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Albumin molecularly imprinted polymer prepared with a semi-rigid crosslinker in mixed organic/aqueous media

Chih-Hsien Hu · Tse-Chuan Chou

Abstract An approach using systematic component optimization for the formation of a molecularly imprinted polymer (MIP) has been adopted to imprint albumin. The result was a copolymer comprising 3-dimethylaminopropyl methacrylate and pentaerythritol tetraacrylate in a mole ratio of 1 to 11. Myoglobin was used in competitive re-binding experiments to compete with the polymer's native template. The effects of monomer to crosslinker mole ratio, polymerization temperature and time were investigated. These results also showed that the MIP possessed a high selectivity and adsorption capacity with respect to albumin in competitive binding protocols when the interfering species was also present in solution. Polymerization temperature and time were all shown to have significant effects on the resulting albumin-MIP's performance. Additionally, higher polymerization temperatures (> 38°C) and extended polymerization times (> 48 h) increased monomer conversion as determined by HPLC, but decreased the selectivity and adsorption capacity of the MIP.

Keywords Molecular imprinted polymer · Albumin · Aqueous media · Synthesis

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Introduction

In recent decades, molecularly imprinted polymers (MIPs) have shown potential applications in many fields, such as sensor design [1–5], chromatograpic separation [6] and catalysis [7, 8]. Because of their easy synthesis, stronger mechanical properties and high selectivities MIPs are potentially useful candidates for many applications. For example, in sensor fabrication, the selectivity of the prepared sensor relies on the recognition element in the sensor device to selectively "catch" the analyte. How to fabricate a recognition element that posses both high selectivity and sensitivity is a key design consideration in sensor fabrication. This is especially true for biosensors, because the antibodies commonly used as recognition elements are labile, the use of stable MIPs as substitute recognition agents is a potential solution [9].

The concept underlying MIP formation is the incorporation of functional monomers into a copolymer to generate interactions with the template molecule present in the pre-polymerization solution. The template molecule is subsequently removed, leaving complimentary cavities, which have high re-binding selectivity with respect to the template molecule. The MIP's recognition ability is attributed to its cavities molecular sizes, shapes and chemical functionalities, which enhance selective adsorption.

The type, and mole ratios, of the monomers and crosslinker used to synthesize the MIP can affect the polymer's performance; due, at least in part, to the resulting stronger or weaker interactions generated between the template molecule and the monomer [10, 11].

In biological systems, molecular recognition process takes place in aqueous media; therefore, it was our aim to synthesize MIPs that can function in this environment [12]. As MIPs are commonly synthesized with organic reagents, it is reasonable to assume that the conformation of protein templates will change if we replace the aqueous media with

an organic solution. Additionally, biological molecules (proteins or peptides) are difficult to dissolve in the organic reagents, this decreases the number of generated cavities and reduces the adsorption ability of the prepared MIPs.

We have developed several procedures for imprinting bio-molecules such as: myoglobin, bilirubin, ribonuclease A and lysozyme [13–16]. Here, we introduce a new method for the preparation of an albumin imprinted polymer using an organic/aqueous mixed media system. We used bovine serum albumin (BSA) as the template molecule, [3-(Dimethylamino) propyl] methacrylamide (DMAPMA) as the functional monomer, pentaerythritol tetraacrylate (PETTA) as the crosslinker, cumene hydroperoxide (CHP) and tetramethylethylenediamine (TMEDA) as a redox initiation pair in this organic/ aqueous synthesis system. We have previously shown that the introduction of a small amount of water into the pre-polymerization solution facilitates the generation of MIPs with excellent recognition and selectivity characteristics [17]. In this paper, we focus on the effect that the structural rigidity of the synthesized polymer has on the selective re-adsorption of the template molecule.

Experimental

Chemicals

Bovine serum albumin (BSA), Applichem (Cheshire, CT, USA); myoglobin, Sigma (St. Louis, MO, USA); 3-dimethylaminopropyl methacrylamide (DMAPMA), Rohm GmbH (Darmstadt, Germany); pentaerythritol tetraacrylate (PETTA), Aldrich (St. Louis, MO, USA); cumene hydroperoxide (CHP) and tetramethyl ethylenediamine (TMEDA), Fluka (Buchs, Switzerland); trifluoroacetic acid (TFA), J.T. Baker (Phillipsburg, NJ, USA); methanol and acetonitrile, Mallinckrodt Baker (Phillipsburg, NJ, USA) were used as received. The water was deionized with a resistance $\geq$ 18.2 MΩ.

