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Communications

Magnetically Separable Nanozeolites: Promising Candidates for Bio-Applications

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Zeolites, microporous crystalline silicates and aluminosilicates, were found to be efficient heterogeneous catalysts in the fields of oil refining and petrochemistry.[1] Recently, nanosized zeolites have attracted the widespread attention of scientists from different disciplines,[2] such as sensing,[3] electronics,[4] and biotechnology.[5−10] The decrease of zeolite crystal size to nanoscale not only brings a large external surface area for the interaction with macromolecules but also makes the uniform and adjustable surface properties of zeolites (i.e., surface charges and hydrophilicity/hydrophobicity)[11] more remarkably exhibited. Therefore, compared to the micrometer-sized ones, nanosized zeolites become more promising candidates for a variety of nonconventional applications, especially in the bio-related fields. Very recently, zeolite nanocrystals have been applied in the enrichment and identification of low-abundance peptides/proteins,[5,6] as well as the immobilization of enzymes for biosensing.[7] The regularly distributed silanols on the surface of nanozeolites also allowed a uniform covalent grafting of enzymes.[8] Moreover, Gd^{3+}-doped zeolite nanoparticles were considered to be good contrast agents for magnetic resonance imaging in medical diagnosis.[9] However, the conventional nanozeolites would inevitably encounter an obstacle when applied in practice, that is, the difficulties in separation and manipulation due to their small size. The usual manipulation processes involving high-speed centrifugation and redispersion by ultrasonication are quite inconvenient and unsuitable for the consecutive processes (e.g., high-speed automatic detection in biotechnology). Although the pre-assembly of nanozeolites on an appropriate substrate may partially solve this problem under some circumstances,[7,10] a high dispersibility and/or a good manipulability of zeolite nanocrystals were the premises of some important applications.[5,6,9]

Magnetic separation provides a very convenient approach for the removal of magnetic particles/composites by applying an appropriate magnetic field and is widely used in biotechnology,[12,13] such as in drug delivery/release,[14,15] DNA probe,[16] and switches for biocatalytic reactions.[17,18] Micrometer-sized

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magnetic zeolites have been synthesized through ion exchange of high-aluminum zeolites followed by reduction[19,20] or surface precipitation of iron oxides on the commercial zeolites.[21] However, both of these methods would cause a serious particle aggregation when employed on nanozeolites. Moreover, the ion-exchange process in the former method is inapplicable to aluminum-free zeolites. Herein, we report a simple and general approach for the synthesis of highly dispersed and magnetically separable nanozeolites. Zeolite nanocrystals were in situ combined with superparamagnetic magnetite (Fe_3O_4) nanoparticles (MNPs) in the hydrothermal synthesis procedure and finally formed magnetite/zeolite composite nanoparticles (MZCNPs). Similar to the conventional zeolite nanocrystals, MZCNPs exhibited a high adsorption amount of enzymes and consequently a good biocatalytic performance, while they could be manipulated or separated simply by a magnet.

The typical synthesis process of MZCNPs was as follows. A total of 0.7 g of tetrapropylammonium hydroxide solution (25 wt %) was mixed with 0.3 g of distilled water. Then 0.5 g of tetraethyl orthosilicate was added, and this precursory solution was kept stirring overnight.[22] Afterward, 0.22 g of MNPs (5−10 nm, synthesized through a coprecipitation method[23] and centrifugated from their aqueous suspension before use, Supporting Information) was dispersed in the aged synthesis solution through ultrasonication. Finally, the synthesis solution was transferred into a 15 mL Teflon lined autoclave, which was then placed in a preheated oven at 140 °C for 3 days. The product was washed with distilled water by repeated centrifugations at 6000 rpm and ultrasonications to remove the uncombined MNPs.

Figure 1a shows the scanning electron microscopy (SEM) image of the typical product. The particle size of MZCNPs is in the range of 200−300 nm, which is suitable for bioapplications.[14] The transmission electron microscopy (TEM) images (Figure 1b−d) reveal that the zeolite component in MZCNPs is polycrystalline, which may greatly expand the external surface area of MZCNPs. According to the nitrogen sorption isotherms, the external surface areas (calculated by the *t* plot method) of MZCNPs and nanosized zeolite single crystals (ca. 150 nm, Supporting Information) are 47.7 and 18.7 $m^2\ g^{-1}$, respectively. The lattice fringes on the high-resolution TEM image (Figure 1e) indicate that the zeolite component in MZCNPs is well-crystallized. A large number of high-contrast dots could be observed on the TEM images and are considered to be MNPs on the basis of their size and elemental analysis by energy-dispersive X-ray (EDX) analysis (Supporting Information). The MNPs were tightly combined with the zeolite component in MZCNPs and could hardly be shaken off under high-power ultrasonication. After treating the MZCNPs with concentrated hydrochloric acid (36 wt %) to remove the MNPs, some low-contrast areas appeared on the TEM image (Supporting Information), indicating that the MNPs may have been occluded in the zeolite particles during the hydrothermal synthesis procedure.[24] Both of the main X-ray diffraction (XRD) peaks of silicalite-1 (siliceous MFI-type zeolite) and MNP could be observed on the XRD pattern of MZCNP (Figure 2a). The room-temperature magnetization curve (Figure 2b) shows that MZCNP inherits the superparamagnetic property from MNP (Supporting Information), and its saturation magnetization value is about 8.9 emu g^{-1}. The weight ratio of MNP in the MZCNP product is calculated to be 20.5% based on

