Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

The Preparation of BHb-Molecularly Imprinted Gel Polymers and Its Selectivity Comparison to BHb and BSA

Qingqing Gai[a]; Feng Qu[a]; Yukui Zhang[b]
[a] School of Life Science and Technology, Beijing Institute of Technology, Beijing, China [b] Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China

Online publication date: 29 November 2010

To cite this Article Gai, Qingqing , Qu, Feng and Zhang, Yukui(2010) 'The Preparation of BHb-Molecularly Imprinted Gel Polymers and Its Selectivity Comparison to BHb and BSA', Separation Science and Technology, 45: 16, 2394 — 2399
To link to this Article: DOI: 10.1080/01496395.2010.484409
URL: http://dx.doi.org/10.1080/01496395.2010.484409

Separation Science and Technology, 45: 2394–2399, 2010

ISSN: 0149-6395 print/1520-5754 online
DOI: 10.1080/01496395.2010.484409

Taylor & Francis
Taylor & Francis Group

The Preparation of BHb-Molecularly Imprinted Gel Polymers and Its Selectivity Comparison to BHb and BSA

Qingqing Gai,[1] Feng Qu,[1] and Yukui Zhang[2]
[1]*School of Life Science and Technology, Beijing Institute of Technology, Beijing, China*
[2]*Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China*

Molecularly imprinted gel polymer (MIP) for the selective imprinting of bovine hemoglobin (BHb) was prepared in aqueous media by bulk polymerization using polyacrylamide matrix. The synthesis conditions of BHb-MIP were investigated, which involved the interaction of functional monomers and template protein in different molar ratio, solution pH, and ionic strength. The adsorption experiments indicated that BHb-MIP had a high affinity for BHb over the non-imprinted polymers. The selectivity of BHb-MIP for BHb and bovine serum albumin (BSA) with similar molecular weight was compared. It was demonstrated that BHb-MIP had better selective adsorption and recognition properties to BHb especially in the presence of BSA as competing protein. It might be helpful for selectively separating template protein with MIP from the proteins mixture with similar molecular weight.

Keywords bovine hemoglobin; bovine serum albumin; molecularly imprinted gel polymer

INTRODUCTION

Molecular imprinting is a promising technique for preparing host compounds for molecule-specific recognition sites in robust network polymers (1–3). Chemically and mechanically stable molecularly imprinted polymer (MIP) was successfully used as sensors, chromatographic stuffing, and mimics for biological receptors, enzymes, and antibodies (4–5). MIP represents a new class of materials that could mimic and possibly replace biological equivalents.

At the beginning, only low molecular weight compounds such as amino acid derivatives, drugs, and pesticides (6–10) were used as imprinted molecules. The imprinted biological macromolecules such as proteins were very limited since imprinting proteins face challenges due to their molecular size, complexity, conformational flexibility, and solubility, which are different from small molecules. The larger molecular size of proteins makes them difficult to slip in and out of a MIP network, and the complexity and variety of their structure make them hard to produce well-defined recognition sites. Moreover, even though most of the proteins have good stability and solubility in water solvent, water is not usually used in conventional molecular imprinting process, which limits the choice of monomers (11). However, imprinting proteins have achieved great success in aqueous media recently (12–19).

Acrylamide (AA) is one of most important monomers used for imprinting proteins in aqueous media. Polyacrylamide (PAA) gel is known to be very inert, hydrophilic, biocompatible, and mild cross-linked. It has a soft and macroporous structure which permits large proteins to pass in and out. Hjerten and coworkers (20,21) pioneered the use of PAA gels and prepared low-density cross-linked PAA gel beds with selective recognition of proteins in affinity chromatography. Afterwards, Hawkins et al. (22) demonstrated the high selectivity of MIP based on PAA hydrogels and explored in detail a variety of template removal strategies. Ou et al. (23) described that PAA gel with electrostatic functional groups had good imprinting effects for lysozyme.

