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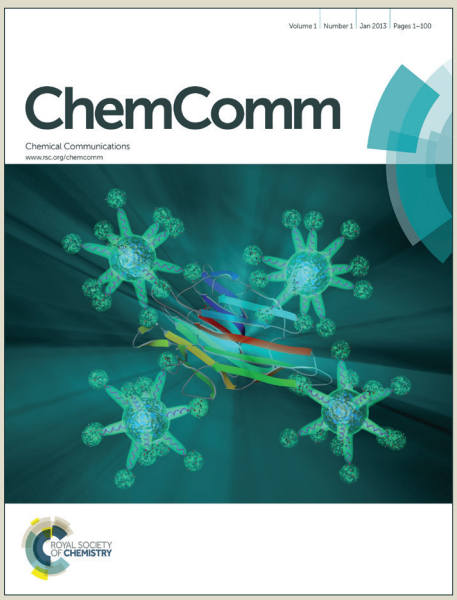

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Efficient synthesis of narrowly dispersed hydrophilic and magnetic molecularly imprinted polymer microspheres with excellent molecular recognition ability in the real biological sample

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A facile and highly efficient approach to obtain narrowly dispersed hydrophilic and magnetic molecularly imprinted polymer microspheres with molecular recognition ability in the real biological sample as good as what they show in the organic solvent-based media is described for the first time.

Molecularly imprinted polymers (MIPs) are tailor-made synthetic receptors with high affinity and specificity towards the targeted analytes.[1,2] Although they have long been recognized as promising alternatives to biological receptors, the presently developed MIPs targeting small organic molecules are normally only organic solvent-compatible and they mostly fail to show specific template bindings in the aqueous solutions, which significantly limits their practical applications in such fields as immunoassay and biomimetic sensors.[2] Many efforts have been devoted to address this issue in the past two decades and some progress has been made in this field.[3-5] In some cases, water-compatible MIPs with specific template bindings in the pure aqueous media[4] (or even in real biological samples[5]) being almost the same as what they show in the organic solvent-based media have been achieved. Unfortunately, their nonspecific template bindings in the aqueous media proved to be typically much higher than those obtained in the organic solvent-based systems, which is very detrimental to their assay and sensor applications. Therefore, the design of MIPs with both their specific and nonspecific template bindings in the aqueous solutions (especially in real biological samples) being the same as those obtained in the organic solvent-based media (which is the ultimate goal of molecular imprinting for developing water-compatible MIPs) remains a great challenge.

Recent years have also witnessed rapidly increasing interest in the magnetic MIPs because their efficient magnetic separation is very useful for practical applications.[6] So far, many magnetic MIPs have been prepared by different approaches. The presently normally used method involves the first preparation of magnetic Fe_3O_4 particles and their subsequent grafting of MIP layers to form magnetic core-shell MIP particles.[7] However, time-consuming surface modification of magnetic particles is necessary prior to their grafting of MIP layers, which largely limits their broad applications. Therefore, the development of facile and versatile approaches for the efficient preparation of magnetic MIPs is highly desirable.

Herein, we report a facile and highly efficient approach to obtain narrowly dispersed hydrophilic and magnetic MIP microspheres with both their specific and nonspecific template bindings in the aqueous solutions (in particular in the real biological sample (i.e., the undiluted bovine serum)) being the same as those obtained in the organic solvent-based medium. It involves the first synthesis of uniform MIP microspheres with surface-grafted hydrophilic poly(glyceryl monomethacrylate) (poly(GMMA) or PGMMA) brushes by the combined use of reversible addition-fragmentation chain transfer (RAFT) precipitation polymerization (RAFTPP)[8] and surface-initiated RAFT polymerization[9] and their subsequent attachment of magnetic Fe_3O_4 nanoparticles via a simple coprecipitation process (Fig. 1). The key to the success of this strategy is the introduction of PGMMA brushes onto the MIP microspheres, which allows the easy and stable immobilization of Fe_3O_4 nanoparticles by their strong multidentate interactions with the 1,2-diol groups of the polymer brushes.[10] The presence of Fe_3O_4 nanoparticles on the polymer brushes of the MIP microspheres

