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Highly selective magnetic polymer particles via molecular imprinting

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Abstract−Magnetic hydrophilic molecularly imprinted polymer (MIP) particles were successfully synthesized via an inverse suspension polymerization in silicone oil, by employing methacryloxypropyltrimethoxysilane(MPS)-modified Fe_3O_4 nanoparticles as magnetic particles, 2,4-dichlorophenoxyacetic acid (2,4-D) as template, hydroxyethyl methacrylate (HEMA) as hydrophilic monomer, and acetonitrile as high polar porogen. The synthesized magnetic hydrophilic MIP particles could be separated rapidly under an external magnetic field. About 94% transmittance of the particle-water suspension could be reached within 20 min by magnetic separation, whereas about 84% transmittance was achieved after at least 180 min by sedimentation. The adsorption capacity of the particles was also studied in pure aqueous environments. These hydrophilic MIP particles had a higher selectivity for templates. Hydrophilic MIP particles took on a higher imprinting factor than hydrophobic MIP particles and 2,4-D were able to rebind hydrophilic MIP particles more easily than 4-Chorophenoxyacetic acid.

Keywords: Molecular Imprinting, Magnetic Particles, Suspension Polymerization, Hydrophilic Particles, Selectivity

INTRODUCTION

Molecular imprinting is a versatile technique for preparing materials containing recognition sites of predetermined selectivity [1,2]. Molecularly imprinted polymers (MIPs) can be synthesized by co-polymerization of functional monomers and cross-linkers in the presence of templates. The prepared MIP has cavities with a three-dimensional structure complementary in shape, size and chemical functionality to that of the templates, and is thereby able to rebind the templates. They have been successfully applied as materials of molecular recognition in a wide range of scientific and technical fields, such as separation processes, immunoassays, antibody mimics, biomimetic sensors, catalysis and artificial enzymes [3-7]. MIP particle was the most commonly used form for various applications and research in this field has attracted greater attention [8,9].

Natural molecular recognition, such as interaction between antigen and antibody, protease and protein substrates, generally occurs in aqueous solutions. However, conventional MIPs have often exhibited poor molecular recognition capability in water since they are usually prepared in organic solvent and have a hydrophobic surface. This preparation method could cause high non-specific binding in aqueous solution, which is undesirable for many practical applications. As "artificial antibody" MIPs face a new challenge in water environment. To solve this problem, MIP particles with hydrophilic external layer have been developed and prepared via multistep reaction [10,11]. Other hydrophilic MIP particles have been synthesized by Pickering emulsion polymerization [12]. Despite the improved hydrophilic property, all of these methods require at least two complicated and time-consuming steps.

Hydroxyethyl methacrylate (HEMA) polymer is a hydrophilic and biocompatible material that has been widely applied for several biological applications [13,14]. Although HEMA particles can be fabricated by suspension polymerization [15,16], its potential for making hydrophilic MIP particles is limited due to the superior solubility of HEMA monomer in water. More HEMA monomers can disperse into water and more water can disperse into pre-polymerization droplets to destroy the interaction between functional monomers and molecular templates.

In addition, a high polar porogen is normally required [17] besides hydrophilic monomer for synthesizing hydrophilic MIPs. As a result, the feasibility of using suspension polymerization in water to prepare hydrophilic MIPs is hindered. To the best of our knowledge, the preparation of hydrophilic MIP particles with HEMA by suspension polymerization has not been reported. A new method should be developed to synthesize hydrophilic MIP particles.

As a competent group of polymer particles, magnetic polymer particles can be easily separated from the system by applying an external magnetic field. They have been widely applied in many fields such as immunoassay, cell-label, drug delivery system and polymer supported-catalysts [18-20]. MIP particles, containing magnetic components such as iron oxide, inherit the intrinsic advantage of easy separation by external magnetic fields. They have already been successfully prepared and applied in many fields such as solid-phase extraction, immunoassay and drug delivery system [21,22].

In this paper, magnetic hydrophilic MIP particles with HEMA as hydrophilic monomer were synthesized by inverse suspension

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Table 1. Typical recipes for the synthesis of magnetic particles

Materials	Hydrophilic MIP	Hydrophilic NMIP	Hydrophobic MIP	Hydrophobic NMIP
EGDMA/mmol	9	9	15	15
4-VP/mmol	3	3	3	3
HEMA/mmol	6	6	0	0
2,4-D/mmol	0.75	0	0.75	0
Acetonitrile/mL	3.18	3.18	3.82	3.82
BPO/mmol	0.03	0.03	0.03	0.03
N,N-dimethylaniline/mmol	0.03	0.03	0.03	0.03
Fe_3O_4 nanoparticles/g	0.15	0.15	0.15	0.15

polymerization for the first time.