Bovine hemoglobin (BHb) is well known for its function in the vascular system of animals, being a carrier of oxygen and also aids the transport of carbon dioxide and regulates the pH of blood (24), which had been imprinted for the selective separation of BHb (25,26). Compared to small proteins often used as imprinted template such as lysozyme (MW 14.4 kDa) (27–29), ribonuclease A (MW 13.7 kDa) (30), cytochrome *c* (MW 12.3 kDa) (31), BHb has nearly five times molecular weight (MW 64.0 kDa) larger than them. It means that BHb possesses more action sites with functional monomers and more flexible conformational transitions in the imprinting process, which bring on more difficulties for BHb to form imprinted sites.

In this work, the synthesis of BHb-MIP was investigated, which involved the interaction of functional monomers and template protein in different molar ratio, solution pH, and ionic strength. The selectivity of BHb-MIP to BHb and bovine serum albumin (BSA, MW 66.0 kDa) with similar molecular weight was compared. BHb-MIP exhibited higher selective adsorption and recognition properties to BHb, especially when BSA existed as a competing protein.

Received 26 January 2010; accepted 7 April 2010.
Address correspondence to Professor Feng Qu, School of Life Science and Technology, Beijing Institute of Technology, Beijing 100081, China. E-mail: qufengqu@bit.edu.cn

We suggested that such competing protein with similar molecular weight only displaced nonspecific binding of template protein in competitive adsorption. It might be helpful to understand the separation of proteins with similar molecular weight with MIP. The competitive adsorption of proteins with similar molecular weight is seldom mentioned by others.

EXPERIMENTAL

Materials and Instrumental

BHb and BSA were purchased from Shanghai Lanji Sci-Technology Company and their solutions were prepared using 10 mmol/L sodium phosphate buffers (PBS). AA and sodium dodecyl sulfate (SDS) were obtained from Tianjin Yongda Chemical Reagent Factory. *N*,*N*′-methylenebisacrylamide (MBA) was supplied from Tianjin Chemical Reagent Institute. *N*,*N*,*N*′,*N*′- tetramethylethylenediamine (TEMED) was Bio-Chemistry Reagent and from Beijing Advanced Chemical Reagent Factory. Ammonium persulfate (APS) was purchased from Tianjin North Tianyi Chemical Reagent Factory. Other chemicals were all analytical grade.

A UV-vis spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) was used for absorbance determination.

Preparation of BHb-MIP

The preparation of BHb-MIP was as follows: BHb (0.0300 g, 0.47 μmol), AA (0.1710 g, 2.4 mmol), and MBA (0.0090 g, 0.058 mmol) were dissolved in 3 mL of 10 mmol/L PBS (pH 5.4), followed by sonication and deoxygenization for 15 min. Then 120 μL of 10% (w/v) APS and 24 μL TEMED were added. The polymerization occurred overnight at room temperature.

The non-imprinted polymer (NIP) was prepared in the same way without adding BHb.

Elution of Template Protein

The resulting gel was granulated by pressing it through a 250-mesh sieve and the template BHb inside gel was removed by 10% (v/v) acetic acid containing 10% (w/v) SDS solution (20), followed by four times with excess ethanol (27,30) to remove the surfactant and finally six times with 10 mmol/L PBS (pH 5.4). The same elution process was applied to both BHb-MIP and NIP.

Adsorption Capacity Determination

To investigate the adsorption dynamics of the gel polymers, 50.0 mg BHb-MIP was mixed with a 5 mL 0.2 mg/mL BHb solution (pH 5.4) at 25°C. At different time intervals, BHb-MIP was centrifuged at 3000 rpm for 4 min. The residual concentration of BHb in the supernatant was determined by the UV–vis spectrophotometer at 280 nm. The amount of adsorbed protein can be determined by the difference in concentration before and after the adsorption.

The adsorption capacity (Q, mg/g) of the protein to the gel polymers is calculated according to

$$Q = (C_0 - C_t)V/m \quad (1)$$

where C_0 and C_t are the initial protein concentration and the residual protein concentration, respectively (mg/mL), V is the volume of initial solution (mL), and m is the weight of the gel polymers (g).

The static equilibrium adsorption was carried out for BHb-MIP as described above with different initial concentrations (0.05–0.45 mg/mL) of BHb solution (pH 5.4) for 2 h, and then the concentration of BHb in the supernatant was determined at 280 nm.

