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Molecularly imprinted polymer microspheres for solid-phase extraction of chloramphenicol residues in foods

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Abstract

Preparation of molecularly imprinted polymer microspheres (MIPMs) for chloramphenicol (CAP) by aqueous suspension polymerization is reported for the first time in this study. The resulting MIPMs had the ability to specifically adsorb CAP, and the molecularly imprinted solid phase extraction (MISPE) based on the MIPMs was shown to be applicable for clean-up and preconcentration of trace CAP in milk and shrimp samples with high recoveries of 92.7% and 84.9%, respectively. Combined with MISPE, the conventional HPLC-UV analysis sensitivity for CAP in foods could be significantly increased.
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Keywords: Molecularly imprinted polymer microspheres; Chloramphenicol; Solid-phase extraction; Suspension polymerization; Foods

1. Introduction

Chloramphenicol (CAP) is a broad spectrum antibiotic derived from the soil bacterium *Streptomyces*, ubiquitously used for treating bacterial diseases in veterinary and aquaculture practice [1]. However, it has the potential to cause serious toxic effects on humans and animals when given by mouth or injection, with non-target effects such as bone marrow depression, aplastic anemia, hypoplastic anemia, thrombocytopenia, as well as granulocytopenia [2]. China, the European Commission, the United States and some other countries have strictly banned the use of CAP in food-producing animals and legislated Maximum Residue Limits (MRLs) [3,4].

The available methods for effectively monitoring and detecting CAP residues in foods include mainly microbiological [5], enzymatic [6], immunological [7], chromatographic [8,9], and sensor methods [10]. Among these, immunological methods and chromatographic methods are widely used, although they have some unavoidable drawbacks. The former is generally used as a screening method with thermal and chemical instability and needs further confirmation of the positive results; the latter is expensive, and laborious with complicated extraction and purification procedures [11]. The difficulty in determining CAP in foods is the extremely low concentrations of 1–10 $\mu g\,kg^{-1}$ in various samples with complex matrices. Therefore, novel, rapid and accurate clean-up and enrichment methods are required for analyses involving CAP monitoring.

Solid-phase extraction (SPE) is routinely used for clean-up and preconcentration in the analysis of biological and environmental samples [12]. Compared with liquid–liquid extraction, SPE has the advantages of simplicity, speed and less consumption of organic solvents. However, generic sorbents usually lack selectivity, and are easily subjected to interference by non-target substances with similar characteristics [13]. Although immunoaffinity chromatography (IAC) is capable of differentially adsorbing target analytes, it still has some disadvantages such as lack of stability and high costs of antibody preparation [14].

With advances in molecularly imprinted technology, molecularly imprinted solid-phase extraction (MISPE) provides a simple and effective pretreatment method in food and food-related products [15,16]. Molecularly imprinted polymers

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(MIPs) are tailor-made materials with high selective recognition properties for a target molecule or analogues, possessing the characteristics of simplicity and stability under extreme conditions of strong acid, alkali, as well as high temperature [17]. MISPE is one of the most important applications of MIPs, displaying higher selectivity than conventional SPE with respect to the binding of target analytes from multiple ingredients [18–21].

Although the reported MIPs against CAP were obtained by bulk polymerization using the 2-(diethylamino) ethyl methacrylate (DEAEM) as the functional monomer, and successfully applied for HPLC and on-line selective SPE sorbents [22,23], these imprinted sorbents of MISPE have some deficiencies particularly with the process of obtaining the appropriate polymeric particles which requires grinding and time-consuming sieving with low yield. This leads to inconvenience and makes the method not feasible for manufacturing uses. Furthermore, the shapes and sizes of the particles are usually irregular, resulting in high pressure and decrease of selectivity [24–26].

To increase the feasibility for MIPMs production and applicability in aqueous samples, we prepared the novel MIPMs against CAP for MISPE analysis through aqueous suspension polymerization, with water as the suspension medium to mimic the natural condition of CAP during the course of polymerization for improving the specificity, and to avoid complicated post-treatment procedures after polymerization. Moreover, the off-line MISPE mode for clean-up and enrichment of CAP in tested food samples was optimized.

2. Experimental

2.1. Chemicals and materials

CAP, as the template molecule, and ethylene glycol dimethacrylate (EGDMA), as a cross-linker, were obtained from Fluka (Steinheim, USA). Florfenicol (FF) and thiamphenicol (TAP) were obtained from Sigma–Aldrich (Steinheim, Germany). 2-(Diethylamino) ethyl methacrylate (DEAEM) as the functional monomer was also from Sigma–Aldrich. 2,2′-Azobis (2-isobutyronitrile) (AIBN), as the initiator,was from the China National Medicines Corporation Ltd. (Shanghai, China). Polyvinyl alcohol 1788 (d.p. = 1788, saponification value = 88%) was from SINOPEC Shanghai Petrochemical Co. Ltd. (Shanghai, China). Methanol of HPLC grade was from Fisher Scientific Co. (USA). Other chemical reagents including chloroform and octanol were of analytical grade. Double distilled water and MilliQ water were provided by local suppliers.