The water content titration reagents used were: Hydranal-coulomat CG and Hydranal-coulomat A, Riedel-deHaen (Buchs, SG, Switzerland).

Apparatus

The HPLC comprised a Shimadzu LC-10AT pump (Kyoto, Japan); SPD-10A detector, SIL-10 AD auto sampler, C-R8A recorder and Phenomenex Jupiter 5u C18 300A column (Torrance, CA, USA). A planetary micro mill, Fritsch (Idar-Oberstein, Germany); and A11 basic analysis mill IKA® (Wilmington, NC, USA); were used to control the particle size of the MIP. The water content was confirmed by Karl Fischer coulometry, AQUATEST™ (Minneapolis, MN, USA).

HPLC analysis

We used HPLC to determine the adsorbed amount of each protein and the extent of conversion of the monomer. HPLC analysis was performed with a constant flow rate of 1.0 mL min^{-1} at ambient temperature and monitored by UV detection at 277 nm. The column was washed with 0.1% TFA$_{(aq)}$ until a stable baseline was achieved. Then, 100 μL of sample was injected into the system. The amount of each protein or monomer in the aqueous solution was determined with a gradient elution, performed using water and acetonitrile both containing 0.1% TFA. Initially, the elutant was 100% aqueous this being reduced by gradient to 55% at 20 min with a total analysis time of 35 min.

Preparation of MIP and NIP

In brief, an MIP having a monomer to crosslinker ratio of 0.09 was prepared as follows: 0.039 g BSA was dissolved in 0.383 g water then 7.966 g DMAPMA was added to form an aqueous DMAPMA-BSA solution. The water content of the solution was adjusted to 6.04%–determined by Karl Fischer coulometry. [Because the DMAPMA monomer is hygroscopic, the water content of DMAPMA-BSA-water mixture cannot easily be estimated by calculation]. The water content was defined as: [the mass of H$_2$O (g) / the mass of the DMAPMA-BSA-water complex (g)] × 100%. The adjusted DMAPMA-BSA-water mixture solution 0.109 g was added to a test tube containing 2.689 g PETTA with mixing to give a homogeneous solution then CHP (0.028 g) and TMEDA (0.028 g) were added to form a clear, aggregate free homogenous solution. This test tube was capped and immersed in a temperature-controlled water bath maintained at (25±0.5°C) for 30 min, after which it was immersed in a water bath maintained at 38±0.5°C, in a reactor that was continuously purged with N$_2$. After 48 h, the opaque and 'stiff' polymer bulk solid was ground and sieved. The synthesis of the NIP (i.e. control polymer) was similar to the MIP except for the absence of the template.

Extraction of the template and un-reacted chemicals

The BSA molecules and un-reacted chemicals in the MIP were removed by Soxhlet extraction with methanol (48 h). The MIP particles were transferred to a vacuum oven (60±0.1°C) for 24 h to remove any residual solvent.

Recovery of BSA and conversion of monomer

The un-extracted MIP 0.210 g and 2 mL water were added to a 10 mL sample vial, and then immersed in a temperature-controlled water bath maintained at (25±0.1°C). After 48 h, this MIP-water mixture was centrifuged (7,000 rpm×

10 min). Then the supernatant was collected and injected in to the HPLC to measure the concentration of extracted BSA and un-reacted DMAPMA in the supernatant.

Determination of the saturation adsorption time of MIP

The MIP particles (0.193 g) were dropped into a sample vial containing 10 mL BSA solution (4×10^{-4} g mL^{-1}), and immersed in a water bath maintained at (25 ± 0.1 °C). The supernatant of this MIP-BSA mixture was sampled periodically to examine the residual concentration of albumin. This testing procedure was repeated until there was no further albumin concentration change.

Adsorption test of MIP

The re-binding ability of the MIP was estimated with an adsorption test. MIP (0.062 g) was dispersed in 2 mL mixed protein solution at 25 ± 0.1 °C. After 3 h, this MIP-protein solution mixture was centrifuged (7,000 rpm× 10 min) and then the supernatant was collected to measure the concentration changes of albumin and the competing proteins.