MATERIALS AND METHODS

4-Vinylpyridine (4-VP), was purchased from Aldrich and further purified by distillation under reduced pressure. Methacryloxypropyltrimethoxysilane (MPS), silicone oil (dimethyl silicone oil), 2,4-dichlorophenoxyacetic acid (2,4-D, >99.0%) and 4-Chorophenoxyacetic acid (>98.0%) were purchased from Chengdu Kelong Chemical Reagent Company (P. R. China) and used without further purification. Ethylene glycol dimethacrylate (EGDMA, Acros) was washed with 5% sodium hydrate solution to remove the inhibitor and distilled under reduced pressure. HEMA (Tokyo Chemical Industry Co. Ltd., Japan) was distilled under reduced pressure. Benzoyl peroxide (BPO) was purchased from Shanghai Chemical Reagent Company, P. R. China and recrystallized from chloroform solution with methanol. Other reagents were purchased from Guangdong Jinhuada Chemical Reagent Company (P. R. China).

1. Preparation of Fe_3O_4 Magnetic Nanoparticles [23,24]

12.2 g $FeCl_3 \cdot 6H_2O$ and 8.4 g $FeSO_4 \cdot 7H_2O$ were added into 100 mL distilled water and temperature was increased to 90 °C. Then 30 mL of ammonium hydroxide and 2 g of oleic acid were sequentially added rapidly. After 3 h, the sediment was washed with distilled water until neutrality was achieved. The magnetic nanoparticles were dried under vacuum at 50 °C for at least 12 h. 0.5 g magnetic nanoparticles were dispersed into 50 mL toluene to form a ferrofluid.

4 mL MPS and 8 mmol dibutylamine were added into 50 mL toluene dispersion of magnetic nanoparticles and were then stirred for 24 h at room temperature. The MPS-modified Fe_3O_4 nanoparticles were precipitated from the dispersion by adding 250 mL petroleum ether and were collected by magnetic separation. Then the magnetic nanoparticles were dispersed in toluene and precipitated by petroleum ether for three times to remove unreacted MPS and replaced oleic acid. Finally, the magnetic nanoparticles were dried under vacuum at 50 °C for at least 12 h.

2. Synthesis of Magnetic Hydrophilic MIP Particles

0.75 mmol 2,4-D, 3 mmol 4-VP, 6 mmol HEMA, 9 mmol EGDMA, 0.15 g Fe_3O_4 magnetic nanoparticles, 0.3 mmol BPO and 0.3 mmol N,N-dimethylaniline were dissolved in 3.18 mL acetonitrile. 50 mL silicone oil was purged with a stream of nitrogen gas for 15 min. The pre-polymerization mixture was added and stirred at 800 rpm for 5 min, and then polymerized under 10 °C for 24 h and 30 °C for 3 h without stir. The synthesized particles were filtered and washed with petroleum ether and toluene. To extract 2,4-D from the polymer networks, the particles were washed with MeOH-pyridine (90/10, v/v) (12×4 h) and MeOH (3×4 h). Finally the particles were dried under vacuum at 50 °C for at least 12 h.

The magnetic hydrophilic non-imprinted polymer (NMIP) particles were prepared and treated in the same manner without 2, 4-D. The magnetic hydrophobic MIP and NMIP particles were prepared with 15 mmol EGDMA and without HEMA. There were the same ratios of 4-VP (mol/mol) in both hydrophilic and hydrophobic particles. Typical recipes used for the synthesis of magnetic particles are shown in Table 1.

Scanning electron micrographs (SEM) were taken by an AMRAY-100 (Amray, USA) scanning electron microscope. The samples were coated under vacuum with a thin layer of gold.

The particles were dispersed in water and diameter analysis was carried out on a Mastersizer 2000 (Malvern, UK).

Atomic emission spectrum was taken on an IRIS 1000 ICP-AES instrument (Thermo Electron. USA).

The magnetic responsivity of the magnetic hydrophilic MIP particles was investigated as follows: the particles were suspended in water solution and the transmittance of the suspension was determined by UV-vis spectrophotometry at 580 nm at a predetermined time under an additional 0.42 T magnetic field or under sedimentation condition.