A stock solution of CAP at 8.0×10^{-3} mol L^{-1} was prepared weekly by dissolving first in 5 mL methanol. Other working solutions of CAP were obtained by dilution with 0.05 mol L^{-1} phosphate buffer (pH 7.0). Solutions of FF and TAP were similarly prepared. Britton-Robinson buffers prepared in 40% methanol with different pH values were used as the washing solutions [22].

2.2. Preparation of the MIPMs

To prepare the MIPMs, 4 g of polyvinyl alcohol 1788 (PVA 1788) was dissolved in 100 mL of ddH$_2$O and stirred at 400 rpm under a stream of nitrogen in a 250 mL reactor flask. Octanol–chloroform (2:1, v/v; 15 mL), 1 mmol of CAP, 5 mL of cross-linker EGDMA, 120 mg of AIBN and the required amounts of functional monomer 2-(diethylamino) ethyl methacrylate (DEAEM), were dissolved together by sonication, and then added to the flask. The polymerization was generated at 70 °C for 24 h under a nitrogen atmosphere, with stirring at 400 rpm. Afterwards, the polymer microspheres were filtered and washed three times sequentially with ddH$_2$O, methanol and acetone, respectively. Subsequently, the imprinted microspheres were washed with methanol–acetic acid (9:1, v/v) in a soxhlet apparatus successively until no CAP could be eluted, with final confirmation by HPLC analysis. The imprinted microspheres were then washed with methanol to remove the residual acetic acid, and dried at 50 °C under vacuum for 12 h. The non-imprinted polymer microspheres (NIPMs) were prepared in the same manner, except that the polymerization mixtures did not contain the template of CAP.

2.3. Preparation for MIPMs cartridges

One hundred milligrams of dried imprinted and non-imprinted polymer microspheres were suspended in 2 mL of isopropanol–methanol (2:1,v/v), packed into the SPE cartridges of 3.0 mL (Supelco, USA), with two glass-wool frits at each end. The cartridges were washed with 5 mL methanol and preconditioned with the 0.05 mol L^{-1} phosphate buffer (pH 7.0) before sample loading. During the SPE operation, the volume of washing solution was 3 mL.

2.4. Adsorption capacity of MISPE cartridges

To evaluate the capacity of MISPE cartridges, 80 mL of 0.05 mol L^{-1} phosphate buffer (pH 7.0) containing 0.025 μmol mL^{-1} of CAP and 30% methanol was successively loaded onto MISPE and NISPE cartridges, respectively. The effluent solutions of each 5 mL loading were collected for detection on a variable-wavelength UV–vis spectrophotometer (UV mini1240, SHIMADZU, Japan). The amount of CAP bound to the polymer was calculated by subtracting the CAP of effluent solutions from the loading solutions [27]. The values were calculated in triplicate and used for accumulative adsorption analysis.

2.5. Sample extraction

The milk and shrimp used in this study were purchased from a local supermarket and confirmed as having no detectable CAP by HPLC-UV. The milk samples were deproteinized [22,28] with minor modifications as follows. The samples of 10 mL milk and 10 g homogenized shrimp in 30 mL polypropylene tubes were spiked with three levels of CAP at 5, 10 and 100 μg kg^{-1}, and placed statically for at least 15 min. Afterwards, 40 mL of 0.05 mol L^{-1} phosphate buffer (pH 7.0) was added to the shrimp samples, vortexed for 2 min, sonicated for 15 min and centrifuged at 1.4×10^3 g for 10 min. The supernatant solutions of treated samples of milk and shrimp were transferred to new tubes. To precipitate the proteins, 1 and 3 mL of 15%

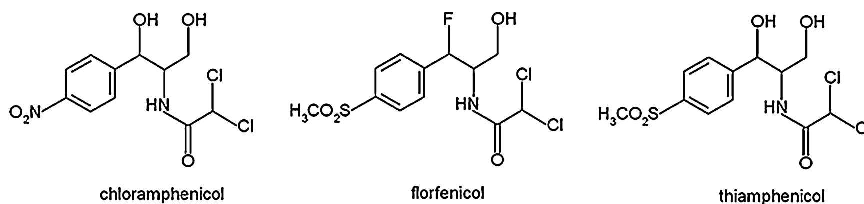

Fig. 1. Chemical structures of chloramphenicol, florfenicol and thiamphenicol.

trichloroacetic acid in water were added, respectively, to milk and shrimp samples. The solutions were vortexed for 2 min, followed by centrifugation at 1.4×10^3 g for 10 min, and, finally, filtered through microfilters with a pore size of 0.45 μm to remove the denatured proteins. The extracts were stored at 4 °C until use.

