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Chinese Chemical Letters 22 (2011) 493–496
CHINESE CHEMICAL LETTERS
www.elsevier.com/locate/cclet

Preparation of a PTE simulacrum based on surface molecular imprinting

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Received 23 August 2010
Available online 15 January 2011

Abstract

Firstly, we synthesized *N*-methacryloyl-histidine monomer and *N*-methacryloyl-histidine-Cu^{2+} complex (MAH-Cu^{2+}). Then the molecular imprinting polymers (MIP) has been prepared by surface grafting on uniform polystyrene (PS) core using reversible addition-fragmentation transfer polymerization (RAFT) with MAH-Cu^{2+} as the functional monomer, methyl paraoxon as the template to simulate phosphotriesterase (PTE). Finally, we have investigated the catalytic hydrolytic activities of MIP and non-imprinting polymers (NIP) to the template methyl paraoxon and the template analogue ethyl paraoxon respectively by UV spectrophotometry. The results showed that the catalytic hydrolytic activity of MIP to the template methyl paraoxon was highest and the value of k is 8.67×10^{-5} mmol L^{-1} min^{-1}, 3.89-fold higher than MIP to the template analogue ethyl paraoxon, 2.79-fold higher than NIP to the template methyl paraoxon. The K_M, r_m of MIP are also determined, and $K_M = 3.95 \times 10^{-4}$ mol/L, $r_m = 2.12$ μmol/min. The MIP can be reused with only lose 7% of catalytic activity for four cycles.

Keywords: Molecular imprinting; Surface grafting; Catalytic hydrolysis; Paraoxon

Organophosphate compounds are the most widely used herbicide and insecticide in agriculture**,** their long-term accumulation in the environment will cause a very serious ecological threat to humans and other animals, and the paraoxon is one of a kind commonly used. Therefore from the perspective of environmental governance that find a clean technology to remove organophosphate compounds is very urgent. Paraoxon hydrolysis equation shown as Scheme 1.

Phosphotriesterase (PTE) is a natural zinc metalloenzyme that catalyzes the hydrolysis of an extensive array of organophosphate pesticides (such as paraoxon, parathion and coumaphos) and mammalian acetylcholinesterase nerve agents (like soman and sarin) [1–3]. Although PTE can quickly and efficiently catalyze the hydrolysis of organophosphate, however, due to its preparation cumbersome, expensive, cannot resist high temperature, poor stability, so its application subject to a certain extent. But the molecular imprinting catalyst simulated PTE is the perfect replacement.

In this work, for the first time, we prepared imprinted mimic enzymes with hydrophobic PS core and hydrogel shell using RAFT technique, it has good recognized performance, can be reused with only lose 7% of catalytic activity for four cycles, most importantly, it can be applied to water system.

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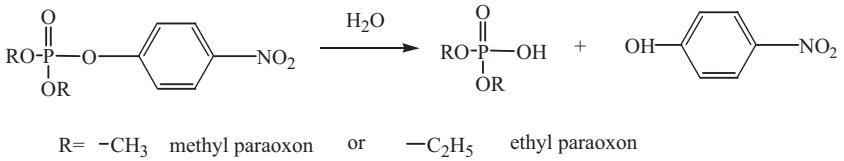
1001-8417/$ – see front matter
doi:10.1016/j.cclet.2010.10.050



Scheme 1. Paraoxon hydrolysis reaction.

1. Experimental

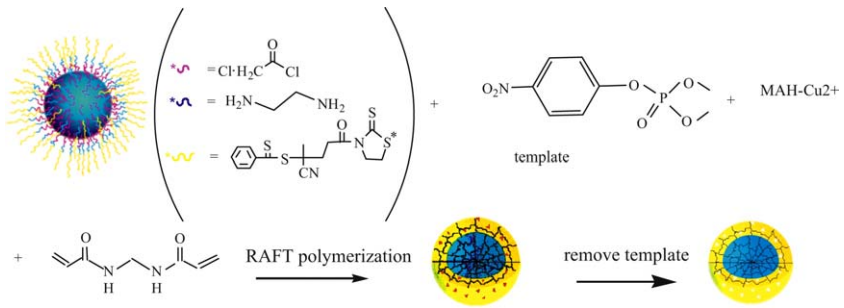
MAH and MAH-Cu^{2+} were prepared according to the reported work [4], the MIP was prepared as follows: a certain amount of disulfide ester modification of polystyrene microspheres (MPS) (containing disulfide ester group 0.1 mmol) suspended in 5.00 mL chloroform and stirred over night at 30 °C. An amount of 0.2 mmol MAH-Cu^{2+} and 0.05 mmol methyl paraoxon were dissolved in 10 mL ethanol and stirred for 2 h. To this solution, 2 mmol *N*,*N*-methylene bisacrylamide was added and dissolved. Together above suspension and solution, then 0.011 mmol AIBN was added. Deoxygenating of the system three times with liquid nitrogen. The polymerization mixture was subsequently polymerized at 70 °C with magnetic stirring for 48 h. After polymerization, centrifugated, obtained yellow solid. Washed and centrifugated with water and ethanol three times respectively. The resulting microbeads were treated with 50/50 (v/v) methanol/water containing 1.0 mol/L of KOH (totally 100 mL) for 24 h to remove the template. The microbeads were cleaned by a water–ethanol mixture and then were dried in a vacuum oven at 70 °C for 48 h. In the same way, NIPs were also prepared but without using template. The schematic presentation of the employed approach for MIP is depicted in Scheme 2.