Competitive adsorption of BHb-MIP and NIP was done at pH 5.4 for 2 h, with a fixed concentration of BHb (0.2 mg/mL) and increasing concentration of BSA (0.1–1.0 mg/mL) as described above. Finally, the concentration of BSA in the supernatant was determined at 280 nm, and BHb was at 405 nm for it had obvious absorption in visible range.

RESULTS AND DISCUSSION

Studies on Interaction Between Functional Monomer and Template Protein

The success of an imprinting system is defined by the ability of the polymers to possess a high affinity for the template, and the success of the system is very much dependent upon the template–monomer interaction (32). The interaction of BHb-AA was investigated when BHb and AA were in different molar ratio, solution pH and ionic strength. The conditions of the molecular imprinting prepolymerization were optimized. According to the equation as follows:

$$\Delta A = A_{added} - A_{obtained} \quad (2)$$

Where A_{added} is the total absorbance of BHb and AA, and $A_{obtained}$ is the absorbance of the BHb-AA complex at 210 nm. So, ΔA denotes the intensity of interaction between template BHb and monomer AA. The bigger the value of ΔA, the stronger the interaction of BHb-AA.

Effect of Molar Ratio of Functional Monomer AA and Template BHb

A template capable of interacting with a defined number of functional monomer molecules should yield MIPs of optimal selectivity (33). The molar ratio of AA to BHb was investigated in the range of 100:1 to 1200:1.

When the molar ratio was below 600:1, ΔA increased with the ratio increase (Fig. 1). When the molar ratio was more than 600:1, ΔA reached a platform, which means a maximum saturation of interaction between AA and BHb. It also indicates that a stable BHb-AA complex

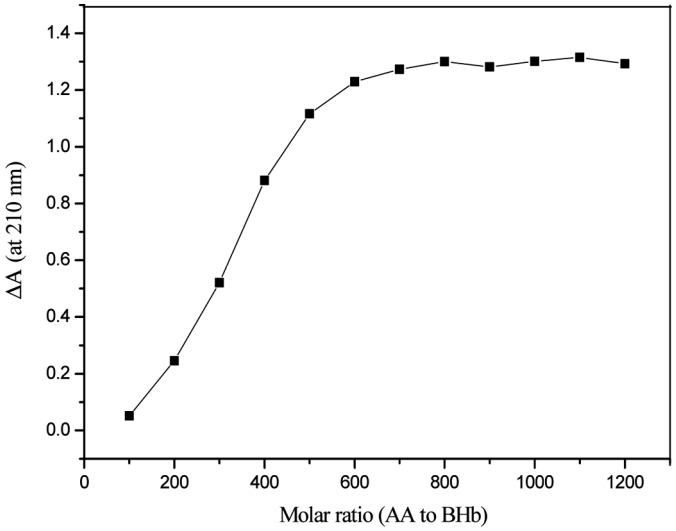

FIG. 1. Effect of molar ratio (AA/BHb) on interaction between functional monomer and protein.

was formed when the molar ratio of monomer AA and BHb was 600:1. In other words, the functional groups of the bare surface of template BHb are completely bound with monomer AA, and the shape, structure, and functional groups of BHb are immobilized by monomer AA. The resulting BHb-MIP should have selective recognition sites and a relatively low nonspecific binding. The saturated value (600:1) of monomer AA to template BHb was distinctly higher than that (about 4:1 (4) or 10:1 (34)) of the monomer to the low molecular weight compound. It is because the functional groups of the low molecular weight compound were much less than that of the complicated protein.

Moreover, in the polymerization process, AA was used not only as a functional monomer, but also the matrix for the gel forming. So the preparation of BHb-MIP needed extra AA.

Effect of pH

The structure and ionic charges of the template protein depended significantly on solution pH. Solution pH is one of the most important factors in the preparation process. When 600 μmol AA and 1 μmol BHb were mixed, ΔA of their complex in the pH range of 4.6–7.4 was between 1.18–1.29. Because the surface of the protein was covered with abundant function groups (-COOH, $-NH_2$), the multiple hydrogen bonding probably could play an important role in the interaction between BHb and AA. Higher ΔA might be considered that the stronger hydrogen-bonding interactions and more recognition sites were formed between AA and BHb. So pH 5.4 ($\Delta A = 1.29$) was chosen as a prepolymerization condition of imprinted polymers.

