Journal of Materials Chemistry A

COMMUNICATION

Cite this: J. Mater. Chem. A, 2014, 2,

Received 14th November 2013 Accepted 13th December 2013

DOI: 10.1039/c3ta14697a www.rsc.org/MaterialsA

2482



View Article Online View Journal | View Issue

Published on 16 December 2013. Downloaded by Universitätsbibliothek Bern on 16/09/2014 06:53:18.

Mesoporous material supported $CoFe_2O_4$ magnetic nanoparticles possess unique peroxidase/oxidase-like activity, and react with luminol to yield a novel chemiluminescence without the need of H_2O_2 . Their oxidase-like activity shows pH and support dependence, and could be reversibly controlled by their pH. This offers a new method for manipulating the enzyme-like activity of nanoparticles.

Recently, engineering nanoparticles (NPs) that mimic enzymes have received great attention due to their potential improvement of properties relative to native enzymes (*e.g.*, greater resistance to extremes of pH and temperature) and potential applications in biochemical analysis, catalysis, sensor technology, and degradation of organic pollutants.¹ Compared with natural enzymes, the NPs mimetics are stable against denaturation, resistant to high concentration of substrate, low in cost, and easy to store and treat. The applications of these novel materials are currently under intensive investigation. And various NPs have been evaluated as catalysts of enzymatic mimetics.²

On the other hand, the mesoporous materials have attracted an extraordinary scientific interest due to their large specific surface area, uniform pore size distribution, tunable porosity, well-defined surface properties and their strong influence on the catalytic efficiency.³ However, their use in enzyme mimetics has not been reported. In this communication, for the first time, we report mesoporous material supported $CoFe_2O_4$ NPs with unique tunable enzyme-like activity. Our results are (i) the mesoporous material supported $CoFe_2O_4$ NPs were successfully synthesized *via* a simple adsorption process; (ii) the $CoFe_2O_4$

Mesoporous material-based manipulation of the enzyme-like activity of CoFe₂O₄ nanoparticles†

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and the supported $CoFe_2O_4$ NPs were found to exhibit significant oxidase activity varied with their pH in luminol chemiluminescence (CL) reaction in the absence of H_2O_2 ; and (iii) the pH tunable catalytic activity of the $CoFe_2O_4$ NPs enzyme mimic was dependent on the used mesoporous materials and could be reversibly controlled by their pH. The results we present herein offer a strategy for the preparation of simple and efficient multifunctional catalysts with magnetism, oxidase-like activity, and pH-sensitivity.

For our first set of experiments, we investigated the kinetic characteristics of the luminol CL system in the presence of CoFe₂O₄ NPs (Fig. S1, ESI[†]). As can be seen, no significant CL was observed for luminol itself. However, CoFe₂O₄ NPs show the enhancement effects of luminol CL. In order to study the role of $CoFe_2O_4$ NPs, the CL spectra of the following systems were studied by F-4500 fluorimetry: (a): luminol solution; (b): $CoFe_2O_4$ + luminol; (c): SBA-15 supported $CoFe_2O_4$ + luminol; (d): SiO₂ supported CoFe₂O₄ + luminol; and (e): Al₂O₃ supported CoFe₂O₄ + luminol. The results demonstrate that all the above systems give one peak situated at about 425 nm, indicating that the role of CoFe₂O₄ NPs or supported CoFe₂O₄ NPs is only an enhancement reagent because there is no new emitter produced in the reaction. In other words, the presence of support materials has not caused the production of the new CL emitter. Therefore, it can be concluded that addition of CoFe₂O₄ NPs or supported CoFe2O4 NPs does not produce a new luminophor of luminol CL reaction. The luminophor for the CL system was still the excited-state 3-aminophthalate anions (AP^{2-}) . The enhanced CL signals were thus ascribed to the possible catalysis from CoFe₂O₄ NPs or supported CoFe₂O₄ NPs. The catalytic activity of CoFe2O4 NPs may be derived from their characteristic of peroxidase enzyme mimic. Recently, Fe₃O₄ nanoparticle, an inorganic nanomaterial, has been discovered to possess an intrinsic enzyme mimetic activity similar to that found in natural peroxidases.4 Compared with the natural HRP, the Fe₃O₄ NPs exhibited higher catalytic activity and were more robust when exposed to a wide range of temperature and pH values. Based on the above advantages of the Fe₃O₄ NPs, Wang