Results and discussion

In this study, we used a simple bulk polymerization method to synthesize the MIP. These chosen chemicals and the optimal water content (6.04%) were obtained from former experimental data [17]. Some studies have focused on the solvent effect with respect to the selectivity of the resulting MIP [18–21]. The selection of the functional monomer, in the context of the solvent system used, is crucial for successfully imprinting the target molecule. For example, a Boc-L-Trp MIP, prepared using acetonitrile as the porogenic solvent, showed good selectivity when an acetonitrile-based adsorption solution was used, however with chloroform instead of the acetonitrile in the adsorption solution, no selectivity was observed [21]. This indicates that the conformation of the molecule is affected by the surrounding environment. Therefore the re-binding media surrounding the template should ideally be similar to the solution when the imprint of the template was originally formed. As we used BSA (a bio-macromolecule) as the template, we therefore chose water as the imprinting medium [17]. The selectivity and adsorption capacity of the synthesized MIP is dependent on the polymerization conditions, e.g. the reaction temperature: where a lower temperature typically gives a polymer with higher selectivity [22]. The redox initiation pair used generates free radicals at low reaction temperatures due to the low initiator-decomposition free energy. Previously, we have used TEGDMA, which has a

linear, flexible molecular structure as a crosslinker to successfully synthesize a BSA imprinted MIP [17], but in this research we chose the relatively rigid PETTA as the crosslinker instead of TEGDMA. We wanted to discover the effect of the crosslinker on the resulting MIP. The molecular structures of PETTA and TEGDMA are shown in Table 2. We assumed that the polymer synthesized with PETTA would have a higher degree of crosslinking, resulting in a more rigid structure than a polymer similarly made with TEGDMA, given the same mole ratio of crosslinker to monomer and reaction conditions.

Determination of the saturation adsorption time of MIP

The uptake of the template protein (BSA) by the MIP is shown in Fig. 1. This shows that the adsorption, of the protein, from a total volume of 10 mL containing 4×10^{-4} g mL^{-1} BSA using 0.193 g MIP, reaches maximum adsorption after 2 h incubation. A 3 h standard sampling time after the commencement of adsorption was used as a standard to ascertain the adsorption capacity of the MIP, which from the graph is shown to be 4.961 mg protein g MIP^{-1}.

Recovery of BSA from the resulted MIP

We determined the recovery of BSA to be about 36.64% (0.013 mg), the equation of recovery is defined as: [the mass of BSA extracted from the MIP (mg) / total mass of BSA in the MIP (mg)]×100%.
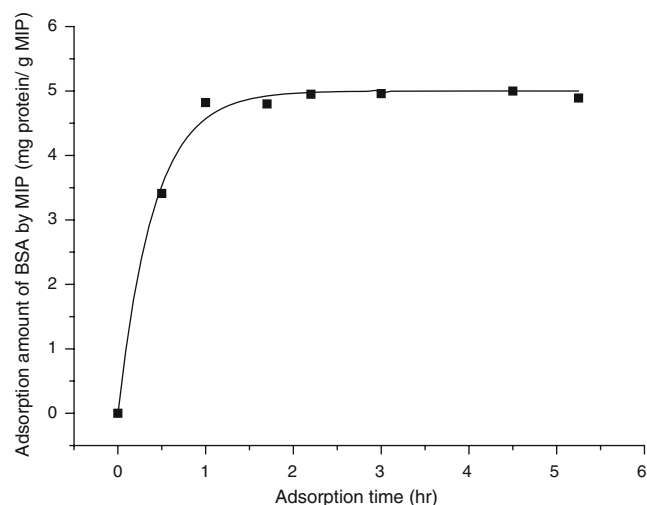

Fig. 1 Adsorption isotherm of MIP. Polymerization conditions: DMAPMA: PETTA=1: 11, water content of monomer complex: 6.04%, polymerization time: 48 h, polymerization temperature: 38± 0.5°C, concentration of BSA: 0.46% and initiator concentration: CHP/ TMEDA: 0.1/ 0.1%. Test condition: adsorption temp.: 25±0.1°C, particle size: 0.105–0.149 mm and concentration of BSA: 0.4 mg mL^{-1}
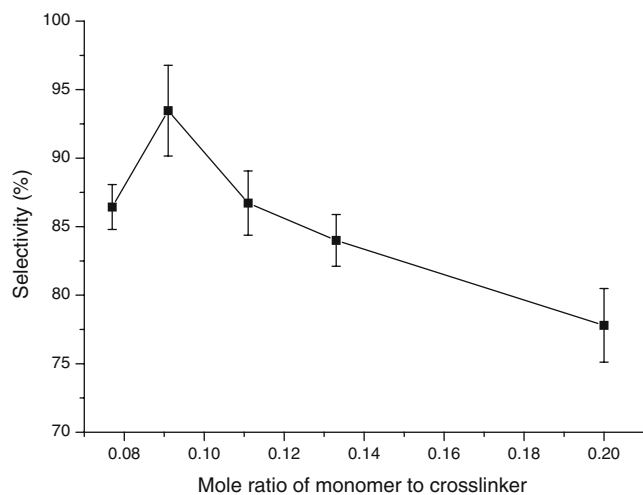
Fig. 2 The effect of crosslinker mole ratio on the selectivity of MIP. Water content of monomer complex: 6.04%, polymerization time: 48 h, polymerization temperature: 38±0.5°C, concentration of BSA: 0.46% and initiator concentration: CHP/ TMEDA: 0.1/ 0.1%. Test condition: adsorption temp.: 25±0.1°C, adsorption time: 3 h, particle size: 0.105–0.149 mm, concentration of each protein in the protein mixture solution: 0.4 mg mL^{-1}