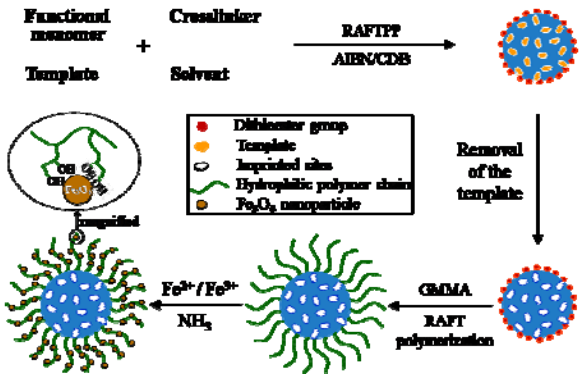

Fig. 1 Schematic protocol for the synthesis of narrowly dispersed hydrophilic and magnetic MIP microspheres with excellent molecular recognition ability in the real biological sample.

proved to not only impart them with proper magnetic properties easily, but also largely improve their surface hydrophilicity, thus leading to the water-compatible and magnetic MIPs with excellent molecular recognition ability both in the pure water and in the real biological sample.

To demonstrate the general principle, a model non-covalent molecular imprinting system was chosen here, which used 2,4-dichlorophenoxyacetic acid (2,4-D), 4-vinylpyridine, ethylene glycol dimethacrylate, and a mixture of methanol and water (4:1 v/v) as the template, functional monomer, crosslinker, and porogenic solvent, respectively. RAFTPP was carried out to prepare 2,4-D-imprinted polymer (2,4-D-MIP) microspheres with AIBN as the initiator and cumyl dithiobenzoate (CDB) as the RAFT agent in the presence of a large amount of porogenic solvent. The corresponding control polymer (CP) microspheres were also prepared similarly but in the absence of the template. The living characteristics of RAFTPP provided "living" 2,4-D-MIP/2,4-D-CP microspheres with surface-bound dithioester groups, as indicated by their light pink color.

Surface-initiated RAFT polymerization of GMMA was then performed to prepare 2,4-D-MIP/2,4-D-CP microspheres with surface-grafted PGMMA brushes (or namely grafted 2,4-D-MIP/2,4-D-CP microspheres) by using the above-obtained "living" MIP/CP microspheres (i.e., the ungrafted MIP/CP microspheres) as the immobilized RAFT agent, CDB as the sacrificial RAFT agent, AIBN as the initiator, and methanol as the solvent. The addition of a certain amount of CDB into the above polymerization systems proved to not only increase the control over the polymerization processes, but also provide some free PGMMA in the reaction solutions, which could be used to evaluate the chemical structures (i.e., molecular weights and dispersities) of the grafted polymer brushes.[11] A weight increase of 8.6% and 8.7% was obtained for the MIP and CP after their surface modification, respectively (Table S1), revealing the successful grafting of PGMMA brushes.

It has been well demonstrated that highly stable PGMMA-coated magnetic Fe_3O_4 nanoparticles can be effectively prepared by the chemical coprecipitation of Fe^{2+} and Fe^{3+} in the presence of PGMMA because of the cooperation of the multidentate interactions of 1,2-diols on PGMMA with iron atoms at the surface of the superparamagnetic iron oxide nanoparticles.[10] Therefore, the magnetic 2,4-D-MIP/2,4-D-CP microspheres were readily prepared by the chemical coprecipitation of Fe^{2+} and Fe^{3+} (1:2 molar ratio) in the presence of the grafted 2,4-D-MIP/2,4-D-CP microspheres in a mixture of water and methanol (4:1 v/v) under mild reaction conditions. The resulting products had a dark brown color even after thorough washing, which is rather different from the light pink color of the grafted MIP/CP microspheres, suggesting the formation of the grafted magnetic MIP/CP microspheres.