Effect of Ionic Strength

When 600 μmol AA and 1 μmol BHb were mixed, ΔA of their complex at NaCl concentration 0–2 mol/L (pH 5.4) remained constant. It indicates that the electrostatic forces nearly do not play the role in the interaction between the protein and function monomers.

The Properties of BHb-MIP

Adsorption Dynamics

Figure 2 shows the different dynamics adsorption of template BHb to BHb-MIP or NIP. Since BHb penetrated into BHb-MIP and easily bound to recognition sites with less resistance, the adsorption capacity of BHb-MIP had a rapid increase in the first 20 min. After that, with time increase, the adsorbed BHb occupied most of the recognition site in BHb-MIP, the adsorption rate slowed down, and eventually reached adsorption equilibrium.

For NIP, because of lacking the imprinting process, the functional groups were randomly distributed, so nonspecific adsorption of BHb onto NIP was observed.

Adsorption Isotherm

The adsorption capacity of BHb-MIP increased steeply within 0.05–0.15 mg/mL of BHb, then reached maximum during 0.2–0.45 mg/mL. Figure 3 shows the equilibrium isotherm for BHb to BHb-MIP.

The adsorption behaviors of BHb-MIP was described by Langmuir adsorption equation as

$$C/Q = C/Q_{max} + 1/KQ_{max} \tag{3}$$

Where C is the concentration of BHb in the equilibrium solution (mg/mL), Q and Q_{max} are the experimental adsorption capacity to BHb and the theoretical maximum adsorption capacity of BHb-MIP, respectively (mg/g), K is

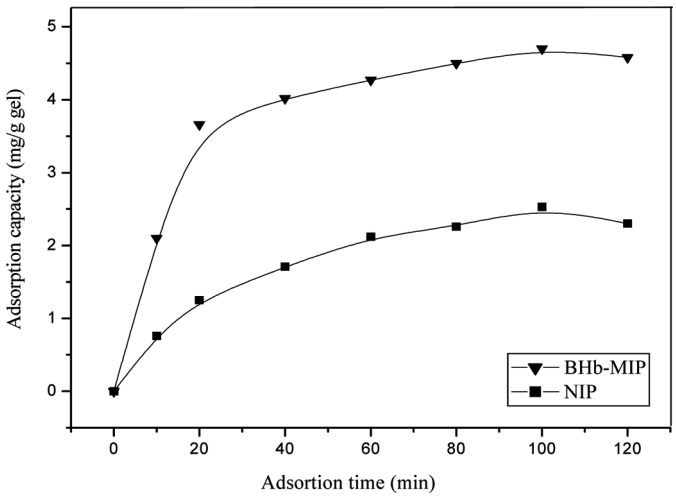

FIG. 2. Adsorption dynamic curve of BHb on BHb-MIP and NIP.

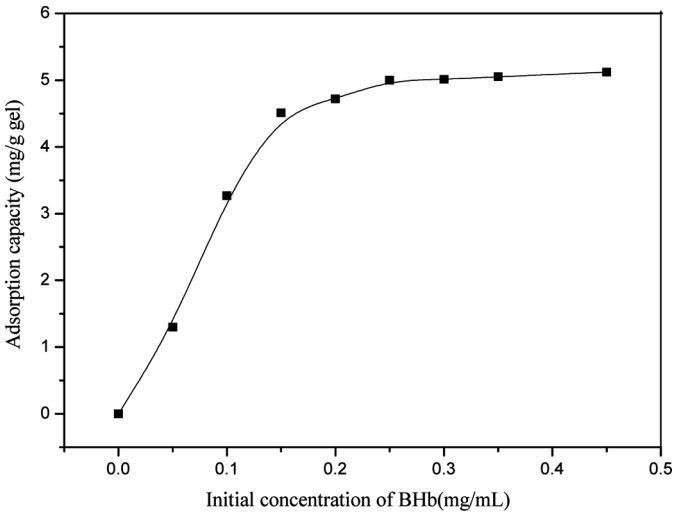

FIG. 3. Adsorption isotherm of BHb on BHb-MIP.