We removed the BSA molecule from the 3D net-work of the MIP with water, which has very high solvation ability toward to BSA, using an extended extraction time (48 h). The experimental data show the BSA was not all removed from the 3D network of the MIP, i.e. there remained about 63.36% (0.022 mg BSA) in the interior of the MIP. The micro-structure of the MIP is constructed with many 3D networks that link the monomer and crosslinker molecules and thereby cause a large mass transfer resistance to any molecule. Therefore a BSA molecule in a position far away from the surface region is hard to extract making the recovery of BSA from the MIP<100%.

The effect of crosslinker mole ratio on the selectivity of MIP

Figure 2 indicates the relationship between the selectivity of the MIP and the mole ratio of monomer to crosslinker. The results show that the maximum selectivity of the MIP appeared at a 0.09 mole ratio of monomer to crosslinker, which shows 93.56% selectivity for the MIP and an uptake of 3.393 mg g-MIP^{-1}. The equation of selectivity of MIP is defined as: [mass (g) of target protein adsorbed / total mass (g) of all proteins adsorbed]×100%. The main topic of this paper is the role of the crosslinker in MIP synthesis, especially the effect of crosslinker structure on the MIP. Myoglobin, another mammalian protein, was chosen as a competitor to evaluate our MIP's performance as it has a pI value of 6.8 and a negative charge in pH7.0 media. This value gives it essentially similar electrostatic properties in

solution to BSA (pI 4.9). The molecular weight of myoglobin is 17.0 KD, which is smaller than that of BSA i.e. 68 KD giving myoglobin a better mass transfer ability. These properties make myoglobin a suitable candidate as a competitor in these experiments. The results show that the selectivity of the synthesized MIP toward to BSA and myoglobin are 93.56% and 6.44% respectively. A polymer optimized with a 0.09 mole ratio of monomer to crosslinker gave the highest selectivity toward to BSA.

Comparison of MIP and NIP

Adsorption capacity comparisons of the MIP and the NIP are shown in Table 1. This shows that the adsorption capacity of the NIP to BSA and myoglobin was 1.980 and 0.388 mg g NIP^{-1}, respectively. Under the same conditions the adsorption capacity of the MIP with respect to BSA and myoglobin was 3.393 and 0.234 mg g MIP^{-1}, respectively. According to these data, the adsorption density of BSA imprinted polymer is about 1.714 times that of the NIP. Additionally, the BSA selectivity of the MIP, i.e. 93.56%, is also higher than the selectivity of the NIP, 83.60%. The selectivity of the MIP is thought to be due to the shape of the BSA imprinted cavities, which can bind BSA but not myoglobin i.e., the conformational differences between the two molecules are responsible for the selectivity of MIP. Since there are no BSA recognition sites in the NIP, the adsorption of protein to the NIP is entirely non-specific.

The effect of polymerization time and synthesis temperature on the adsorption capacity, selectivity and conversion of monomer

Figures 3 to 6 show the relationships between the polymerization time, conversion of monomer, selectivity and adsorption capacity of the resulting MIP at different temperatures. All data shown in Figs. 3 and 4 represents the

Table 1 Comparison of MIP and NIP made with DMAPMA and PETTA

	Selectivity of polymer (%)	Adsorption amount of BSA by polymer (mg-BSA g-MIP^{-1})	Adsorption amount of myoglobin by polymer (mg-myoglobin g-MIP^{-1})
MIP	93.56	3.393	0.234
NIP	83.60	1.980	0.388

Polymerization conditions: DMAPMA: PETTA=1: 11, water content of monomer complex: 6.04%, polymerization time: 48 h, polymerization temperature: 38±0.5°C and initiator concentration: CHP/ TMEDA: 0.1/ 0.1%