3. Adsorbing Experiments

A buffer solution was first prepared by dissolving 25 mmol $Na_2HPO_4 \cdot 12H_2O$, 25 mmol $NaH_2PO_4 \cdot 2H_2O$, 0.1 g sodium dodecyl sulfate (SDS) and 40 mL ethanol into 1,000 ml deionized water. 100 mg of MIP particles was placed in a 25 mL conical flask, and then mixed with 10 ml 2,4-D buffer solution with specific initial concentrations ranging from 0.2 to 5.0 mmol/L. After the conical flasks were shaken for 24 h, the solution was separated under an additional 0.42 T magnetic field. The concentration of free 2,4-D in the solution was measured by UV-vis spectrophotometry (VARIAN CARY 100 Conc, USA) at 285 nm. In addition, the adsorbing amount of 2,4-D to the non-imprinted polymer particles was also determined.

The same method was used in recognition selectivity experiments, and the solutions were 4.0 mmol/L of 2,4-D and 4-Chorophenoxyacetic acid in buffer solution respectively.

RESULTS AND DISCUSSION

1. Synthesis of Magnetic Hydrophilic MIP Particles

To prepare magnetic hydrophilic MIP particles, HEMA (hydro-

philic monomer) and CH_3CN (high polar porogen) were used in this paper. Suspension polymerization in water could not be achieved because HEMA and CH_3CN have superior solubility in water and they weighted about 77% (w/w) of the pre-polymerization mixture. Moreover, since all materials of the pre-polymerization mixture are soluble in oil phase such as toluene, conventional inverse suspension polymerization could also not be performed.

We adapted a special inverse suspension polymerization method to prepare the magnetic hydrophilic MIP particles [25]. Silicone oil was used as disperse phase since pre-polymerization mixture did not dissolve in it. After stirring only 5 min, pre-polymerization droplets were dispersed in silicone oil well and steadily. Thereby, magnetic hydrophilic MIP particles could be obtained by polymerization via one step. Fig. 1 shows SEM images of magnetic hydrophilic MIP particles and magnetic hydrophilic NMIP particles. Both of these two types of particles exhibit spherical shape in the microscale range.

The particles were dispersed in water and their diameter was measured. Fig. 2 shows diameter and distribution of magnetic hydrophilic MIP and NMIP particles. The particles are both polydisperse. There are two peaks on distribution curves of magnetic hydrophilic particles. Two parts of particles were in accordance with Gaussian distribution, respectively. One part was particles with 1.5 μm in diameter and its volume fraction was about 7.94%. Another part was particles with 18 μm in diameter and its volume fraction was about 92.06%. The reason for giving two peaks was probably because stir was not employed during the polymerization, which was different with the conventional suspension polymerization and inverse suspension polymerization. When hydrophilic particles disperse in water solution, it will take a long time that all particles with 1.5 μm in diameter go down to the bottom under sedimentation condition. Generally, magnetic separation is a method to solve this problem.

To compare the selectivity characters of magnetic hydrophilic particles in water, magnetic hydrophobic MIP and NMIP particles without HEMA were also synthesized by the same methods. Fig. 3 shows SEM images of magnetic hydrophobic MIP particles. As can be seen from Fig. 1 and Fig. 3, the morphology and size between hydrophobic magnetic MIP particles and hydrophilic mag-