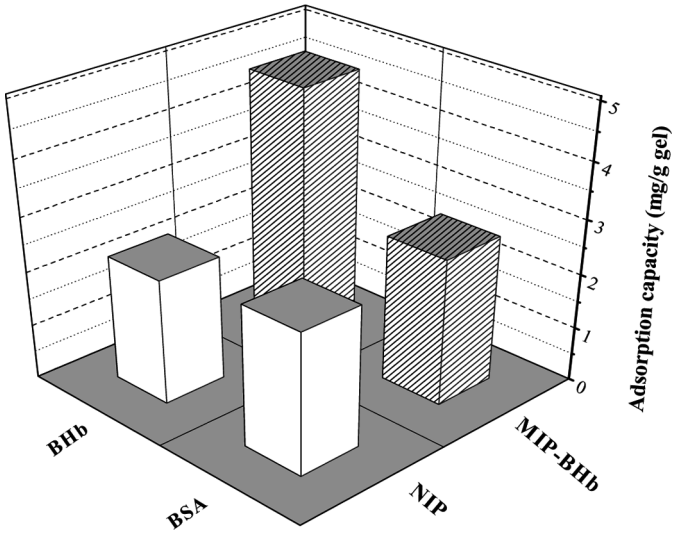

FIG. 4. Adsorption capacities of BHb and BSA on BHb-MIP and NIP.

the Langmuir adsorption equilibrium constant for BHb to BHb-MIP (mL/mg). According to the linearized plot of C/Q versus C, K and Q_{max} of BHb to BHb-MIP were 27.68 mL/mg and 5.71 mg/g, respectively. It can be seen that the Langmuir equation fits well for BHb adsorption on BHb-MIP under the concentration range studied ($R = 0.9969$).

Selectivity of BHb-MIP

Adsorption Selectivity to BHb and BSA

Although BSA is similar to the template BHb in size, the spatial arrangement of the effective groups on its surface is different from BHb, and the recognition sites in the BHb-MIP cavities are not complementary in shape to BSA. So there is no selectivity to BSA on BHb-MIP. Figure 4 shows that BHb-MIP possessed a distinctly higher adsorption capacity to template BHb than to control protein BSA, which is an indication of the imprinting effect of the template protein. For NIP, it has similar nonspecific adsorption to both BHb and BSA. It illustrates that randomly nonspecific interaction between proteins and polymers could play a role in the physical adsorption processes of proteins to polymers.

Adsorption Selectivity to the Mixture of BHb and BSA

With an increasing concentration of BSA, the adsorption of BHb on MIP-BHb was studied (Fig. 5). When BHb concentration was fixed at 0.2 mg/mL, adding 0.1 mg/mL BSA caused the adsorption capacities of BHb on BHb-MIP and NIP to be decreased obviously compared to without BSA, which means that the adsorptive BHb was displaced by the competing BSA. With more BSA (0.2–0.5 mg/mL) being added, its competition to BHb reached saturation point and the adsorption capacity of BHb to BHb-MIP and NIP did not change. In other words, more addition of BSA had no effect on the adsorption capacity. Since the size and structure of the imprinted cavities should resemble more accurately the structure of the original imprinted molecular, it is reasonable that the addition of BSA as competing protein should not match the BHb imprinted cavities as well as the BHb template. However, BSA can displace the nonspecific recognition sites of cavities and the nonspecific binding of BHb to BHb-MIP. That is to say, the template protein BHb to BHb-MIP consisted of both specific recognition and nonspecific binding. For NIP, the adsorption of BHb and BSA were all nonspecific and random without selectivity. Therefore, the addition of BSA made the adsorption capacity of BHb to BHb-MIP and NIP decrease.

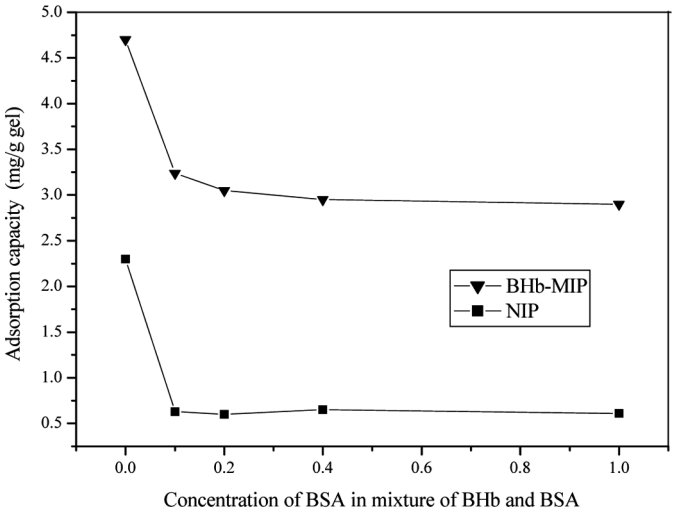

FIG. 5. Competitive adsorption of BHb in the presence of BSA.

