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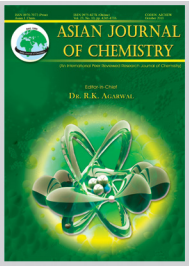

Synthesis and Evaluation of Restricted Access Material-Molecularly Imprinted Polymer Based on Self-Assembly and Sol-Gel Technique

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A new method for synthesis of the restricted access material-molecularly imprinted polymer was developed. Based on self-assembly and sol-gel technique, restricted access material-molecularly imprinted polymer was prepared by self-hydrolyzed, self-condensed and co-condensation of tetraethoxysilane and (octyl)-trimethoxysilane (C8-TMS) in the presence of lomefloxacin and surfactant micelles and with the covalently anchored organic groups in the mesoporous silica matrix. The surface of the silica matrix was modified by γ-(2,3-epoxypropoxy) propyltrimethoxysilane (KH-560) to enhance the hydrophilic properties. The recognition and hydrophilic properties of the restricted access material-molecularly imprinted polymer were evaluated by binding study and the swelling degree measurements and the molecularly imprinted mesoporous silica was used for comparison. Both specific interactions between template and restricted access material-molecularly imprinted polymer and the recovery of bovine serum albumin increased obviously. The recovery of bovine serum albumin was higher than 99 %.

Key Words: Restricted access materials, Molecularly imprinted polymers, Self-assembly, Lomefloxacin, Bovine serum albumin.

INTRODUCTION

In the bioanalytical field, considerable interest has been focused on sample preparation for it is often considered as the time-limiting step in bioanalytical process[1,2]. The extraction techniques commonly used for sample preparation such as solid-phase extraction[3-5], however, proteins can destructive accumulate in the surface of traditional extraction sorbent in the process of sample preparation[6]. The development of special and selective extraction sorbent, which proteins are eluted in the void volume without destructive accumulation, was required. The restricted access materials (RAM)[7-9] are considered, it designates a family which allows direct injection of biological sample by limiting the accessibility of interaction sites within the pores to small molecules only and the macro-molecules are excluded, which minimizes the adsorption of matrix proteins[10].

Nowadays, restricted access materials were usually prepared as follows[11,12]: grafting of the hydrophobic groups onto the surface of silica gel and then only the groups on the external surface are removed, so that hydrophilic groups or silanol groups are solely exposed on the external surface. Although this method has been used for a design of new adsorbents and catalysts, it has two major shortcomings[13]: (1) It is difficult to control the loading of the hydrophobic groups; (2) The undesired polycondensation by-products can be easily formed during surface modification or grafting. In order to overcome this problem, we direct incorporate the hydrophobic groups onto mesoporous silica adsorbents in the process for preparation of it. Unlike the grafting process, direct incorporation allows the functionalized mesoporous adsorbents to retain high surface area and pore volume, to have a uniform functional group distribution inside the pore channels and to avoid the local clustering of the functional groups and the necking of the pore channels.

To improve the selectivity of the material, we introduced the molecularly imprinted technique (MIT). The molecularly imprinted technique is an increasingly developing technique for preparing materials with desired and pre-determined selectivity and provides specific binding sites or catalytic sites in molecularly imprinted polymers (MIP)[14-16]. The material combining the characteristics of restricted access materials and molecularly imprinted polymers is an ideal, because the analytical efficiency will be greatly improved by simultaneous protein removal and selective[12,17,18]. In this work, we used a new method to prepare the restricted access material-molecularly imprinted polymer and the recognition and hydrophilic properties of restricted access material-molecularly imprinted polymer were evaluated.

EXPERIMENTAL

Tetraethoxysilane (TEOS) and cetyl trimethylammonium bromide (CTAB) were purchased from Kermel Reagent Co. of Tianjin. Lomefloxacin was purchased from Fluka (Buchs, Switzerland). Lomefloxacin (LOM), bovine serum albumin (BSA) and enrofloxacin (ENR) were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), KH-560, (Octyl)-trimethoxysilane (C8-TMS). All the other chemicals were used without further disposal. The adsorption capacity was measured by T6 UV-VIS spectrophotometer (Beijing purkinje general instrument Co. Ltd. China).