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[†] Electronic supplementary information (ESI) available: Experimental details and supporting figures. See DOI: 10.1039/c3ta14697a

and co-workers used Fe₃O₄ as an artificial peroxidase to determine H₂O₂ and glucose.^{2b} The peroxidase enzyme mimetic activity of CoFe2O4 NPs or supported CoFe2O4 NPs was demonstrated by using TMB, which is typically HRP substrate in various bioassays, and most recently was used to demonstrate the peroxidase-like activity of iron oxide NPs.2b,4 Upon the addition of H₂O₂ and TMB, CoFe₂O₄ NPs or supported CoFe₂O₄ NPs can produce a blue color reaction (Fig. S2, ESI[†]), indicating that CoFe₂O₄ NPs or supported CoFe₂O₄ NPs have peroxidaselike catalytic activity. Interestingly, the supported CoFe₂O₄ NPs catalyze the fast oxidation (within minutes) of TMB even in the absence of H_2O_2 (Fig. 1a), which is a commonly used electron acceptor or oxidizing agent in the peroxidase-catalyzed reactions. This demonstrated that CoFe2O4 NPs or supported CoFe₂O₄ NPs also exhibit oxidase-like activity. Furthermore, results in Fig. 1b suggest that the catalytic activity of the supported CoFe₂O₄ NPs is pH-dependent and support-dependent clearly. The ability of the supported CoFe₂O₄ NPs to oxidize TMB decreases with increase of solution pH from 4.0 to 6.0.

To investigate the effect of supporting materials on the CL response, three mesoporous materials, namely Al_2O_3 , SiO_2 , and SBA-15, were used. Microscope images suggest sphere-like shape of SiO_2 and Al_2O_3 particles with diameter of micrometer range (Fig. S3A, ESI†). SEM micrographs (Fig. 2A) show that the disordered wormhole like arrangement of pores is observed on the surface of SiO_2 particles, indicating the formation of the mesoporous structure. The Al_2O_3 particle morphology is layer-



Fig. 1 (a) Images of production of colored product upon addition of $CoFe_2O_4$ and supported $CoFe_2O_4$ NPs to TMB at pH 3.5 (0.2 mol L⁻¹ acetate buffer), and subsequently being quenched by H_2SO_4 . (b) Effect of pH on oxidation of TMB catalyzed by the supported $CoFe_2O_4$ NPs in the absence of H_2O_2 , (A): SBA-15/CoFe_2O_4 NPs; (B): SiO_2/CoFe_2O_4 NPs; and (C): Al_2O_3/CoFe_2O_4 NPs.



Fig. 2 (A) SEM images of the commercial SiO₂, the commercial Al₂O₃, and SBA-15 (from left to right). (B) N₂ adsorption–desorption isotherm curves of three mesoporous materials. Inset: pore size distribution curves of SBA-15, the commercial SiO₂, and the commercial Al₂O₃.

like with ordered porous structure. SBA-15 has the typical wheat-like morphology and consists of aggregates of uniform rope-like particles, in agreement with the results reported elsewhere.5 The N2 adsorption-desorption isotherms of the three supports (Fig. 2B) exhibiting a type IV isotherm with a hysteresis loop, clearly demonstrate the mesoporous characteristics of the three supports. The pore size distribution curves of the three supports are shown in the inset of Fig. 2B. The pore sizes of Al₂O₃, SBA-15 and SiO₂ calculated using the BJH method (Table S1, ESI[†]) were 4.47 nm, 5.85 nm, and 13.40 nm, respectively. The surface areas of Al₂O₃, SBA-15 and SiO₂ are 183.8 m² g^{-1} , 320.2 m² g⁻¹ and 354.5 m² g⁻¹, respectively. Compared with SBA-15 and Al₂O₃, SiO₂ shows significant elevation of the adsorption plateau in the isotherm, indicating large pore volume. However, the pore volume of Al₂O₃ is lower than that of SBA-15. This may be due to its more condensed network construction. Due to the mesoporous characteristics of the three supports, good adsorbing capability for CoFe₂O₄ NPs is expected. The results of FTIR indicate that the spectrum of CoFe₂O₄-coated support is very similar to that of the support (Fig. S3B[†]). The difference spectrum shows characteristic CoFe₂O₄ absorption bands (inset of Fig. S3B[†]), suggesting unambiguously that CoFe₂O₄ was coated on the surface of the support, which was further confirmed by the very similar particle size distribution between coated particles and uncoated ones (Fig. S3A†).

The as-prepared particles were characterized *via* CL to study the response to their pH. In order to do this, first, we performed pH optimization of the luminol solution on the CL response. Due to the nature of the luminol reaction, which is more favored under basic conditions, the effects of pH of the luminol solution on the CL intensity were investigated in the range of pH 10.9– 12.3 (Fig. S4, ESI[†]). As shown, the highest CL intensity was obtained at pH 11.7 (0.1 mol L⁻¹ carbonate buffer). It was found that the pH of the whole CL reaction was close to the pH of the luminol solution, revealing that the pH of the luminol-dissolved oxygen CL reaction system was dominated by the pH of the luminol solution. So, we selected 11.7 as optimal luminol solution pH.