A SEM study revealed that all the above-obtained MIPs/CPs were narrowly dispersed spherical particles with their number-average diameters around 2.7 μm (Fig. 2a-f) and the grafted MIP/CP microspheres showed somewhat larger diameters than their corresponding ungrafted ones (Table S1). This, together with the presence of the characteristic peaks for the hydroxyl O-H stretching band around 3540 cm^{-1} in the FT-IR spectra of the grafted MIPs/CPs (Fig. S1) as well as their considerably reduced water contact angles (Fig. 2g, Table S1) and much higher water dispersion stability (Fig. 2h, Fig. S2) in comparison with the ungrafted ones, strongly verified the successful grafting of hydrophilic PGMMA brushes. In addition, the GPC study indicated that the number-average molecular weights ($M_{n,GPC}$) of the esterified polymers of PGMMA generated in the solutions of the above surface-initiated RAFT polymerization systems (or the esterified polymer brushes) for the MIP and CP particles were 68700 and 68800, respectively, and their dispersities ($Đ$) were 1.24 and 1.21, respectively (Table S1), suggesting the well-controlled characteristics of the polymerizations.

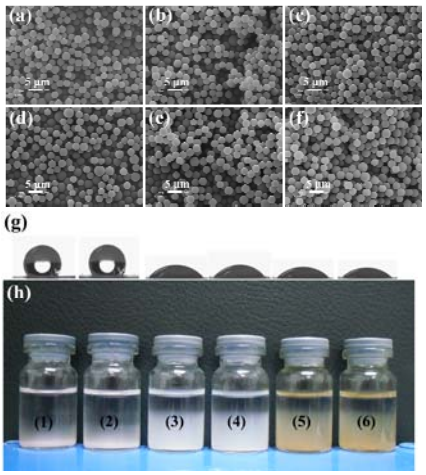

Fig. 2 (a-f) SEM images of the ungrafted MIP (a)/CP (d) microspheres, the grafted MIP (b)/CP (e) microspheres, and the grafted magnetic MIP (c)/CP (f) microspheres (The scale bar is 5 μm). (g,h) The profiles of a water drop on the films of the ungrafted, grafted, and grafted magnetic MIPs/CPs (g) and their dispersion photographs in pure water (1 mg/mL) at 25 °C after settling down for 2 h (h). The samples located from left to right in the above two figures are the ungrafted MIP (1)/CP (2), the grafted MIP (3)/CP (4), and the grafted magnetic MIP (5)/CP (6) microspheres.

The successful immobilization of the magnetic Fe_3O_4 nanoparticles on the PGMMA brushes was confirmed by the presence of the characteristic band around 584 cm^{-1} related to the Fe-O bond of the naked Fe_3O_4 in the FT-IR spectra of the grafted magnetic MIP/CP particles (Fig. S1), the existence of Fe_3O_4 nanoparticles on the MIP particle surfaces as shown by the TEM images (Fig. 3a and b), and the observation of six characteristic peaks for Fe_3O_4 in their XRD patterns (Fig. 3c). The measurement with a magnetometer revealed the superparamagnetic feature of our grafted magnetic MIP microspheres because no obvious magnetic hysteresis was observed for them (Fig. 3d). Note that a relatively low saturation magnetization value (i.e., 2.4 emu/g) was obtained for the grafted magnetic MIP particles, which might be improved by grafting PGMMA brushes with higher molecular weights or grafting densities onto the MIP particles prior to the attachment of Fe_3O_4 nanoparticles. Anyway, their efficient separation from the aqueous solution was realized under the external magnetic field (Fig. S3). In addition, the attachment of Fe_3O_4 nanoparticles onto the PGMMA brushes proved to largely enhance the surface hydrophilicity of the MIP/CP microspheres, as suggested by their obviously lower static contact angles in comparison with the grafted ones (Table S1).