Preparation of molecularly imprinted polymers-MS (MIP-MS): To prepare the MIP-MS, 0.058 g of lomefloxacin (template), 4.37 g of CTAB and 0.84 mL TMB were dissolved in 120 mL of water while stirring, then 5.2 mL of OCTEO was added into the mixture. After stirring the mixture for 0.5 h, 18 mL of tetraethoxysilane solution (dissolved in 150 mL of ethanol and stirred for 30 min) and 3.50 mL of 2 mol L^{-1} NaOH were added. The mixture was stirred for 24 h. The solid product was recovered by filtration, washed with water and methanol and dried in the vacuum at 80 ºC for 24 h. The resultant particles were washed with copious diluted HCl (2 mol L^{-1}) in ethanol for several times to remove CTAB and lomefloxacin templates. To ensure the complete removal of the surfactant and lomefloxacin templates, the material was Soxhlet extracted with a solution of acetic acid in methanol (20 %, v/v) for 24 h and then washed with methanol and copious water in turn until lomefloxacin could no longer be detected at 283 nm in the eluent and the pH was higher than 6. The material was finally dried at 80 ºC in the vacuum for 24 h. then MIP-MS was obtained.

The non-imprinted polymer based on mesoporous silica (NIP-MS) was prepared with the same method without adding the lomefloxacin.

Preparation of the restricted access material-molecularly imprinted polymer (RAM-MIP): 50 g of the MIP-MS was mixed with both 150 mL of 10 % KH570 in dry toluene and 4.8 g (61 mmol) of dry pyridine under nitrogen. The mixture was refluxed for 16 h, then cooled, washed sequentially with toluene and methanol and dried under vacuum at 90 ºC for 4 h. The resultant particles were added into 25 mL of a perchloric acid solution (10 % v/v) and agitated (200 rpm) for 24 h at room temperature. At the end of the reaction, the particles were filtered, washed with 100 mL of ethanol, 100 mL of acetone, 100 mL of diethyl ether and then dried under vacuum overnight at 90 ºC. The restricted access media-molecularly non-imprinted polymer (RAM-NIP) was prepared with same method without adding the lomefloxacin.

Binding experiments of lomefloxacin on RAM-MIP and RAM-NIP: Binding experiments were performed in water media, 50 mg of polymer particle were mixed with 5 mL lomefloxacin (2 m mol/L), solution pH was adjusted with HCl and NaOH solutions. The mixtures were incubated for 8 h with continuous shaking in a horizontal shaker at room temperature. After incubating, the mixtures were filtered through 0.45 mm microporous membranes, the amount of the unbonded lomefloxacin was determined by UV-VIS spectrophotometer at 283 nm. Moreover, the equilibrium adsorption capacity (Q_e, μ mol g^{-1}) was calculated according to eqn. $Q_e = (C_0 - C_e)$ v/w.

Swelling degree measurements of RAM-MIP and RAM-NIP: 50 mg of the particles were packed into a 1 mL empty SPE columns. 10 mL water was passed through the cartridges. The weights recorded after 12 h and used to give the water content percent (WR %) by the following eqn.: WR % = $(W_s-W_d)/ W_d$, where W_s and W_d are weights of swollen and dried particles, respectively. Each experiment was carried out in triplicate.

Recovery of bovine serum albumin: 50 mg of MIP-MS, NIP-MS, RAM-MIP and RAM-NIP, respectively, were packed into a 1.0 mL empty SPE columns. Before use, the columns were preconditioned by successive washings with water, HCl (0.07 M), water, methanol, water and 10 mM phosphate buffer (pH = 6.8). The adsorption test was performed by loading the cartridge with 2.0 mL of bovine serum albumin standard solution in 10 mM phosphate buffer (pH=6.8). The amount of adsorbed protein after loading step was calculated by UV-VIS spectrophotometer at 290 nm and experiments were repeated three times.

RESULTS AND DISCUSSION

Preparation of the restricted access material-molecularly imprinted polymer: The synthesis protocol of the restricted access material-molecularly imprinted polymer was expressed in Fig. 1. In the synthesis process, the surfactant micelle solution was first formed between the CTAB and the lomefloxacin and then the OCTEO and tetraethoxysilane were independently self-hydrolyzed and self-condensed. Both partially self-condensed silane was added to the surfactant micelle solution and co-condensed in the surface of micelle, with the covalently anchored organic groups in the mesoporous sorbent matrix. After removing the surfactant template and lomefloxacin, the MIP-MS was formed. The organic groups and Si-OH can rebind template via hydrophobic interaction and hydrogen bond interaction. The KH-560 was grafted to the surface of the mesoporous silica matrix in order to enhance the hydrophilic properties.