Next, we investigated the response to pH of the as-prepared particles from 2.0 to 7.0 and from 7.0 back to 2.0 (Fig. 3). Interestingly, the CL emission clearly decreased when the pH was increased from 2.0 to 7.0. As seen, the pH of the CL reaction system increased slightly with an increase in the pH of the mesoporous material supported CoFe₂O₄ NPs (Fig. S5, ESI⁺). Thus, it was expected that the CL response would increase somewhat with the increase in the pH of the CL reaction due to the nature of the luminol CL reaction. This result suggests that the pH of the mesoporous material supported $CoFe_2O_4$ NPs could affect not only the pH of the CL reaction, but also their activities. Result of the control experiment indicated that this pH-dependent catalytic activity is not caused by the nature of the luminol CL reaction, but the mesoporous material supported CoFe₂O₄ NPs themselves (Fig. 3). This was confirmed by the difference of the color variation of TMB oxidation at different pH values in the presence of the supported CoFe₂O₄ NPs (Fig. 1).

In a reversibility experiment, the pH of the SiO_2 supported $CoFe_2O_4$ NPs was changed from 7.0 to 2.0 and back to 7.0 three times, and the CL emission reached the expected value in all cases (Fig. 4), indicating a good reversibility of this sensing material. The effect of the nanoparticle size on the present CL reaction in the size (hydrated diameter) from 152 to 606 nm was investigated. As seen, the CL intensity decreased with increasing the diameter of $CoFe_2O_4$ NPs (Fig. S6, ESI†). The supported NPs samples showed good dispersion of $CoFe_2O_4$ NPs on the surface of the mesoporous supports (Fig. S7, ESI†).



Fig. 3 Reversible variation of the CL response with pH of CoFe₂O₄ NPs (A), SBA-15 supported CoFe₂O₄ NPs (B), SiO₂ supported CoFe₂O₄ NPs (C) and Al₂O₃ supported CoFe₂O₄ NPs (D). Conditions: 2.0×10^{-6} mol L⁻¹ luminol in 0.1 mol L⁻¹ Na₂CO₃-NaHCO₃ buffer (pH 11.7); CoFe₂O₄ concentration: 2.14 mg L⁻¹; SBA-15, SiO₂, Al₂O₃ supported CoFe₂O₄ were made by 2.14 mg L⁻¹ CoFe₂O₄ and 20 mg L⁻¹ SiO₂, 20 mg L⁻¹ Al₂O₃, and 8 mg L⁻¹ SBA-15, respectively.



Fig. 4 Reversibility of the pH tunable catalytic activity of CoFe₂O₄ NPs supported on SiO₂. The pH of SiO₂ supported CoFe₂O₄ NPs was changed from 7.0 to 2.0 repeatedly. The CL emission intensity was plotted for each measurement. Conditions: 2.0×10^{-6} mol L⁻¹ luminol in 0.1 mol L⁻¹ Na₂CO₃–NaHCO₃ buffer (pH 11.7); SiO₂ supported CoFe₂O₄ were made by 2.14 mg L⁻¹ CoFe₂O₄ and 20 mg L⁻¹ SiO₂.

The size of $CoFe_2O_4$ NPs on three supports is almost the same. Clearly, the size of the supported $CoFe_2O_4$ NPs on three supports is smaller than that of the unsupported $CoFe_2O_4$ NPs. The dispersion of $CoFe_2O_4$ NPs on the supports made them more stable and exposed more active sites to participate in the CL reaction, leading to much better catalytic activities than the unsupported NPs. This size effect on the CL response is consistent with that reported by Cui *et al.*⁶

Fig. 5 illustrates the dependency of CL response on superficial charge of the as-prepared NPs. As seen, the catalytic activity increased dramatically in varying the zeta potential from negative to positive. The catalytic ability of the $CoFe_2O_4$ NPs increased with increasing zeta potential and varied with support in the order $Al_2O_3 > SiO_2 > SBA-15$. This can be tentatively explained by the affinity improvement by enhancing electrostatic interaction between the substrate and the nanoparticle surface.⁷ Luminol carries two amine groups with negative charge under strong basic conditions,^{2d} likely yielding stronger affinity towards positively charged support. Hence, the supported $CoFe_2O_4$ NPs should be of positive charge and the employed SiO₂ or Al_2O_3 might provide the source of positive charge at appropriate pH values. Hence, the rationale for varied



Fig. 5 Plots of CL responses versus zeta potential for different $CoFe_2O_4$ NPs with luminol as the substrate. Zeta potentials were from Fig. S8.†

CL response was that affinity was alerted by changes in electrostatic interaction between supported $CoFe_2O_4$ NPs and luminol. Furthermore, the intensity of superficial charge on NPs appeared to influence the degree to which CL response was alerted, as the CL response varied with the varied zeta potential. Among three studied mesoporous materials, Al_2O_3 supported $CoFe_2O_4$ NPs had the highest positive charge at given pH. Hence, Al_2O_3 supported $CoFe_2O_4$ NPs showed the highest catalytic activity in the luminol CL reaction.