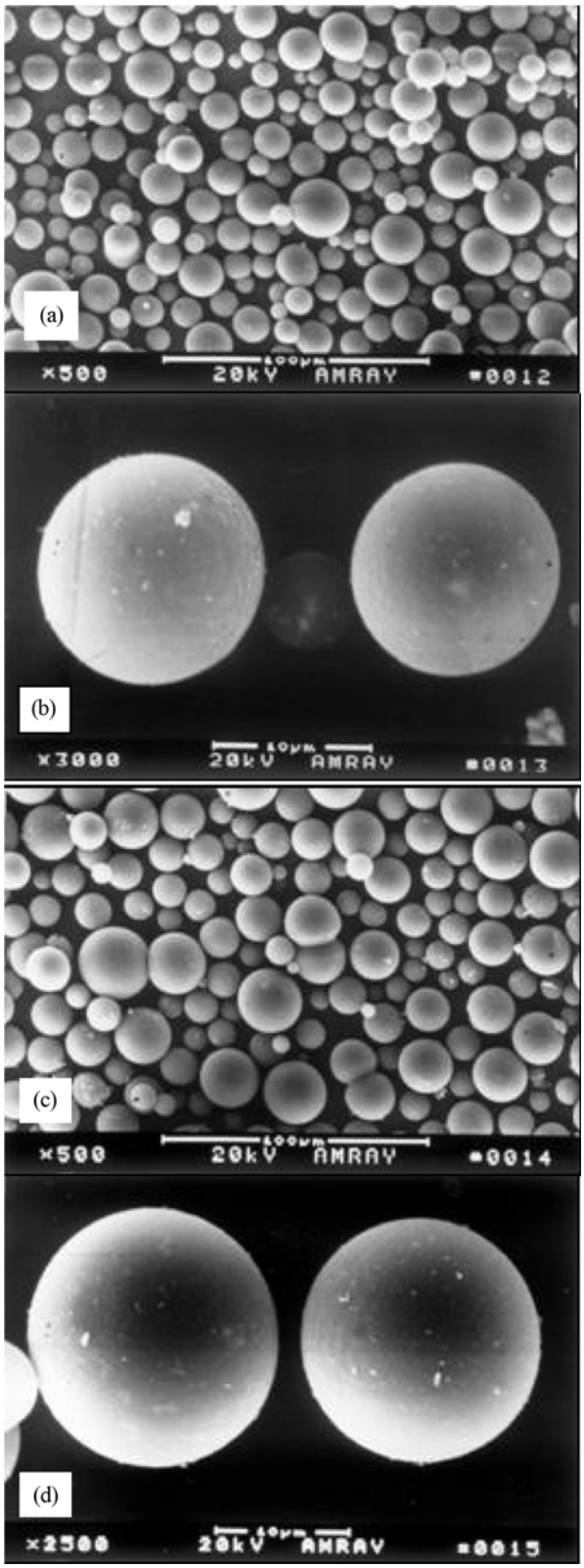

Fig. 1. SEM images of magnetic hydrophilic MIP (a), (b) and NMIP particles (c), (d).

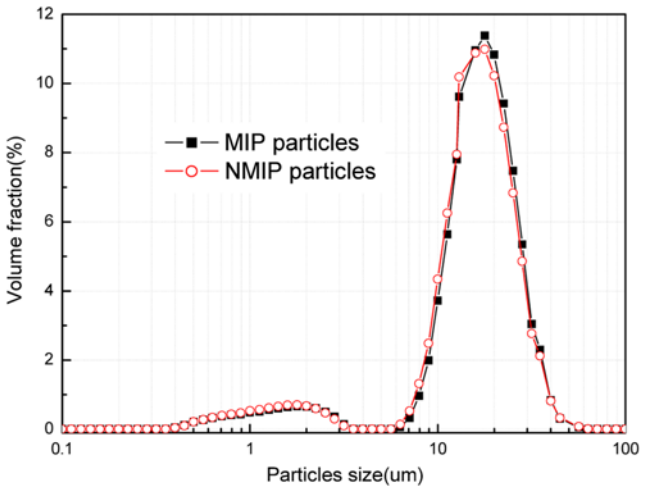

Fig. 2. Diameter and distribution of magnetic hydrophilic MIP and NMIP particles.

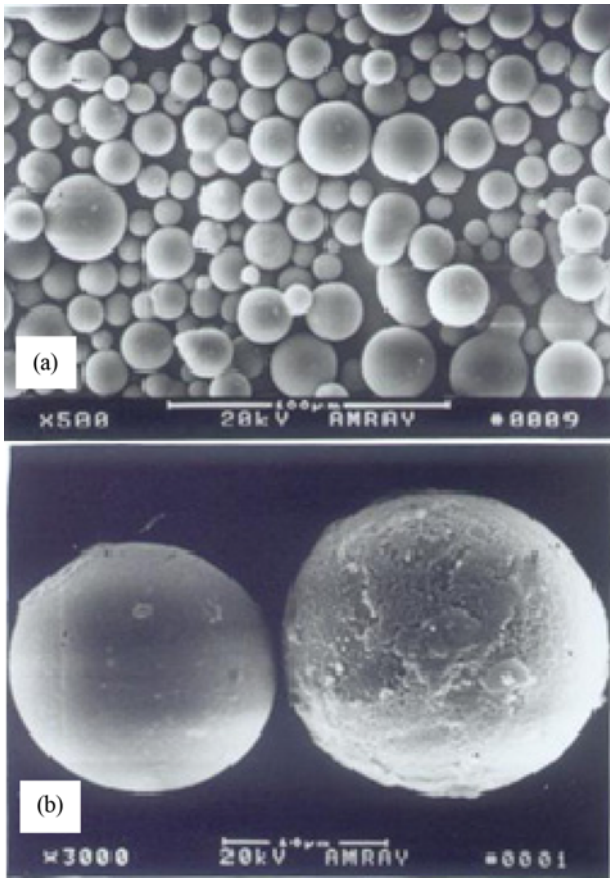

Fig. 3. SEM images of magnetic hydrophobic MIP particles.