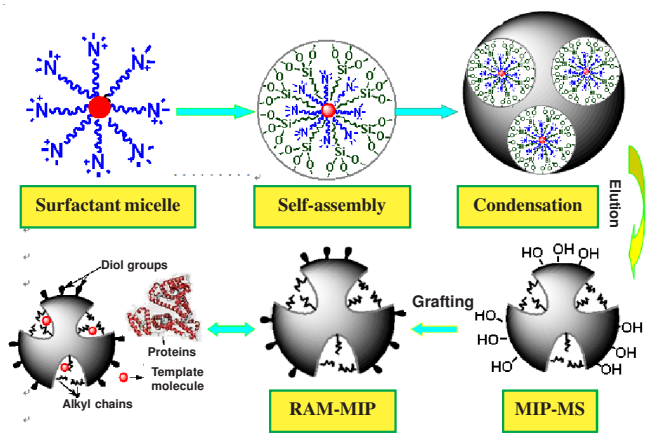

Fig. 1. Synthesis protocol of restricted access materials-molecularly imprinted polymers

Pore-size distribution, pore volumes and surface area were studied using the nitrogen adsorption-desorption experiments.

Nitrogen adsorption-desorption analysis date were shown in Table-1. Average pore size of the particles are most the same, this shown that KH-560 modification did not change the pore size apparently.

TABLE-1
NITROGEN ADSORPTION-DESORPTION ANALYSIS DATE

Polymers	Average pore size (nm)	Average pore volume ($cm^3 g^{-1}$)	Average surface area ($m^2 g^{-1}$)
NIP-MS	5.84	0.3581	236.8
MIP-MS	6.25	0.3898	243.2
RAM-NIP	5.54	0.3207	255.5
RAM-MIP	5.98	0.3542	297.2

Characteristic of the FT-IR spectra and TGA analysis: The structure of MIP-MS and restricted access material-molecularly imprinted polymer were analyzed by FTIR (Fig. 2), compared with the IR spectrum of SiO_2, the peaks at 2925 cm^{-1} (C-H stretching vibration) and 2855 cm^{-1} (-CH_2- stretching vibration) appear in the spectrum of MIP-MS and restricted access material-molecularly imprinted polymer, which demonstrates that OCTEO has been bond on the silica matrix. To ascertain the presence of KH-560 on the surface of the mesoporous silica matrix, the thermogravimetric analysis was analyzed. Fig. 3 shows the results of differential thermogravimetric analysis of MIP-MS and restricted access material-molecularly imprinted polymer. After 200 ºC, MIP-MS and restricted access material-molecularly imprinted polymer present abrupt decrease in weight. The weight of residue at 470 ºC is the inorganic silica content. The weight retention of the MIP-MS is about 60 %, while the weight retention of restricted access material-molecularly imprinted polymer is about 50 %, which shows that the surface modification of the mesoporous silica matrix is successful.

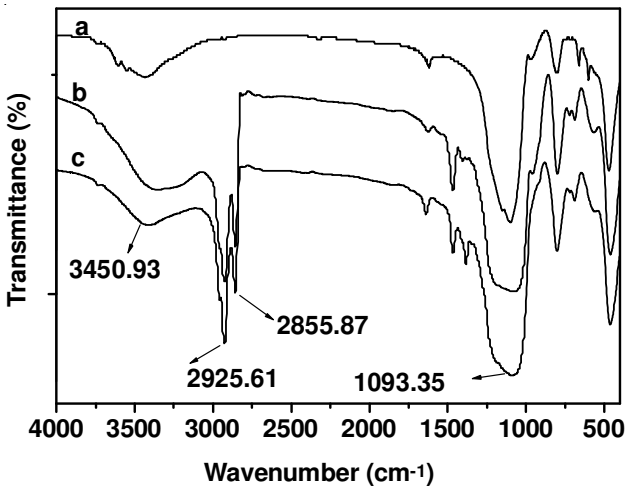

Fig. 2. FT-IR spectra of SiO_2 (a), MIP-MS (b) and RAM-MIP (c)

Effect of pH on the restricted access material-molecularly imprinted polymer: The optimization of pH value for adsorption medium plays a vital role in the adsorption studies. The effect of solution pH on the equilibrium adsorption capacity is shown in the Fig. 4. The result may be attributed to the hydrophobic interaction and electrostatic attractions between lomefloxacin and restricted access material-molecularly imprinted polymer. As the pH value increasing, the hydrophobic interaction of lomefloxacin becomes stronger. Another reason is that as the carboxyl group of the lomefloxacin protonates, the electrostatic attraction between the lomefloxacin and the restricted access material-molecularly imprinted polymer will become stronger. At pH > 7, the surface of the restricted access material-molecularly imprinted polymer and lomefloxacin turned to negative charge and the electrostatic repulsions were not benefited for the adsorption system.