The equilibrium template binding properties of the above-obtained 2,4-D-MIPs/2,4-D-CPs were first studied in an organic solvent-rich medium (i.e., methanol/water (4:1 v/v)). Fig. 4a shows that all the ungrafted, grafted, and grafted magnetic MIP microspheres can exhibit obvious specific template bindings (i.e., the binding differences between the MIP and its CP) in methanol/water. This,

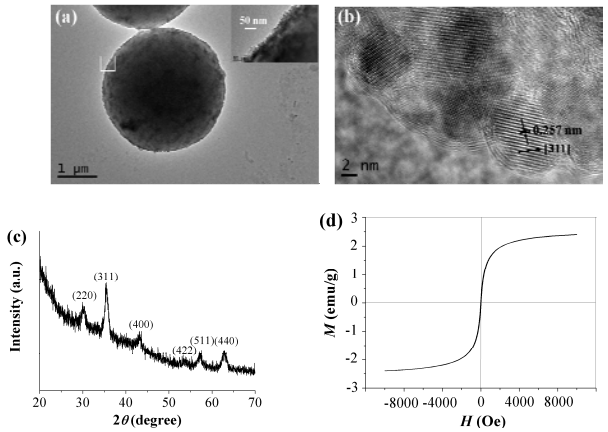

Fig. 3 (a) TEM image of the grafted magnetic MIP microspheres and the magnified image of the area within the box (see the insert figure). (b) High-resolution TEM (HRTEM) image of the immobilized Fe_3O_4 nanoparticles on the polymer brushes. (c) X-ray powder diffraction pattern of the grafted magnetic MIP microspheres. (d) SQUID magnetization of the grafted magnetic MIP microspheres at 300 K.

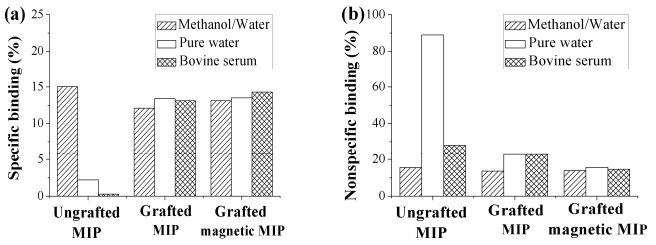

Fig. 4 Specific (a) and nonspecific (b) template bindings of the ungrafted, grafted, and grafted magnetic MIP microspheres in different media ($C_{2,4\text{-D}}$ = 0.02 mM, polymer concentration: 12 mg/mL).

together with their high template selectivity (Fig. S5a, Table S2), indicates the presence of specific binding sites in the obtained MIPs.

We then performed the equilibrium binding experiments in the aqueous solutions including both pure water and the real biological medium (i.e., the undiluted bovine serum). As expected, the specific template bindings of the ungrafted MIP almost completely disappeared in pure water and in bovine serum (Fig. 4a), mainly due to their high surface hydrophobicity.[5] In sharp contrast, both the grafted and grafted magnetic MIPs could exhibit specific template bindings in these aqueous media almost the same as what they showed in methanol/water (Fig. 4a). This, together with their high template selectivity (Fig. S5b and c, Table S2), demonstrated their good specific molecular recognition ability in such aqueous media. Nevertheless, the nonspecific template bindings of the grafted MIP in pure water and in bovine serum were apparently higher than that obtained in methanol/water (Fig. 4b), just as reported previously.[4,5] To our delight, the nonspecific template bindings of the grafted magnetic MIP particles in such aqueous media proved to be essentially the same as that obtained in methanol/water (Fig. 4b), which could be attributed to their largely enhanced surface hydrophilicity because of the attachment of very hydrophilic Fe_3O_4 nanoparticles in comparison with the grafted MIP (Table S1).

In conclusion, we have demonstrated for the first time the efficient synthesis of the narrowly dispersed hydrophilic and magnetic MIP microspheres with excellent molecular recognition ability both in the pure aqueous solution and in the real biological sample by the facile and efficient immobilization of magnetic and very hydrophilic Fe_3O_4 nanoparticles onto the PGMMA brushes of the MIP microspheres. The obtained MIP microspheres showed not only proper magnetic properties, but also the same specific and nonspecific template bindings in the real biological sample as those obtained in the organic solvent-based medium because of their significantly enhanced surface hydrophilicity. We believe this finding represents a major breakthrough for the molecular imprinting technology, since it has realized the ultimate goal of molecular imprinting for the development of ideal water-compatible MIPs and opens the door to the facile and effective preparation of such advanced water-compatible and magnetic MIP particles with great potential in a wide range of applications such as MIP immunoassay and biomimetic sensors.