Results shown in Fig. S1[†] clearly demonstrated that luminol was oxidised to AP²⁻ during the CL reaction because the luminophor for the CL system was still AP²⁻. In order to understand the possible mechanism of the enhanced CL reaction, the influence of O2 on the present CL reaction was studied. When the solutions were purged with O_2 , the relative CL response increased by 30%. However, when purged with N2, it decreased by 35% (Fig. S9, ESI[†]). These results implied that O₂ participated in the CL process, indicating the important role of the dissolved O₂ in the luminol CL reaction. However, enhancement of the CL signal by CoFe₂O₄ or supported CoFe₂O₄ NPs suggested that the oxidative activity of the dissolved O2 was activated by CoFe₂O₄ or supported CoFe₂O₄ NPs, which was further confirmed by TMB oxidation by O2 catalyzed by CoFe2O4 NPs under acidic conditions (Fig. 1). Also, the scavengers of various reaction oxygen species were used to confirm the emitting species. As shown in Table 1, the CL intensity was almost totally quenched by the addition of 1 μ mol L⁻¹ ascorbic acid (scavengers of 'OH radical and O2' radical) or 1 mM thiourea (scavengers of 'OH radical), showing that abundant 'OH radicals were generated in the reaction.8 More than 99% of the CL emission was inhibited by the addition of 0.5 mg mL⁻¹ SOD, suggesting that O2^{•-} was produced and played a role in the CL emission process. O_2 .⁻ may come from the oxygen dissolved in the solution.9 After production, the recombination reaction of OH' and O2' would generate 1O2,10 which was confirmed with the significantly quenching effects upon the addition of NaN₃ (scavenger of singlet oxygen). The reaction of the produced ${}^{1}O_{2}$ with luminol might be responsible for the CL emission at 425 nm. A ¹O₂-induced luminol CL mechanism was also suggested recently by Huang et al.9,10

When mesoporous materials, such as Al_2O_3 , SiO_2 and SBA-15, were used to support $CoFe_2O_4$ NPs, strong electrostatic interaction between positively charged mesoporous material

 $\label{eq:table_$

Scavengers	Intermediates	Concentration	Percent inhibition ^{b} (%)
H ₂ O	/	/	0
Ascorbic acid	$OH', O_2'^-$	$1 \ \mu mol \ L^{-1}$	97.2
Thiourea	OH.	$1 \text{ mmol } L^{-1}$	99.3
NaN ₃	$^{1}O_{2}$	$10 \text{ mmol } L^{-1}$	44.2
SOD	O_2 .	$0.5~\mathrm{mg~mL}^{-1}$	99.1

^{*a*} Solution conditions were 2×10^{-6} mol L⁻¹ luminol in 0.1 mol L⁻¹ Na₂CO₃-NaHCO₃ buffer (pH 11.7) and 2.14 mg L⁻¹ CoFe₂O₄ in 20 mg L⁻¹ Al₂O₃. ^{*b*} Mean value of three measurements.



Scheme 1 Possible CL mechanism from the reaction between ${\rm CoFe_2O_4}$ or SBA-15 supported ${\rm CoFe_2O_4}$ NPs and luminol.

support $CoFe_2O_4$ NPs and negatively charged luminol anions occurred, this could produce much more active radical species, such as 'OH and O_2 '⁻, and lead to yield more 1O_2 on the surface of mesoporous material support $CoFe_2O_4$ NPs accompanied by strong CL emission. Based on the above discussion, the possible CL mechanism, using SBA-15 as a model mesoporous material, could be expressed in a simple form as shown in Scheme 1.

In summary, we have successfully synthesized three mesoporous material (SBA-15, SiO₂ and Al₂O₃) supported CoFe₂O₄ NPs through a simple adsorption method, and demonstrated that the mesoporous material supported CoFe₂O₄ NPs exhibited different levels of unique oxidase-like activity in the order of Al₂O₃ > SBA-15 > SiO₂ in terms of TMB color reaction, showing support dependence; this order was probably related to their superficial charge. Their oxidase-like activity also showed pH dependence, and could be reversibly controlled by their pH. The result clearly demonstrated the effective manipulation of the enzyme-like activity of NPs by the mesoporous materials. The unique properties of the as-prepared CoFe₂O₄ NPs offer a strategy for the preparation of simple and efficient multi-functional catalysts with magnetism, oxidase-like activity, and pHsensitivity.

Acknowledgements

The financial support of the research by the Natural Science Foundation of China (no. 21075099, no. 21275021) and the Fundamental Research Funds for the Central Universities (XDJK2013C114, XDJK2013A022) are acknowledged.

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