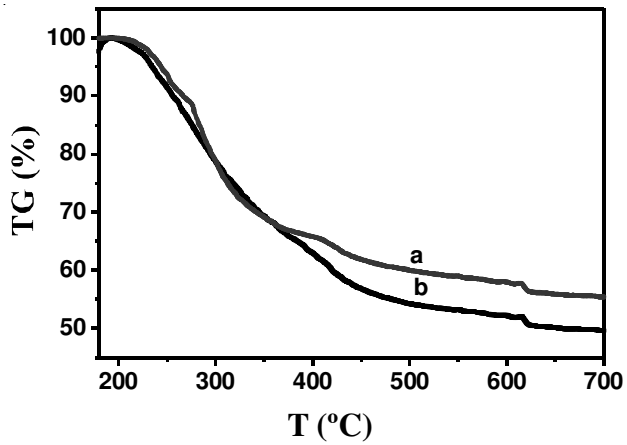

Fig. 3. TG curves of MIP-MS (a) and RAM-MIP (b)

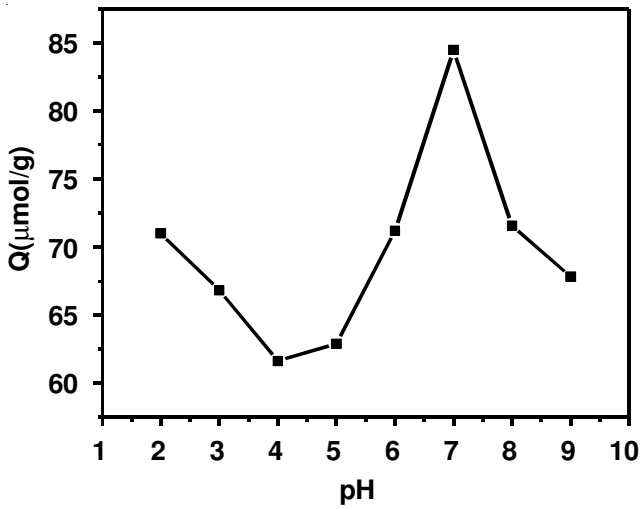

Fig. 4. Effect of the solution pH on the adsorption

Binding study: The imprinting efficiency (α) is the easiest way to highlight the recognition properties in a MIP-MS. In our work, α(LOM) was determined as the ratio between the amount (%) of lomefloxacin bound by the materials, the results were shown in Table-2, the α(LOM) of the MIP-MS and RAM-MIP almost the same, which were high than 1.7, this confirmed that after hydrophilic surface modification, the material did not change the imprinting properties. The α(NOR) was lower than 1.2, The very low values of α(NOR) show the high chemical and spatial complementarity of binding sites toward the template.

The swelling characteristics of restricted access material-molecularly imprinted polymer: According to the literature[17], the swelling characteristics of polymers were determined in order to check increased hydrophilic affinity of RAM-

MIP and RAM-NIP compared to MIP-MS and NIP-MS, polymeric microparticles with the external hydrophilic layer were able to absorb the relevant amount of water. The WR (%) for all prepared materials was reported in Table-3. Water content of MIP-MS and NIP-MS were only 12.34 % and 10.89 %, that of restricted access material-molecularly imprinted polymer and RAM-NIP reached 45.00 % and 48.36 %, the experimental date confirmed that restricted access material-molecularly imprinted polymer and RAM-NIP have the external hydrophilic layer.