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Notes and references

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1 K. Haupt and K. Mosbach, *Chem. Rev.*, 2000, **100**, 2495; B. Sellergren, *Angew. Chem. Int. Ed.*, 2000, **39**, 1031; G. Wulff, *Chem. Rev.*, 2002, **102**, 1; C. Alexander, H. S. Andersson, L. I. Andersson, R. J. Ansell, N. Kirsch, I. A. Nicholls, J. O'Mahony and M. J. Whitcombe, *J. Mol. Recognit.*, 2006, **19**, 106; H. Zhang, L. Ye and K. Mosbach, *J. Mol. Recognit.*, 2006, **19**, 248; Y. Hoshino and K. J. Shea, *J. Mater. Chem.*, 2011, **21**, 3517; K. Haupt, A. V. Linares, M. Bompart and B. T. Sum Bui, *Top Curr. Chem.*, 2012, **325**, 1.

2 G. Vlatakis, L. I. Andersson, R. Müller and K. Mosbach, *Nature*, 1993, **361**, 645; K. Haupt, *Chem. Commun.*, 2003, 171; Y. Ge and A. P. F. Turner, *Chem. Eur. J.*, 2009, **15**, 8100; J. L. Bowen, P. Manesiotis and C. J. Allender, *Mol. Imprint.*, 2012, 35.

3 B. Dirion, Z. Cobb, E. Schillinger, L. I. Andersson and B. Sellergren, *J. Am. Chem. Soc.*, 2003, **125**, 15101; Y. Watabe, K. Hosoya, N. Tanaka, T. Kubo, T. Kondo and M. Morita, *J. Chromatogr. A*, 2005, **1073**, 363; C. E. Hunt, P. Pasetto, R. J. Ansell and K. Haupt, *Chem. Commun.*, 2006, 1754; P. Manesiotis, C. Borrelli, C. S. A. Aureliano, C. Svensson and B. Sellergren, *J. Mater. Chem.*, 2009, **19**, 6185; X. Shen and L. Ye, *Macromolecules*, 2011, **44**, 5631; X. Shen and L. Ye, *Chem. Commun.*, 2011, **47**, 10359.

4 G. Pan, Y. Zhang, X. Guo, C. Li and H. Zhang, *Biosens. Bioelectron.*, 2010, **26**, 976; G. Pan, Y. Zhang, Y. Ma, C. Li and H. Zhang, *Angew. Chem. Int. Ed.*, 2011, **50**, 11731; Y. Ma, Y. Zhang, M. Zhao, X. Guo and H. Zhang, *Chem. Commun.*, 2012, **48**, 6217.

5 Y. Ma, G. Pan, Y. Zhang, X. Guo and H. Zhang, *Angew. Chem. Int. Ed.*, 2013, **52**, 1511.

6 L. Chen and B. Li, *Anal. Methods*, 2012, **4**, 2613; X. Li, G. Zhu, Y. Luo, B. Yuan and Y. Feng, *TrAC Trends Anal. Chem.*, 2013, **45**, 233.

7 L. Li, X. He, L. Chen and Y. Zhang, *Chem. Asian J.*, 2009, **4**, 286; C. Lu, Y. Wang, Y. Li, H. Yang, X. Chen and X. Wang, *J. Mater. Chem.*, 2009, **19**, 1077; C. Gonzato, M. Courty, P. Pasetto and K. Haupt, *Adv. Funct. Mater.*, 2011, **21**, 3947.

8 H. Zhang, *Eur. Polym. J.*, 2013, **49**, 579.

9 R. Barbey, L. Lavanant, D. Paripovic, N. Schüwer, C. Sugnaux, S. Tugulu and H.-A. Klok, *Chem. Rev.*, 2009, **109**, 5437.

10 S. Wang, Y. Zheng, Y. Liu, H. Yan and K. Liu, *J. Mater. Chem.*, 2005, **15**, 3424.

11 M. D. Rowe, B. A. G. Hammer and S. G. Boyes, *Macromolecules*, 2008, **41**, 4147.