Recognition selectivity of BHb-MIP can be evaluated by the imprinted factor (α), α is defined as follows:

$$\alpha = Q_{imprinted}/Q_{non\text{-}imprinted} \quad (4)$$

$Q_{imprinted}$ and $Q_{non\text{-}imprinted}$ were the adsorption capacity of proteins in BHb-MIP and NIP, respectively. α_{BHb} in competitive adsorption keeps around 5 and is more than two times as much as α_{BHb} in single adsorption (Fig. 6, ratio of BSA/BHb = 0), while α_{BSA} was primarily invariable about 1 (Fig. 6). It can be seen that BHb-MIP had higher recognition selectivity to the template BHb in the presence of BSA. The competitive adsorption of proteins with similar molecular weight is seldom mentioned by others.

The above results further indicate that BHb-MIP distinguishes proteins not only based on molecular size, but also basically on the synergistic effect of shape memory and multiple weak hydrogen bonding interactions provided by the functional monomers with the template protein in macromolecular recognition. Therefore, the shape, conformation, and/or amino acid composition of proteins is essential to recognition selectivity of imprinted gel polymers.

Proteins present a large number of potential recognition sites over a relatively large surface area compared to smaller molecules. The interactions between the template and the monomer AA in acrylamide matrix were mainly hydrogen bonds. A large number of weak hydrogen bonds formed between the imprinted gel polymer and the protein produced an overall strong interaction and led to the success of the imprinting, which is in agreement with Hjerten's opinion (20,21). The opinion is against the traditional imprinting theory that multiple weak interactions are unlikely to produce a highly specific MIP for small molecules.

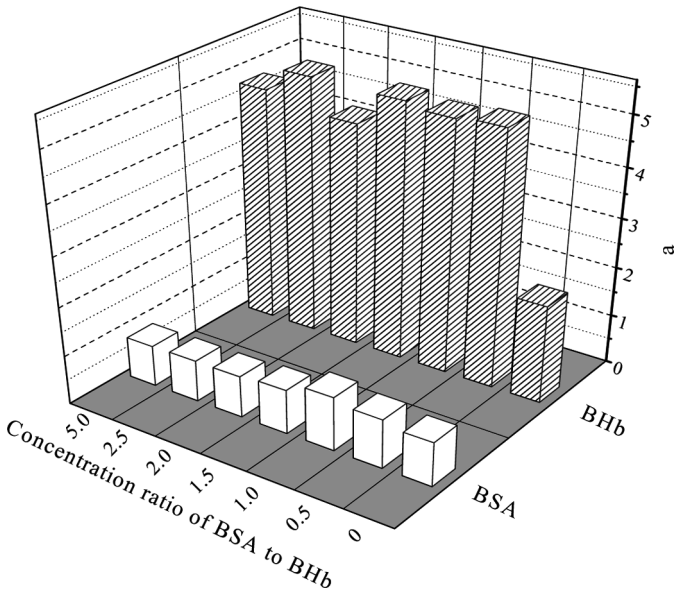

FIG. 6. Imprinted factors of BHb and BSA in competitive adsorption.

CONCLUSIONS

The interaction between monomers AA and template BHb was discussed, and the optimization conditions of BHb-MIP preparation by bulk polymerization with polyacrylamide matrix were achieved. Due to the double functions of AA as both the functional monomer and the gel matrix, the preparation of BHb-MIP needed more AA than the optimal molar ratio of AA to BHb. BHb-MIP had better adsorption and higher affinity for BHb when BSA was present. BHb-MIP exhibited selective recognition for BHb not simply dependent on molecular weight or size separation but mainly on the synergistic effect of shape complementarity and multiple hydrogen bonding interactions between the functional monomers and the template protein in imprinting cavities. It might be helpful for selectively separating the template protein with MIP from the proteins mixture with similar molecular weight.