TABLE-2
PERCENTAGE OF BOUNDING LOM AND NOR BY RAM-MIP, RAM-NIP, MS-MIP AND MS-NIP

Polymers	Bound LOM (%)	RSD (%)	α (LOM)	Bound NOR (%)	RSD (%)	α (NOR)
MIP-MS	42.19	3.27	1.70	29.87	1.96	0.98
NIP-MS	29.10	1.92		30.41	3.47	
RAM-MIP	42.47	1.89	1.80	29.57	1.15	1.17
RAM-NIP	23.58	3.02		25.29	2.36	

TABLE-3
HYDROPHILIC PROPERTIES OF POLYMERS: WATER CONTENT (%) OF THE POLYMER

Polymers	W_d (g)	W_s (g)	Water content (%)	RSD (%)
MIP-MS	505	561	12.34	2.68
NIP-MS	499	546	10.89	3.16
RAM-MIP	501	726	45.00	4.53
RAM-NIP	504	741	48.36	2.50

Recovery of bovine serum albumin from restricted access material-molecularly imprinted polymer: Table-4 shown the recovery bovine serum albumin from MIP-MS, NIP-MS, RAM-MIP and RAM-NIP, bovine serum albumin was recovery only 48.63 % and 52.34 % from MIP-MS and NIP-MS, after hydrophilic surface modification of the MIP-MS and NIP-MS, bovine serum albumin was most completely recovered. This agrees with the expected order of decreasing hydrophobicity where polymers of RAM series possessed the most hydrophilic character and could be less susceptible to fouling by proteins. The result described that restricted access material-molecularly imprinted polymer and RAM-NIP can direct to direct used in preparation of biological sample.

TABLE-4
HYDROPHILIC PROPERTIES OF POLYMERS: RECOVER OF BSA FROM THE POLYMERS

Polymers	Concentration of BSA (g/L)	Recovery of BSA (%)	RSD (%)
MIP-MS	0.13	48.63	2.30
NIP-MS	0.12	52.34	5.35
RAM-MIP	0.13	100.12	3.91
RAM-NIP	0.13	99.93	3.05

Conclusion

In this study, a new method for preparation of restricted access material-molecularly imprinted polymer (RAM-MIP) based on self-assembly and sol-gel technique was successfully developed. It direct incorporated hydrophobic organic groups in the process of the mesoporous silica matrix. Meanwhile we evaluated the recognition properties and hydrophilic properties of restricted access material-molecularly imprinted polymer, the result shown that restricted access material-molecularly imprinted polymer have well selective properties and external hydrophilic properties, it can be direct used in preparation of biological sample.

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REFERENCES

1. M. Gilar, E.S.P. Bouvier and B.J. Compton, *J. Chromatogr. A*, **909**, 111 (2001).
2. M.C. Hennion, *J. Chromatogr. A*, **856**, 3 (1999).
3. A.K. Malik, V. Kaur and N. Verma, *Talanta*, **68**, 842 (2006).
4. H. Liang, M.B. Kays and K.M. Sowinski, *J. Chromatogr. B*, **772**, 53 (2002).
5. X.L. Sun, X.W. He, Y.K. Zhang and L.X. Chen, *Talanta*, **79**, 926 (2009).
6. Y.Q. Cai and S.F. Mou, *Chin. J. Anal. Chem.*, **33**, 1647 (2005).
7. C.P. Desilets, M.A. Rounds and F.E. Regnier, *J. Chromatogr. A*, **544**, 25 (1991).
8. F. Gasparrini, A. Ciogli, I.D. Acquarica, D. Misiti, E. Badaloni, F. Giorgi and A. Vigevani, *J. Chromatogr. A*, **1176**, 79 (2007).
9. H.S. Wang, P. Jiang, M. Zhang and X.C. Dong, *J. Chromatogr. A*, **1218**, 1310 (2011).
10. S. Souverain, S. Rudaz and J.L. Veuthey, *J. Chromatogr. B*, **801**, 141 (2004).
11. I.H. Hagestam and T.C. Pinkerton, *Anal. Chem.*, **57**, 1757 (1985).
12. W.J. Xu, S.F. Su, P. Jiang, H.S. Wang, X.C. Dong and M. Zhang, *J. Chromatogr. A*, **1217**, 7198 (2010).
13. Z.J. Wu, J. Hyeonwoo and L. Kangtaek, *Chem. Eng. J.*, **112**, 227 (2005).
14. G. Wulff, *Angew. Chem. Int. Ed. Eng*., **34**, 1812 (1995).
15. A.S. Abu-Surrah and Y.S. Al-Degs, *J. Appl. Polym. Sci*., **117**, 2316 (2010).
16. T. Alizadeh, *Anal. Chim. Acta*, **669**, 94 (2010).
17. G.Z. Kyzas, D.N. Bikiaris and N.K. Lazaridis, *Chem. Eng. J.*, **149**, 263 (2009).
18. S. Haruyo and J. Haginaka, *Analyst*, **128**, 593 (2003).