2.6. HPLC-UV analysis

Prior to analysis, the eluent samples from the MISPE cartridges were evaporated to dryness under a stream of nitrogen (Pressured gas blowing concentrator, Quandao, China) and re-dissolved with an appropriate volume of mobile phase. HPLC analysis was performed with a Beckman HPLC system (USA) equipped with a UV detector. A 7725I sample injection valve with a 20 μL sample loop and a Beckman ODS C18 column ODS C18 cartridge (4.6 mm id × 250 mm, 5 μm) were used. The column temperature was kept at ambient. The mobile phase consisted of methanol and water (40:60, v/v) for CAP. For FF and TAP, the proportion of methanol to water was 35:65 (v/v). The flow rate was constant at 1.0 mL min^{-1}, and the injection volume was 20 μL. The wavelengths for detection of CAP, FF and TAP were at 278, 223 and 226 nm, respectively (Fig. 1).

3. Results and discussion

3.1. Preparation of the MIPMs

To overcome the shortcomings of the reported MIPs against CAP, we prepared the MIPMs against CAP using the modified methods of preparation for MIPMs [29–32]. In the present study, three dispersants were used; Glutin and PEG 4000 at 4%, and PVA1788 at three concentrations of 2%, 4% and 6% during the polymerization were first tested (Table 1). PVA1788 at concentration of 4% was selected for further use since the appropriate

Table 1
The effect of the type and concentration of dispersants on the figuration and size of MIPMs

MIPMs code	Type and concentration of dispersants	Agglomerate	Average size (μm)
1	PVA1788 (2%)	Little	120
2	PVA1788 (4%)	Little	80
3	PVA1788 (6%)	Little	50
4	Glutin (4%)	Block	na
5	PEG 4000 (4%)	Severe	na
6	PEG 6000 (4%)	Severe	na

na, no polymers available.

MIPMs were obtained using PVA 1788 with diameter distributions from 50 to 120 μm (Fig. 2A), and a porous surface could be clearly observed in SEM photographs (Fig. 2B).

Generally, proper molar ratios of functional monomer to template are very important to enhance specific affinity of polymers and number of MIPs recognition sites. High ratios of functional monomer to template result in high non-specific affinity, while low ratios produce fewer complexation due to insufficient functional groups [33]. Five molar ratios of the monomer DEAEM to the template of 2:1, 3:1, 4:1, 6:1 and 8:1 were used in the experiments. The optimum ratio of functional monomer to template for the specific rebinding of CAP was 4:1 (Fig. 3), which had the best specific affinity and the highest recovery of 81.6%, while that of the corresponding NIPMs was low at 28.5%. The specific adsorption recovery of CAP at 4:1 was 53.1%, while those at 2:1, 3:1, 6:1 and 8:1 were 33.5%, 25.9%, 20.1% and 17.6%, respectively. For the polymers with a ratio of 8:1, an excess of the functional monomer with respect to the template yielded higher non-specific affinity. The adsorption recovery and specificity of those at 4:1 were both higher than those at 2:1 and 3:1, indicating that lower ratios of functional monomer to template may not have sufficient specific complexation in pre-polymerization.

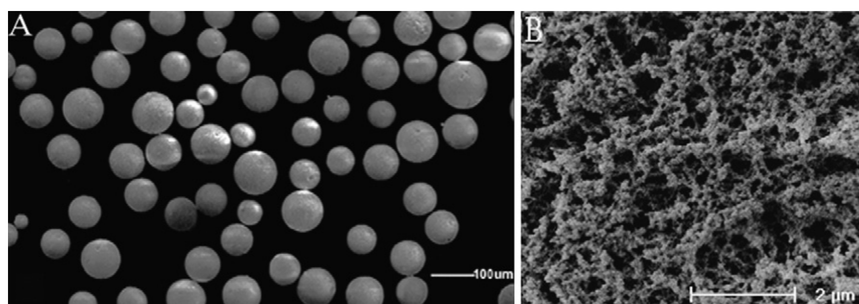

Fig. 2. Scanning electron micrographs of the MIPMs by aqueous suspension polymerization method. A, ×300; B, ×20000.