ACKNOWLEDGEMENTS

This study was supported by the National Basic Research Program of China (973 Program, No. 2007CB914101) and the National Natural Science Foundation of China (No. 20875009).

REFERENCES

1. Wulff, G. (1995) Molecular imprinting in cross-linked materials with the aid of molecular templates-a way towards artificial antibodies. *Angew. Chem. Int. Ed. Engl.*, 34: 1812.
2. Mosbach, K. (1994) Molecular imprinting. *Trends. Biochem. Sci.*, 19 (1): 9.
3. Haupt, K.; Mosbach, K. (2000) Molecularly imprinted polymers and their use biomimetic sensors. *Chem. Rev.*, 100 (7): 2495.
4. Hu, S.G.; Wang, S.W.; He, X.W. (2003) An amobarbital molecularly imprinted microsphere for selective solid-phase extraction phenobarbital form human urine and medicines by high-performance liquid chromatography. *Analyst*, 128 (12): 1485.
5. Hu, S.G.; Li, L.; He, X.W. (2005) Solid-phase extraction of esculetin from the ash bark of Chinese traditional medicine by using molecularly imprinted polymers. *J. Chromatogr. A*, 1062 (1): 31.
6. Yoshida, M.; Hatate, Y.; Uezu, K.; Goto, M.; Furusaki, S. (2000) Chiral-recognition polymer prepared by surface molecular imprinting technique. *Colloids Surf. A*, 169 (11): 259.
7. Quaglia, M.; Chenon, K.; Hall, A.J.; De Lorenzi, E.; Sellergren, B. (2001) Target analogue imprinted polymers with affinity for folic acid and related compounds. *J. Am. Chem. Soc.*, 123 (10): 2146.
8. Anderson, L.; Sellergren, B.; Mosbach, K. (1984) Imprinting of amino acid derivatives in macroporous polymers. *Tetrahedron Lett.*, 25 (45): 5211.
9. Vlatakis, G.; Andersson, L.I.; Mueller, R.; Mosbach, K. (1993) Drug assay using antibody mimics made by molecular imprinting. *Nature*, 361 (6413): 645.
10. Fisher, L.; Muller, R.; Ekberg, B. (1991) Direct enantioseparationary phase prepared by molecular imprinting. *J. Am. Chem. Soc.*, 113 (24): 9358.
11. Ramström, O.; Ansell, R.J. (1998) Molecular imprinting technology: Challenges and prospects for the future. *Chirality*, 10 (3): 195.
12. Shi, H.; Tsai, W.B.; Garrison, M.D.; Ferrari, S.; Ratner, B.D. (1999) Template-imprinted nanostructured surfaces for protein recognition. *Nature*, 398 (6728): 593.