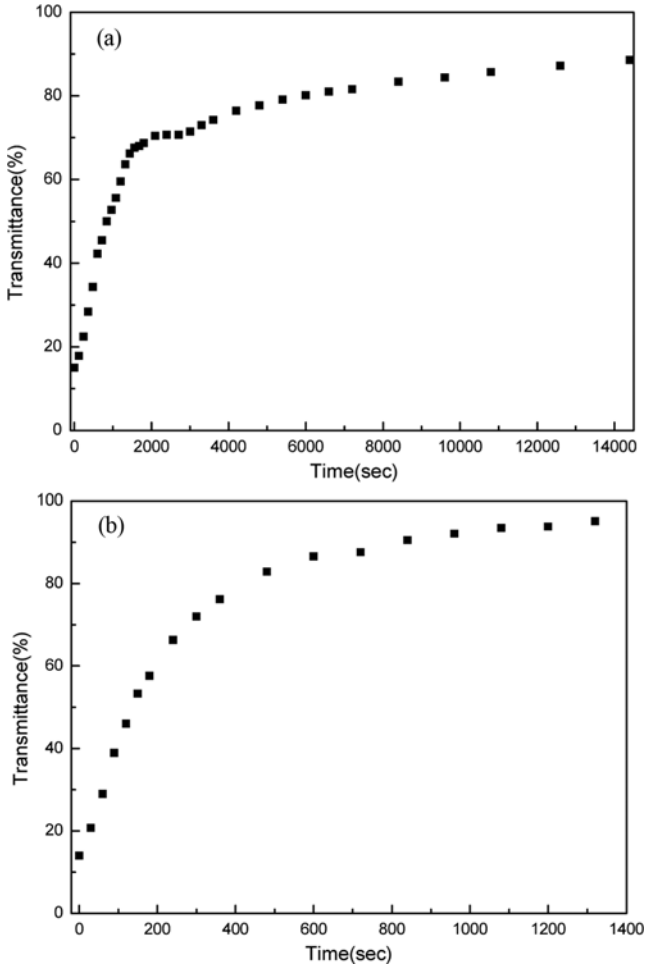

Fig. 4. Transmittance of suspension of magnetic hydrophilic MIP particles after being separated for a certain time: (a) Sedimentation (b) magnetic separation (0.42 T).

netic MIP particles are similar.

2. Magnetic Responsivity of Magnetic Hydrophilic MIP Particles

Magnetic responsivity is an important property of magnetic hydrophilic MIP particles. With a better magnetic responsivity, magnetic hydrophilic MIP particles can be separated and purified fast. In our study, Fe_3O_4 nanoparticles were prepared by co-precipitation method using oleic acid as surfactant [23] and were modified with MPS by ligand exchange reaction. MPS molecules were connected with Fe_3O_4 nanoparticles through Si-O-Fe bonds. C=C bonds at another end of MPS could be connected with cross-linker, functional monomer or hydrophilic monomer by polymerization [24]. Amounts of Fe in the magnetic MIP particles were obtained by atomic emission spectrum. And then the amounts of Fe_3O_4 were calculated by formula (Fe_3O_4%=Fe%/0.7235). As shown in Table 2, the amount of Fe_3O_4 in the magnetic MIP particles did not decrease after being immersed in water and shaken for a week. This result suggests that the Fe_3O_4 nanoparticles did not leak from the magnetic MIP particles, even though the crosslink of hydrophilic particles was lower than that of hydrophobic particles since these magnetic nanoparticles were fixed within the network of polymer particles via covalent bonds.

Table 2. The amounts of Fe_3O_4 in the magnetic MIP particles

Times (day)	0	1	2	3	5	7
Fe_3O_4[a] (% w/w)	1.11	1.10	1.08	1.09	1.09	1.08
Fe_3O_4[b] (% w/w)	1.35	1.35	1.33	1.33	1.34	1.33

[a]Hydrophobic particles
[b]Hydrophilic particles

Fig. 4 shows the magnetic separation property of magnetic hydrophilic MIP particles, which was determined through transmittance of the particle suspension after being separated by 0.42 T magnetic field for a certain time. The experimental results indicated that magnetic hydrophilic MIP particles could be separated easily and rapidly under magnetic field. About 94% transmittance of the particles-water suspension could be reached within 20 min by magnetic separation, whereas about 84% transmittance was achieved after at least 180 min by sedimentation. Under sedimentation condition, the particles with 18 μm in diameter went to the bottom quickly, but the particles with 1.5 μm in diameter fell very slowly. Under 0.42 T magnetic field, all the particles could go to the bottom very quickly. The magnetic hydrophilic MIP particles had excellent magnetic responsiveness.

