 60 nm. Table 1 shows the apparent kinetic parameters of Ag, Fe$_3$O$_4$ and Ag@Fe$_3$O$_4$ samples, we can see that the K_m (H$_2$O$_2$) value of Ag@Fe$_3$O$_4$ nanowire

Table 1 Apparent kinetic parameters for the Ag nanowire, Fe_3O_4 nanoparticles and Ag@Fe_3O_4 nanowire to H_2O_2

	K_m (mM)	V_{max} (Ms^{-1})	K_{cat} (s^{-1})	K_{cat}/K_m ($M^{-1}s^{-1}$)
Ag nanowire	108	2.84×10^{-9}	0.635	5.87×10^2
Fe_3O_4 nanoparticles	117	3.63×10^{-9}	0.811	6.13×10^2
Ag@Fe_3O_4 nanowire	75.2	22.8×10^{-9}	4.72	6.75×10^2

is lower those of pure Fe_3O_4 and Ag, indicating that the Ag@Fe_3O_4 nanowire have a much higher affinity to H_2O_2. As a result, Ag@Fe_3O_4 nanowire exhibit a larger K_{cat} value than that of pure Ag nanowires and pure Fe_3O_4 nanoparticles suggesting a higher peroxidase-like activity.

4 Conclusion

In conclusion, we have demonstrated highly efficient peroxidase-like catalyst activity of Ag@Fe_3O_4 nanowire. The peroxidase-like activity was H_2O_2, pH, TMB dependent and the optimal reaction condition for the whole system was pH of 5.0, TMB concentration of 0.3 mM and H_2O_2 concentration of 0.1 mM. On the other hand, catalyzed by Ag@Fe_3O_4 nanowire conform to a typical Michaelis–Menten kinetics. The kinetic parameter investigation indicated that the nanocomposite has a higher affinity for H_2O_2. The excellent peroxidase-like properties and the easily scaled-up preparation route make Ag@Fe_3O_4 nanowire promising for real application.

Acknowledgement. This research was supported by the projects of National Natural Science Foundation (Nos. 51072072, 51272095, 51203069), Natural Science Foundation of Jiangsu Province (No. BK2012276), Startup Fund for Distinguished Scholars (No. 35211103, 33211103).

Key words. Ag nanowire, Ag@Fe_3O_4 nanowire, Peroxidase-like activity.

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Cryst. Res. Technol., 1–6 (2014) / DOI 10.1002/crat.201300440

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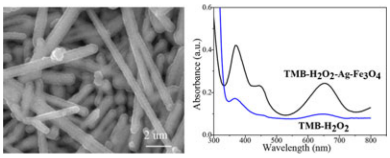

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