The activity of MIP was assayed using 1 mmol/L of methyl paraoxon as substrate. As paraoxon is hydrolyzed to diethylphosphate and *p*-nitrophenol, the hydrolysis activity of polymers was measured spectrophotometrically by monitoring the increase of *p*-nitrophenol. An amount of 20 mg of polymer were suspended in 4.876 mL (20 mmol/L) Tris–HCl buffer (pH 9.0). The dispersion system under a constant temperature 25 °C for 5 min and 124 μL paraoxon in ethanol were added. Then, the activity of the MIPs was determined in batch mode using magnetic agitation at 25 °C. An amount of 100 μL of the reaction solution was sampled, diluted with 20 mmol/L Tris–HCl buffer (pH 9.0) and the absorbance at 400 nm was determined using UV spectrophotometry.

2. Results and discussion

Fig. 1 shows the SEM images of the MPS and MIP. As shown in Fig. 1, the surface of MPS microsphere is porous, while the surface of the MIP microsphere is smooth; in addition, the diameter of MPS microsphere is about 5.36 μm, while the diameter of the MIP microsphere is about 6.10 μm. So it can be concluded that the MIP shell has been successfully grafted onto the surface of MPS microsphere.

The kinetic curves (Fig. 2) illustrate the catalytic hydrolytic activity of MIP and NIP to the template methyl paraoxon and the template analogue ethyl paraoxon respectively, the result initial reaction rates (*k*) was summarized in Table 1.



Scheme 2. Schematic presentation of the fabrication of MIP.

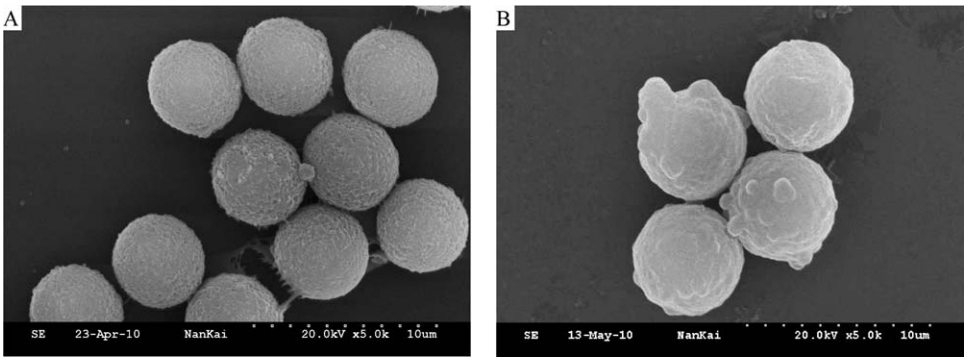


Fig. 1. SEM images of the MPS(A) and MIP(B).

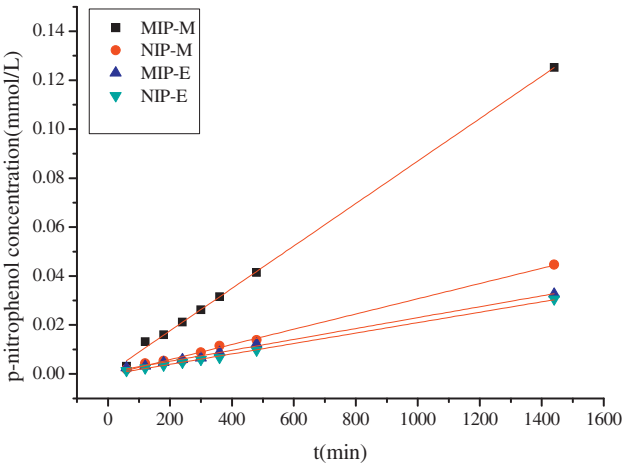


Fig. 2. The catalytic hydrolytic kinetic curves of MIP and NIP for the template methyl paraoxon (MIP-M and NIP-M) and the template analogue ethyl paraoxon (MIP-E and NIP-E), respectively.