3. Adsorption Capacity of Magnetic Hydrophilic MIP Particles

The adsorbing curves of the synthesized particles were measured in 2,4-D buffer solution with a concentration ranging from 0.2 to

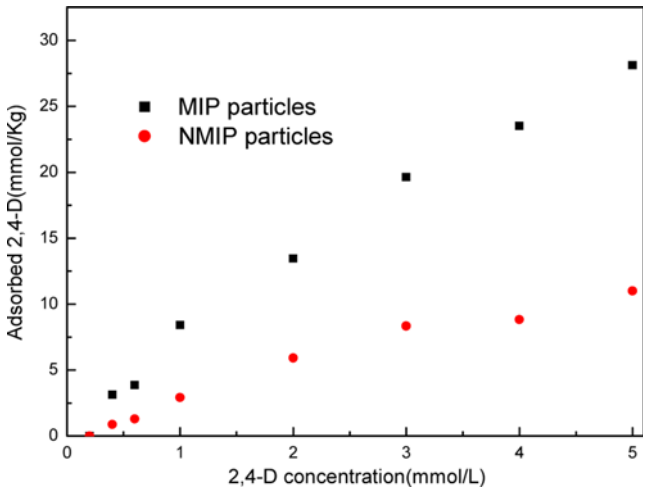

Fig. 5. Adsorbing curves of magnetic hydrophilic particles in 2,4-D buffer solution: MIP particles (■) and NMIP particles (●).

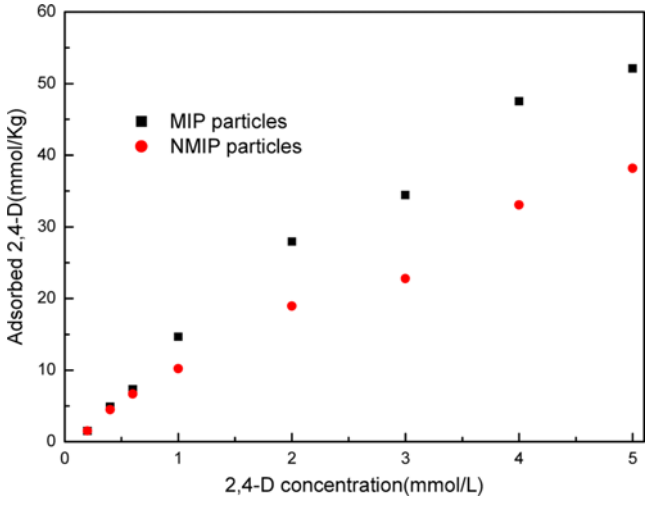

Fig. 6. Adsorbing curves of magnetic hydrophobic particles in 2,4-D buffer solution: MIP particles (■) and NMIP particles (●).

5.0 mmol/L. The experimental results of magnetic hydrophilic particles and magnetic hydrophobic particles are shown in Fig. 5 and Fig. 6, respectively.

The adsorbing amount of hydrophobic MIP particles was 47.5 mmol/kg while that of hydrophobic NMIP particles was 33.0 mmol/kg when concentration of 2,4-D was 4.0 mmol/L. In comparison, the adsorbing amount of hydrophilic MIP particles was 24.5 mmol/kg while that of NMIP particles was 8.4 mmol/kg when concentration of 2,4-D was 4.0 mmol/L. It can be seen that the adsorbing amounts of MIP particles were more than that of NMIP particles. The reason was probably that MIP particles were synthesized with 2,4-D as template and there were many cavities with functional groups, fixed size and shape complemented to 2,4-D according to the principle of molecular imprinting.

In addition, the adsorbing amounts of hydrophilic MIP and NMIP particles were greatly reduced compared with hydrophobic particles. The recognition selectivity of hydrophilic magnetic MIP particles and NMIP particles was evaluated by the static distribution coefficient K_D and imprinting factor (IF). The IF indicates the recognition selectivity of MIP particles for templates, compared with NMIP particles. The greater the IF value is, the higher the selective capacity will be. As shown in Table 3, the IF of hydrophobic particles is 1.50, while the IF of hydrophilic particles is 3.03. It suggests that hydrophilic MIP particles could better selectively recognize 2,4-D in buffer solution.