Test condition: adsorption temp.: 25±0.1°C, particle size: 0.105–0.149 mm, concentration of each protein in the protein mixture solution: 0.4 mg mL^{-1}
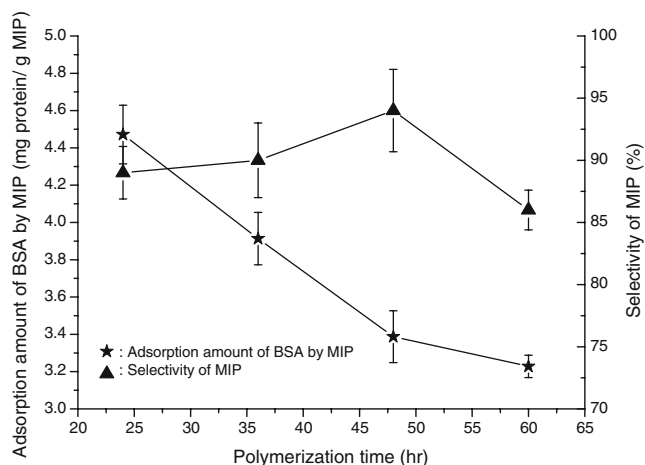
Fig. 3 Effect of polymerization time on the adsorption amount of BSA and selectivity of MIP under 38°C of polymerization temperature. Polymerization conditions: DMAPMA: PETTA=1: 11, water content of monomer complex: 6.04%, polymerization temperature: 38±0.5°C, concentration of BSA: 0.46% and initiator concentration: CHP/ TMEDA: 0.1/ 0.1%. Test condition: adsorption temp.: 25±0.1°C, adsorption time: 3 h, particle size: 0.105–0.149 mm, concentration of each protein in the protein mixture solution: 0.4 mg mL^{-1}
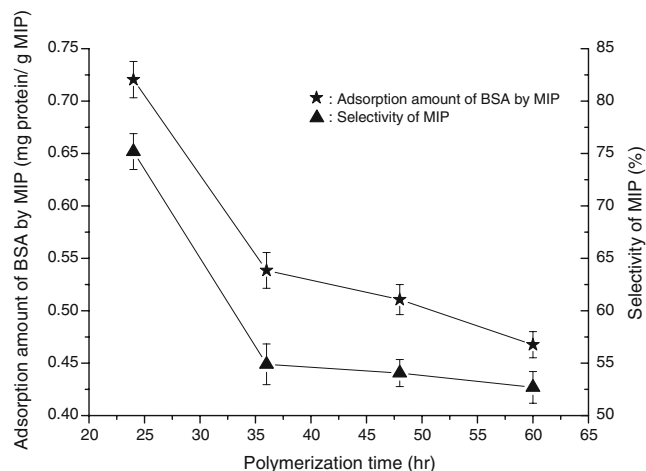

Fig. 5 Effect of polymerization time on the adsorption amount of BSA and selectivity of MIP under 68°C of polymerization temperature. Polymerization conditions: DMAPMA: PETTA=1: 11, water content of monomer complex: 6.04%, polymerization temperature: 68±0.5°C, concentration of BSA: 0.46% and initiator concentration: CHP/ TMEDA: 0.1/ 0.1%. Test condition: adsorption temp.: 25±0.1°C, adsorption time: 3 h, particle size: 0.105–0.149 mm, concentration of each protein in the protein mixture solution: 0.4 mg mL^{-1}

MIP, which was synthesized at a set temperature of 38°C, while Figs. 5 and 6 show results with the MIP synthesized at 68°C. The conformation of the BSA is strongly affected by the temperature. In using polymerization temperatures of 38 and 68°C we assumed the protein's conformation would change in a stable manner. At higher temperatures amino

acids in the primarily structure of proteins have more mobility than that at lower temperatures. The degree of conformation changing of the molecule can be observed by measuring the proportional changing of the α-helical and β-structures, which exist in the secondary structures of proteins[23]. Previous research indicated that BSA would
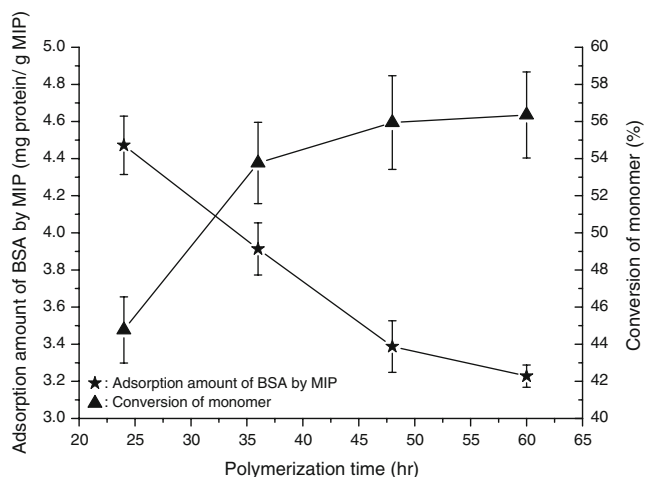