13. Rachkov, A.; Minoura, N. (2001) Towards molecularly imprinted polymers selective to peptides and proteins. The epitope approach. *Biochim. Biophys. Acta*, 1544 (1–2): 255.
14. Yang, H.H.; Zhang, S.Q.; Yang, W.; Chen, X.L.; Zhuang, Z.X. (2004) Molecularly imprinted sol-gel nanotubes membrane for biochemical separations. *J. Am. Chem. Soc.*, 126 (13): 4054.
15. Pang, X.S.; Cheng, G.X.; Lu, S.L.; Tang, E.J. (2006) Synthesis of polyacrylamide gel beads with electrostaticfunctional groups for the molecular imprinting of bovine serum albumin. *Anal. Bioanal. Chem.*, 384 (1): 225.
16. Mi, H.F.; Guo, M.J.; Zhao, Z.; Fan, Y.G.; Wang, C.H.; Shi, L.Q. (2006) Protein-imprinted polymer with immobilized assistant recognition polymer chains. *Biomaterials*, 27 (24): 4381.
17. Lin, T.Y.; Hu, C.H.; Chou, T.C. (2004) Determination of albumin concentration by MIP-QCM sensor. *Biosens. Bioelectron.*, 20 (1): 75.
18. Bossi, A.; Bonini, F.; Turner, A.P.F.; Piletsky, S.A. (2007) Molecularly imprinted polymers for the recognition of proteins: The state of the art. *Biosens. Bioelectron.*, 22 (6): 1131.
19. Tan, C.J.; Chua, H.G.; Ker, K.H.; Tong, Y.W. (2008) Preparation of bovine serum albumin surface-imprinted submicrometer particles with magnetic susceptibility through core-Shell miniemulsion polymerization. *Anal. Chem.*, 80 (3): 683.
20. Hjerten, S.; Liao, J.L.; Wang, Y. (1996) A novel support with artificially created recognition for the selective removal of proteins and for affinity chromatography. *Chromatographia*, 42 (5–6): 259.
21. Hjerten, S.; Tong, D.; Hetenyi, C.; Bikadi, Z.; Gao, J.P. (2001) Some studies of the chromatographic properties of gels (artifical antibodies/receptors) or selective adsorption of proteins. *Chromatographia*, 54 (1–2): 7.
22. Hawkins, D.H.; Stevenson, D.; Reddya, S.M. (2005) Investigation of protein imprinting in hydrogel-based molecularly imprinted polymers (HydroMIPs). *Anal. Chim. Acta*, 542 (1): 61.
23. Ou, S.H.; Wu, M.C.; Chou, T.C.; Liu, C.C. (2004) Polyacrylamide gels with electrostatic functional groups for the molecular imprinting of lysozyme. *Anal. Chim. Acta*, 504 (1): 163.
24. Wang, Y.Q.; Zhang, H.M.; Zhou, Q.H. (2009) Studies on the interaction of caffeine with bovine hemoglobin. *Eur. J. Med. Chem.*, 44 (5): 2100.
25. Guo, T.Y.; Xia, Y.Q.; Wang, J.; Song, M.D.; Zhang, B.H. (2005) Chitosan beads as molecularly imprinted polymer matrix for selective seperation of proteins. *Biomaterials*, 26 (28): 5737.
26. Guo, T.Y.; Xia, Y.Q.; Hao, G.J.; Song, M.D.; Zhang, B.H. (2004) Adsorptive seperation of hemoglobin by molecularly imprinted polymers. *Biomaterials*, 25 (27): 5905.
27. Zhang, W.; Qin, L.; He, X.W.; Li, W.Y.; Zhang, Y.K. (2009) Novel surface modified molecularly imprinted polymer using acryloyl-cyclodextrin and acrylamide as monomers for selective recognition of lysozyme in aqueous solution. *J. Chromatogr. A*, 1216 (21): 4560.
28. Qin, L.; He, X.W.; Zhang, W.; Li, W.Y.; Zhang, Y.K. (2009) Macroporous thermosensitive imprinted hydrogel for recognition of protein by metal coordinate interaction. *Anal. Chem.*, 81 (17): 7206.
29. Qin, L.; He, X.W.; Zhang, W.; Li, W.Y.; Zhang, Y.K. (2009) Surface-modified polystyrene beads as photografting imprinted polymer matrix for chromatographic separation of proteins. *J. Chromatogr. A*, 1216 (5): 807.
30. Tan, C.J.; Tong, Y.W. (2007) Preparation of superparamagnetic ribonuclease A surface-imprinted submicrometer particles for protein recognition in aqueous media. *Anal. Chem.*, 79 (1): 299.
31. Rick, J.; Chou, T.C. (2005) Imprinting unique motifs formed from protein–protein associations. *Anal. Chim. Acta*, 542 (1): 26.
32. Bures, P.; Huang, Y.B.; Oral, E.; Peppas, N.A. (2001) Surface modifications and molecular imprinting of polymers in medical and pharmaceutical applications. *J Control. Release*, 72 (1–3): 25.
33. Anderson, H.S.; Nicholls, I.A. (1997) Spectroscopic evaluation of molecular imprinting polymerization systems. *Bioorg. Chem.*, 25 (3): 203.
34. Piletsky, S.A.; Karim, K.; Piletska, E.V.; Day, C.J.; Freebairn, K.W.; Legge, C.; Turner, A.P.F. (2001) Recognition of ephedrine enantiomers by molecularly imprinted polymers designed using a computational approach. *Analyst*, 126 (10): 1826.