Table 3. K_D of 2,4-D on magnetic MIP and NMIP particles

Particles	K_D[a] (MIP) L/kg	K_D (NMIP) L/kg	IF[b]
Hydrophobic	13.5	8.99	1.50
Hydrophilic	6.52	2.15	3.03

[a]K_D=Qe/Ce, where Qe (mmol/kg) was the equilibrium binding amount; Ce (mmol/L) was the equilibrium concentration
[b]IF=K_D (MIP)/K_D (NMIP)

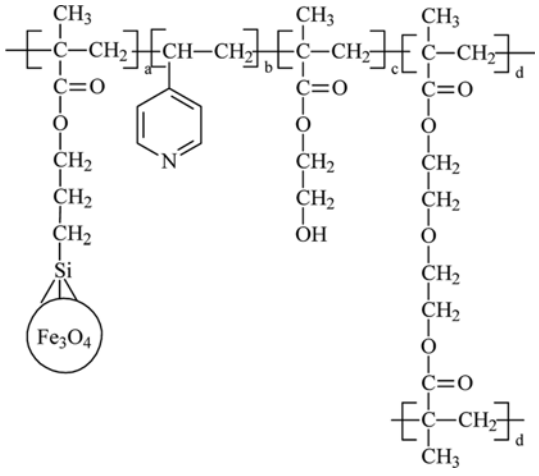

Fig. 7. Chemical Structure of MIP.

There are two types of interactions for the adsorption of MIP particles in buffer solution: 1) selective adsorption caused by the complementary three-dimensional structure of MIP particles and ion interaction between poly-4-VP and 2,4-D; and 2) non-selective adsorption by the interaction between the hydrophobic surface of particles and hydrophobic groups of 2,4-D. The hydrophobic surface was formed basically by C-C main chains and hydrophobic side groups (-CH_3) in MIP particles (Fig. 7).

In hydrophilic particles with hydrophilic HEMA groups, the non-selective hydrophobic interaction between particles hydrophobic surface with hydrophobic groups of 2,4-D was suppressed. Therefore, the adsorbing amounts of hydrophilic MIP particles and hydrophilic NMIP particles were both greatly reduced; however, the selective adsorption capacity of hydrophilic MIP particles was still preserved and the molecular recognition selectivity was thus improved, compared with hydrophobic MIP and NMIP particles.

The recognition selectivity of hydrophilic magnetic MIP particles was also evaluated by K_D and α. The parameterαindicates the recognition selectivity of hydrophilic magnetic MIP particles for templets, compared with other substrates. The greater the α value is, the higher the selective capacity will be. If α approximates 1, the

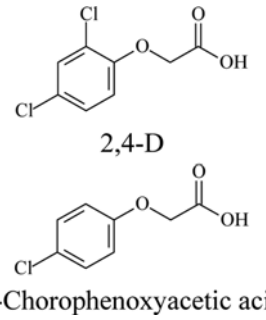

Fig. 8. Structural formula of substrates.

Table 4. K_D of tested substrates on magnetic hydrophilic MIP and NMIP particles

Substrates	2,4-D	4-Chorophenoxyacetic acid	α^a
K_D (MIP) L/kg	6.52	3.45	1.89
K_D (NMIP) L/kg	2.15	1.99	1.08

[a] $\alpha = K_D$ (2,4-D)/K_D (4-Chorophenoxyacetic acid)

particles are not able to distinguish templets from other substrates.

2,4-D and 4-Chorophenoxyacetic acid, which have similar structure, were chosen to indicate recognition selectivity of hydrophilic magnetic MIP particles. Their structural formulas are shown in Fig. 8.

As shown in Table 4, the α of magnetic hydrophilic MIP particles is 1.89, while the α of magnetic hydrophilic NMIP particles is 1.08. The results suggests that hydrophilic magnetic MIP particles could recognize 2,4-D selectively. The reason is probably attributed to the fact that the cavities in MIP particles were more suitable for 2,4-D because MIP particles were synthesized with 2,4-D as template. After removing 2,4-D from MIP particles, these cavities remained and 2,4-D was able to rebind MIP particles more easily than other substrate such as 4-Chorophenoxyacetic acid. NMIP particles, in which there were no such cavities, could not recognize 2,4-D.