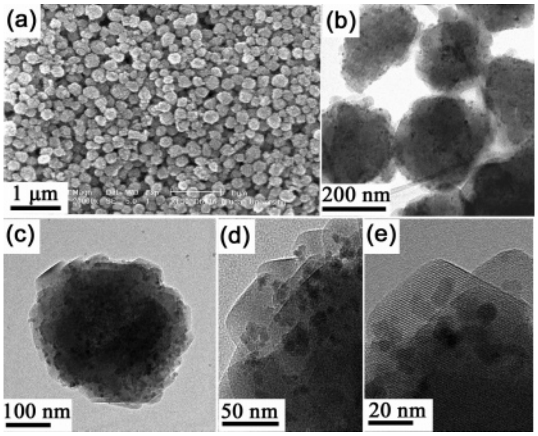

Figure 1. SEM (a) and TEM (b−e) images of MZCNPs.

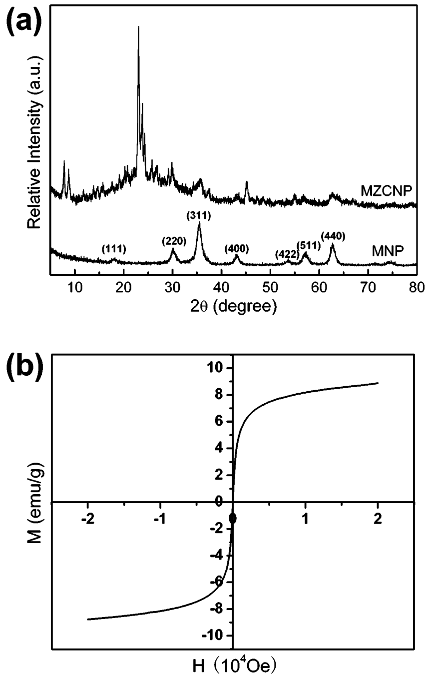

Figure 2. (a) XRD patterns of MNP and MZCNP; (b) magnetization curve of MZCNP at 300 K.

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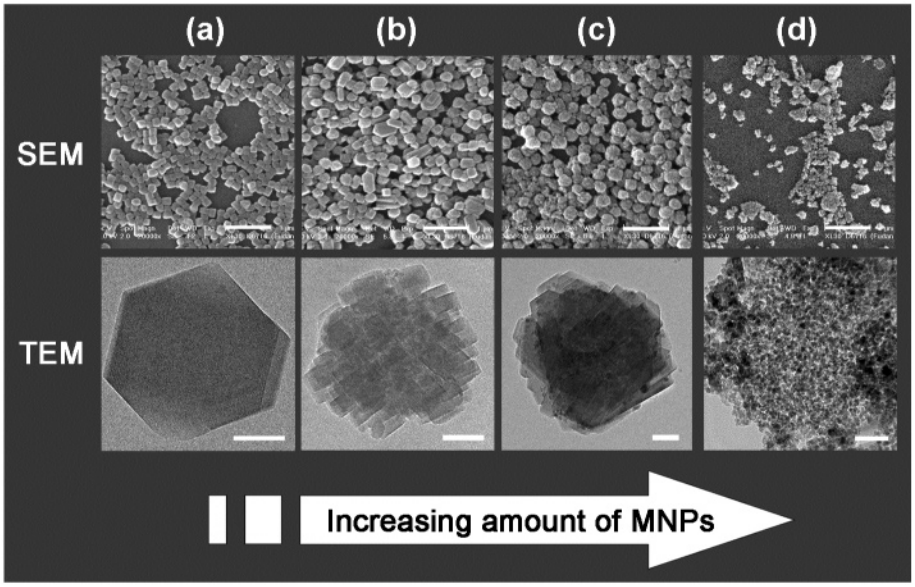

Figure 3. Morphological and textural transformation of products with increasing amount of MNPs added in the typical synthesis solution: (a) 0.05, (b) 0.15, (c) 0.24, and (d) 0.30 g. Scale bars in SEM and TEM images represent 1 μm and 50 nm, respectively. Note: The product in part d is the aggregate of MNPs, and no obvious zeolite crystals were observed under TEM.

the saturation magnetization values of MZCNP and pure MNP. When a magnet was placed near the glass bottle containing the ethanol suspension of MZCNPs, all MZCNPs could be attracted by the magnet within 90 s (Supporting Information).