Fig. 4 Effect of polymerization time on the adsorption amount of BSA and conversion of monomer of the MIP under 38°C of polymerization temperature. Polymerization conditions: DMAPMA: PETTA=1: 11, water content of monomer complex: 6.04%, polymerization temperature: 38±0.5°C, concentration of BSA: 0.46% and initiator concentration: CHP/ TMEDA: 0.1/ 0.1%. Test condition: adsorption temp.: 25±0.1°C, adsorption time: 3 h, particle size: 0.105–0.149 mm, concentration of each protein in the protein mixture solution: 0.4 mg mL^{-1}, the adsorption test was used 0.06 g dried, extracted MIP particles and 0.2 g un-extracted MIP particles was applied to the conversion of monomer measuring
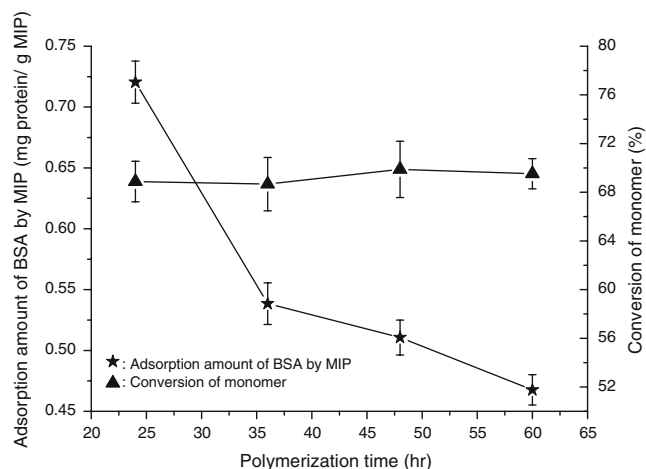

Fig. 6 Effect of polymerization time on the adsorption amount of BSA and conversion of monomer of the MIP under 68°C of polymerization temperature. Polymerization conditions: DMAPMA: PETTA=1: 11, water content of monomer complex: 6.04%, polymerization temperature: 68±0.5°C, concentration of BSA: 0.46% and initiator concentration: CHP/ TMEDA: 0.1/ 0.1%. Test condition: adsorption temp.: 25±0.1°C, adsorption time: 3 h, particle size: 0.105–0.149 mm, concentration of each protein in the protein mixture solution: 0.4 mg mL^{-1}, the adsorption test was used 0.06 g dried, extracted MIP particles and 0.2 g un-extracted MIP particles was applied to the conversion of monomer measuring
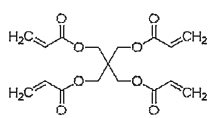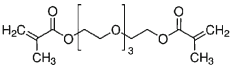
totally denature at temperatures >68 °C and generate an almost constant proportion of α-helical and β-structures under 40 °C. Accordingly, we chose the temperatures 38 and 68 °C as our synthetic temperatures to give us the most contrastive, stable molecular conformations without any unstable or transitional conformations. In Fig. 3, the results indicate the polymerization time is inversely proportional to the adsorption capacity of the MIP, i.e. from 24 to 60 h adsorption capacity decreased from 4.472 mg g-MIP^{-1} to 3.228 mg g-MIP^{-1}. However, the increased polymerization time leads to an increased conversion of monomer (from 44.77% to 56.35%) shown in Fig. 4. The polymerization time is inversely proportional to the adsorption capacity of the MIP from 24 to 60 h. This may be explained as follows; the shape of the cavities that are created becomes more defined with increasing polymerization time i.e., the increasing conversion of monomer increases the selectivity of the MIP but it also makes the mass transfer resistance gradually become larger and causes a decrease in adsorption. Thus, the most structurally complementary microstructure with respect to the template may not be ideal for macromolecular imprinting due to the mass transfer problem. We observed the highest selectivity of the MIP (93.56%) to appear at 48 h polymerization time, and then decreased as the polymerization time increased.