CONCLUSION

Magnetic hydrophilic MIP particles were prepared by inverse suspension polymerization in silicone oil. The magnetic hydrophilic MIP particles could be separated rapidly under magnetic field. The adsorption capacity of the particles was studied in pure aqueous environments. These hydrophilic MIP particles had a higher selectivity for templates. Hydrophilic MIP particles took on a higher imprinting factor than hydrophobic MIP particles and 2,4-D were able to rebind hydrophilic MIP particles more easily than 4-Chorophenoxyacetic acid. More importantly, they are hydrophilic particles which have a promising potential for the recognition and separation of natural and water-soluble molecules and species such as antibody and protein.

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REFERENCES

1. G. Wulff, *Chem. Rev.*, **102**, 1 (2002).
2. K. Haupt and K. Mosbach, *Chem. Rev.*, **100**, 2495 (2002).
3. G. Wulff, *Microchim Acta*, **180**, 1359 (2013).
4. A. Martín-Esteban, *Trends in Analytical Chemistry*, **45**, 169 (2013).
5. Y. Yin, Z. Dong, Q. Luo and J. Liu, *Progress in Polymer Sci.*, **37**, 1476 (2012).
6. K. Yang, J. Liu, S. Li, Q. Li, Q. Wu, Y. Zhou, Q. Zhao, N. Deng, Z. Liang, L. Zhang and Y. Zhang, *Chem. Commun.*, **50**, 9521 (2014).
7. J. Wang, J. Li, H. Li and L. Ding, *Mater. Lett.*, **131**, 9 (2014).
8. E. Asadi, S. Azodi-Deilami, M. Abdouss, D. Kordestani, A. Rahimi and S. Asadi, *Korean J. Chem. Eng.*, **31**, 1028 (2014).
9. L. Piscopo, C. Prandi, M. Coppa, K. Sparnacci, M. Laus, A. Laganà, R. Curini and G. D'Ascenzo, *Macromol. Chem. Phys.*, **203**, 1532 (2002).
10. M. Zhao, X. Chen, H. Zhang, H. Yan and H. Zhang, *Biomacromolecules*, **15**, 1663 (2014).
11. T. Zhou, L. Jørgensen, M. Mattebjerg, I. Chronakis and L. Ye, *RSC Adv.*, **4**, 30292 (2014).
12. X. Shen, C. Xu and L. Ye, *Soft Matter*, **8**, 7169 (2012).
13. V. Bulmus, Y. Chan, Q. Nguyen and H. Tran, *Macromol. Biosci.*, **7**, 446 (2007).
14. F. Xu, E. Kang and K. Neoh, *Biomaterials*, **27**, 2787 (2006).
15. B. Gao, Y. Yang, J. Wang and Y. Zhang, *J. Biochem. Molecular Toxicology*, **22**, 166 (2008).
16. C. Gomez, G. Pastrana, D. Serrano, E. Zuzek, M. Villar and M. Strumia, *Polymer*, **53**, 2949 (2012).
17. B. Dirion, Z. Cobb, E. Schillinger, L. Andersson and B. Sellergren, *J. Am. Chem. Soc.*, **125**, 15101 (2003).
18. H. Daniel, B. Michal, M. Hana and J. B. Milan, *J. Sep. Sci.*, **30**, 1751 (2007).
19. C. Gonzato, M. Courty, P. Pasetto and K. Haupt, *Adv. Funct. Mater.*, **21**, 3947 (2011).
20. M. Ding, X. Wu, L. Yuan, S. Wang, Y. Li, R. Wang, T. Wen, S. Du and X. Zhou, *J. Hazard. Mater.*, **191**, 177 (2011).
21. X. Wang, L. Wang, X. He, Y. Zhang and L. Chen, *Talanta*, **78**, 327 (2009).
22. Y. Zou, C. Zhao, J. Dai, Z. Zhou, J. Pan, P. Yu, Y. Yan and C. Li, *Colloid Polym. Sci.*, **292**, 333 (2014).
23. W. Zheng, F. Gao and H. Gu, *J. Magn. Magn. Mater.*, **288**, 403 (2005).
24. Y. Sun, X. Ding, Z. Zheng, X. Cheng, X. Hu and Y. Peng, *Chem. Commun.*, **26**, 2765 (2006).
25. X. Wang, X. Ding, Z. Zheng, X. Hu, X. Cheng and Y. Peng, *Macromol. Rapid Commun.*, **27**, 1180 (2006).