The amount of MNPs added in the typical synthesis solution has a remarkable effect on the morphology and texture of product, as displayed in Figure 3. The existence of a relatively small amount ($<$0.10 g) of MNPs in the synthesis system hardly influenced the crystallization behavior of zeolites, and nanosized zeolite single crystals combined with few MNPs were obtained. As the amount of MNPs was increased from 0.10 to 0.20 g, the product gradually transformed from single-crystalline to polycrystalline nanozeolites. Nevertheless, the MNPs still showed a low tendency to be incorporated under such conditions. A further enhancement of the amount of MNPs led to the formation of polycrystalline nanozeolites with a high loading of MNPs, as described in the above section. However, an excessive amount ($>$0.25 g) of MNPs would hinder the crystallization of zeolites.

To study whether the combination of MNPs would influence the interaction between zeolite nanocrystals and biomolecules, a protein adsorption experiment was first carried out (Supporting Information). It was found that the saturated adsorption amount of hemoglobin on MZCNPs at pH 7.0 was 148 mg g^{-1}, indicating that MZCNPs have a satisfactory adsorption capacity for biomolecules. To further evaluate the biocatalytic activity and stability of enzymes immobilized on MZCNPs, hemoglobin-immobilized MZCNPs (H-MZCNPs) were applied in the decomposition reaction of H_2O_2 (Supporting Information). As shown in Figure 4a, the H-MZCNPs still retain a high relative activity in the fourth cycle of reaction, indicating the high stability of enzymes immobilized on the MZCNPs. More importantly, the removal of H-MZCNPs from the reaction system could be realized by simply applying a magnetic field, which is convenient and more suitable for the consecutive processes in bio-applications than the conventional centrifugation. Figure 4b displays the different amperometric responses to the decomposition of H_2O_2 in an electrochemical system with and without H-MZCNPs on the surface of an indium tin oxide (ITO) electrode (Supporting Information). The blank experiment showed that the bare ITO electrode had a weak catalytic activity for the reaction (green line). In contrast, a large amperometric response was observed when the H-MZCNPs were immobilized on the surface of the ITO electrode by a magnet (red line), indicating the good catalytic activity of H-MZCNPs. Notably, if the H-MZCNPs were

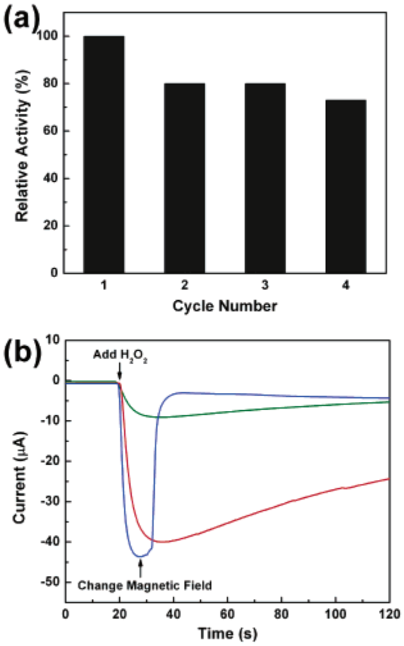

Figure 4. (a) Catalytic activity of H-MZCNPs in the decomposition of H_2O_2; (b) amperometric response to the decomposition of H_2O_2 under different surface conditions of the ITO electrode: blank (green line), H-MZCNPs adsorbed (red line), and H-MZCNPs first adsorbed then removed by a magnet (blue line).

suddenly removed off the surface of the ITO electrode during the reaction by changing the orientation of the magnetic field, the large amperometric response would be immediately quenched (blue line), further proving the excellent manipulability of MZCNPs in bio-applications.

In conclusion, magnetically separable nanozeolites have been fabricated through a simple route. The combination of the surface properties of zeolites and the superparamagnetic property of Fe_3O_4 nanoparticles endows this material with a bright perspective in various novel bio-applications such as magnetically controllable biosensors and microfluidic biochips. Moreover, this work also provided a facile and general method for synthesizing nanocomposites of zeolites and other functional nanoparticles, which may find wider applications besides in biotechnology.

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Supporting Information Available: SEM/TEM images, EDX analysis result, magnetization curve, photographs, and detailed experimental section (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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