The experimental data shown in Figs. 5 and 6 are for the MIP synthesized at 68 °C. According to these data, we can see the higher polymerization temperature results in a conversion of monomer higher than when carried out at 38 °C (average value ~69.24%). But the larger resistance generated by increasing monomer conversion makes the adsorbed amount of the MIP quite low (the variation of the adsorption amount of MIP are from 0.720 mg g-MIP^{-1} to 0.468 mg g-MIP^{-1} during the period of polymerization time 24 to 60 h). Due to the BSA denaturation at 68 °C, which was the synthesis temperature of this MIP, the resulting imprinted polymer was almost without any selectivity toward the template molecule. All the results mentioned above were found with an adsorption temperature of 25 °C.

Here, we also compared the experimental data from a MIP synthesis system which employed DMAPMA and TEGDMA as the functional monomer and crosslinker, respectively [17] these data are shown in Table 2. According to the former experimental data, the crosslinker TEGDMA can make a resulting polymer, which has higher adsorption ability and selectivity of MIP than that with PETTA. The molecular structures of TEGDMA and PETTA are quite different: TEGDMA has a flexible main chain which has high freedom of conformation, but the PETTA instead has a more rigid chain which has low freedom of conformation. Comparing these data, we may deduce that the crosslinker which has linearly structure is more suitable for macromolecular imprinting. The interactions generated between

Table 2 Comparison of MIP made with different kind of crosslinker under the same polymerization time and temperature

Kind of crosslinker	Selectivity of MIP (%)	Adsorption amount of BSA by MIP (mg-BSA g-MIP^{-1})	Conversion of monomer (%)
PETTA	93.56	3.393	55.9
TEGDMA	98.67	6.020	23.2

Polymerization conditions: Water content of monomer complex: 6.04%, polymerization time: 48 h, polymerization temperature: 38± 0.5 °C and initiator concentration: CHP/ TMEDA: 0.1/ 0.1%

Test condition: adsorption temp.: 25±0.1 °C, particle size: 0.105– 0.149 mm, concentration of each protein in the protein mixture solution: 0.4 mg mL^{-1}

functional monomer and template molecule are constructed with Van der Waals forces and the electrostatic force. The differences caused by using different kinds of crosslinkers can contribute to the amount of the generated Van der Waals forces between the monomer, or crosslinker, and the template, but the major component in the MIP synthesis system is the crosslinker. TEGDMA, which has a flexible main chain can easily change its conformation to match the template molecule but PETTA, which has a more rigid main chain structure cannot adapt in a similar manner. Therefore, the linearly structured crosslinker can assemble with the BSA in the polymerization solution in a way determined by the relatively strong Van der Waals forces, between the template and the crosslinker, to create the desired conformational structure of the imprinted cavities [24].

Whereas, previous studies have tended to emphasize functional monomer selection, our results clearly show that the polymerization conditions need to be optimized for all the MIP's components and that with proteins the choice of crosslinker is especially important as a determinant of the template's transport function into and out of the polymer.

Conclusions

An albumin imprinted MIP was successfully synthesized using DMAPMA-PETTA as the functional monomer and crosslinker respectively. The results show that the synthesized MIP possesses both a high selectivity and adsorption capacity with respect to albumin, in comparison to our

chosen competing protein. The polymerization temperature and the time allowed for polymerization were shown to have a significant effect on the resulting albumin imprinted MIP's performance. Also of interest is the observation, that having optimized the preparation conditions for our chosen template, the polymer's affinity for the competing protein was minimal. Higher polymerization temperatures ($>38\,^\circ$C) and extended polymerization times (>48 h) increased monomer conversion but decreased the selectivity and adsorption capacity of the MIP. Our optimized MIP with 93.56% selectivity and 3.393 mg g-MIP^{-1} adsorption capacity was obtained with the following polymerization conditions: 0.09 mole ratio of monomer to crosslinker, 6.04 wt% water content of monomer complex, 48 h of polymerization time, polymerization at $38\,^\circ$C and 0.46% albumin in the pre-polymerization monomer complex.