As can be seen from Fig. 2 and Table 1, the activity of MIP to the template methyl paraoxon is highest and the value of k is 8.67×10^{-5} mmol L^{-1} min^{-1}, 3.89-fold higher than MIP to the template analogue ethyl paraoxon, 2.79-fold higher than NIP to the template methyl paraoxon.

Hydrolytic activity of MIP was evaluated in the framework of Micheaelis-Menten kinetics. For this purpose, keep MIP unchanged, the methyl paraoxon substrate concentrations at 0.2 mmol/L, 0.5 mmol/L, 2 mmol/L, 4 mmol/L were used in each case and initial reaction rates (k) of hydrolysis were determined. Then, $1/k$ versus reciprocal of the initial substrate concentration ($1/M_0$) for MIP was plotted which is called a Lineweaver–Burk plot (Fig. 3). The values of r_m and K_m were obtained from the plots as 2.12 μmol/min and 3.95×10^{-4} mol/L, respectively.

Reusability was necessary to evaluate the regeneration and recatalytical efficiency of the mimic enzyme polymer after each cycle. In order to examine the reusability of the MIP, the catalytic cycle was repeated four times using the same MIP for methyl paraoxon degradation. As can be seen from Fig. 4, after repeated four times, the catalytic ability decreased only 7%.

Table 1
The result initial reaction rates.

Entry	Methyl paraoxon (mmol L^{-1} min^{-1})	Ethyl paraoxon (mmol L^{-1} min^{-1})
MIP	8.67×10^{-5}	2.23×10^{-5}
NIP	3.11×10^{-5}	2.13×10^{-5}

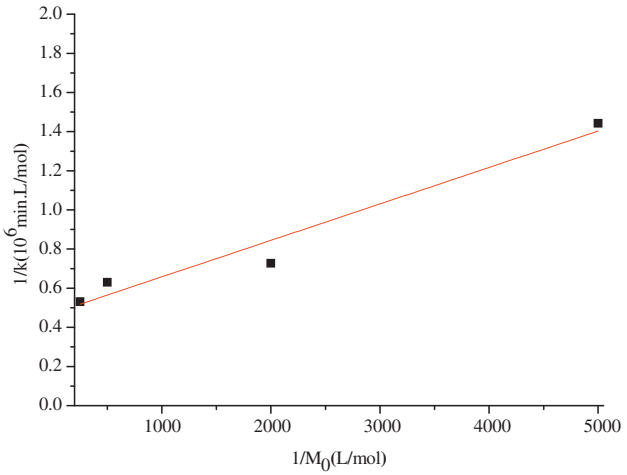


Fig. 3. Lineweaver-Burk plot of kinetics data of hydrolysis for paraoxon.

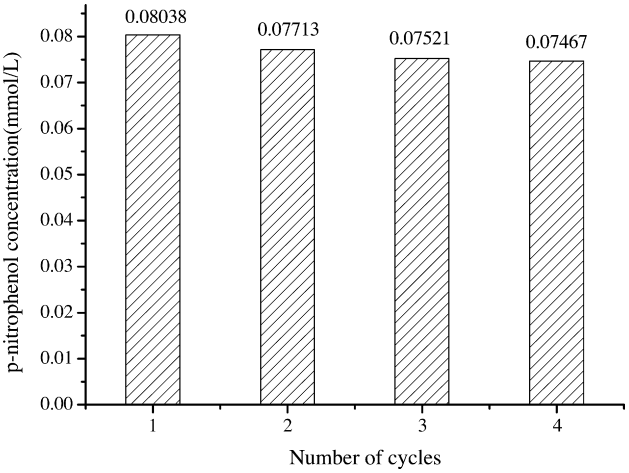


Fig. 4. Reusability of MIP.

3. Conclusion

In summary, this letter reports the preparation and catalytic activity evaluation of a MIP with hydrophobic PS core and hydrogel shell for methyl paraoxon. The results indicate that the synthetic MIP can catalyze hydrolysis of the methyl paraoxon effectively. The MIP is expected to apply in the field of environmental governance.

Acknowledgments

We gratefully acknowledge the support of the National Natural Science Foundation of China (No. 50978138).

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