References

1. Du J, Shen L, Lu J (2003) Flow injection chemiluminescence determination of epinephrine using epinephrine-imprinted polymer as recognition material. Anal Chim Acta 489:183

2. Hayden O, Bindeus R, Haderspo̎ck C, Mann K, Wirl B, Dickert FL (2003) Mass-sensitive detection of cells, viruses and enzymes with artificial receptors. Sens Actuators B 91:316

3. Dickert FL, Hayden O, Halikias KP (2001) Synthetic receptors as sensor coatings for molecules and living cells. Analyst 126:766

4. Ye L, Mosbach K (2001) Polymers recognizing biomolecules based on a combination of molecular imprinting and proximity scintillation: a new sensor concept. J Am Chem Soc 123:2901

5. Liang C, Peng H, Bao X, Nie L, Yao S (1999) Study of a molecular imprinting polymer coated BAW bio-mimic sensor and its application to the determination of caffeine in human serum and urine. Analyst 124:1781

6. Rezeli M, Kilár F, Hjertén S (2006) Monolithic beds of artificial gel antibodies. J Chromatogr A 1109:100

7. Cheng Z, Zhang L, Li Y (2004) Synthesis of an enzyme-like imprinted polymer with the substrate as the template, and its catalytic properties under aqueous conditions. Chem Eur J 10:3555

8. Takeuchi T, Mukawa T, Matsui J, Higashi M, Shimizu KD (2001) Molecularly imprinted polymers with metalloporphyrin-based molecular recognition sites coassembled with methacrylic acid. Anal Chem 73:3869

9. McCluskey A, Holdsworth CI, Bowyer MC (2007) Molecularly imprinted polymers (MIPs): sensing, an explosive new opportunity? Org Biomol Chem 5:3233

10. Fish WP, Ferreira J, Sheardy RD, Snow NH, O'Brien TP (2005) Rational design of an imprinted polymer: maximizing selectivity by optimizing the monomer-template ratio for a cinchonidine MIP, prior to polymerization, using microcalorimetry. J Liq Chromatogr Relat Technol 28:1

11. Cooper AI, Hems WP, Holmes AB (1999) Synthesis of highly cross-linked polymers in supercritical carbon dioxide by heterogeneous polymerization. Macromolecules 32:2156

12. Janiak DS, Kofinas P (2007) Molecular imprinting of peptides and proteins in aqueous media. Anal Bioanal Chem 389:399

13. Lin HY, Rick J, Chou TC (2007) Optimizing the formulation of a myoglobin molecularly imprinted thin-film polymer—formed using a micro-contact imprinting method. Biosens Bioelectron 22:3293

14. Hsu CY, Lin HY, Thomas JL, Wu BT, Chou TC (2006) Incorporation of styrene enhances recognition of ribonuclease A by molecularly imprinted polymers. Biosens Bioelectron 22:355

15. Syu MJ, Nian YM, Chang YS, Lin XZ, Shiesh SC, Chou TC (2006) Ionic effect on the binding of bilirubin to the imprinted poly(methacrylic acid-co-ethylene glycol dimethylacrylate). J Chromatogr A 1122:54

16. Ou SH, Wu MC, Chou TC, Liu CC (2004) Polyacrylamide gels with electrostatic functional groups for the molecular imprinting of lysozyme. Anal Chim Acta 504:163

17. Hu CH, Chou TC (2009) Albumin molecularly imprinted polymer with high template affinity — prepared by systematic optimization in mixed organic/ aqueous media. Microchem J 91:53

18. Xu Z, Liu L, Deng Q (2006) Study on the mechanism of binding specificity of metoclopramide-imprinted polymers. J Pharm Biomed Anal 41:701

19. Zhu QZ, Haupt K, Knopp D, Niessner R (2002) Molecularly imprinted polymer for metsulfuron-methyl and its binding characteristics for sulfonylurea herbicides. Anal Chim Acta 468:217

20. Idziak I, Benrebouh A, Deschamps F (2001) Simple NMR experiments as a means to predict the performance of an anti-17α-ethynylestradiol molecularly imprinted polymer. Anal Chim Acta 435:137

21. Yu C, Mosbach K (2000) Influence of mobile phase composition and cross-linking density on the enantiomeric recognition properties of molecularly imprinted polymers. J Chromatogr A 888:63

22. Piletsky SA, Piletska EV, Karim K, Freebairn KW, Legge CH, Turner APF (2002) Polymer cookery: influence of polymerization conditions on the performance of molecularly imprinted polymers. Macromolecules 35:7499

23. Takeda K, Wada A, Yamamoto K, Moriyama Y, Aoki K (1989) Conformational change of bovine serum albumin by heat treatment. J Protein Chem 8:653

24. Hart BR, Shea KJ (2002) Molecular imprinting for the recognition of N-Terminal Histidine peptides in aqueous solution. Macromolecules 35:6192