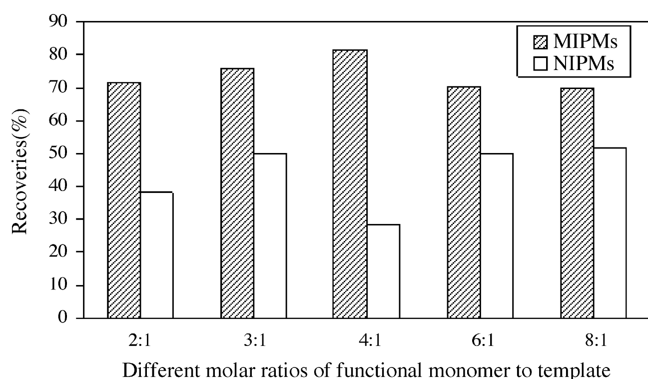

Fig. 3. Effect of different molar ratios of the functional monomer to the template on the retention of CAP. The PBS buffer (0.05 mol L^{-1}, pH 7.0) was used as loading solution. The concentration of loading solution was 0.1 μmol mL^{-1} and the volume of loading solution was 1 mL, washing with 3 × 1 mL MeOH–PBS(4:6), elution with 2 × 1 mL MeOH.

Levi et al (1997) obtained the imprinted polymers of CAP using the functional monomer DEAEM by bulk polymerization, and only the polymer prepared at the ratio of functional monomer to template of 2:1 worked [23]. The reasons why the ratio of functional monomer to template of 4:1 showed the best efficiency may be ascribed to the distribution of some functional monomer to the water [34]. These MIPMs were selected for further experiments.

3.2. Solid-phase extraction analysis

Retention of imprinted molecules can be achieved in aqueous-rich solutions [33]. Specific recognition of CAP molecules in aqueous-rich media on SPE cartridges depends mainly on selective interactions, such as hydrogen bonding, ionic interactions, and hydrophobic effects.

To set up the optimized conditions for MISPE, the influence of pH on retaining for CAP was first investigated, with a range of 2 to 9 (Fig. 4). Five milliliters of solutions spiked with 0.01 μmol mL^{-1} of the CAP were percolated through the MIPMs and NIPMs cartridges. Almost all the CAP had the ability to rebind, and the retained CAP could be eluted over the pH values ranging from 2.0 to 7.0, which was mainly attributed to non-specific binding because of similar results observed for the NIPMs. However, we also observed that while the pH values of loading solution were at 8.0 or 9.0, the recoveries on the MIPMs showed more decrease than those on the NIPMs. The p*K*a values of CAP and DEAEM were 5.5 and 7.3, respectively, and so it was assumed that the interactions of CAP and imprinted polymers was mainly via hydrogen bonding and ionic interaction between correctly arranged diethylamino groups on the polymer and the hydroxyl groups on the CAP, meanwhile the CAP could be retained on the NIPMs by hydrophobic strength.

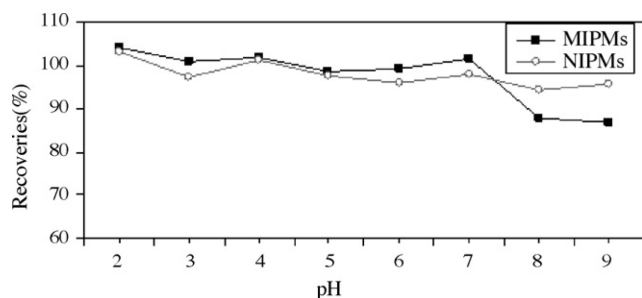

Fig. 4. Influence of pH values on the retention of CAP by MIPMs and NIPMs cartridges. The Britton-Bobinson buffers at different pH values were used as loading solution. The concentration of each loading solution was 0.01 μmol mL^{-1} and the volume of loading solution was 5 mL.

Although the recovery was approximately 100% when the pH values of loading solution were at the range of 2.0 to 7.0 (Fig. 4), non-specific adsorption of CAP on MIPMs was higher than expected. In order to reduce the non-specific adsorption and improve the selective binding of the CAP, the washing solution of MIPMs and NIPMs cartridges was adjusted by optimizing the ratio of methanol in water. There was almost no difference in the recoveries of MIPMs and NIPMs cartridges after washing by the majority of solutions, where the non-specific bindings could not be disrupted between the imprinted polymers and CAP (Fig. 5A). With increased methanol in the washing solution, the recoveries of CAP decreased precipitously in the NIPMs cartridges. When washing with 40% methanol, the recovery of CAP in the NIPMs cartridges was reduced to 28.5%, while the recovery of the MIPMs was 81.6%, indicating stronger retention of CAP by the MIPMs than the NIPMs. These confirmed that MIPMs had higher specificity for CAP than NIPMs and also showed that the hydrophobic interaction is one of the main factors to the retention of CAP on MIPMs.

The higher proportion of methanol in washing solution caused a large decrease of CAP retention on the MIPMs car-

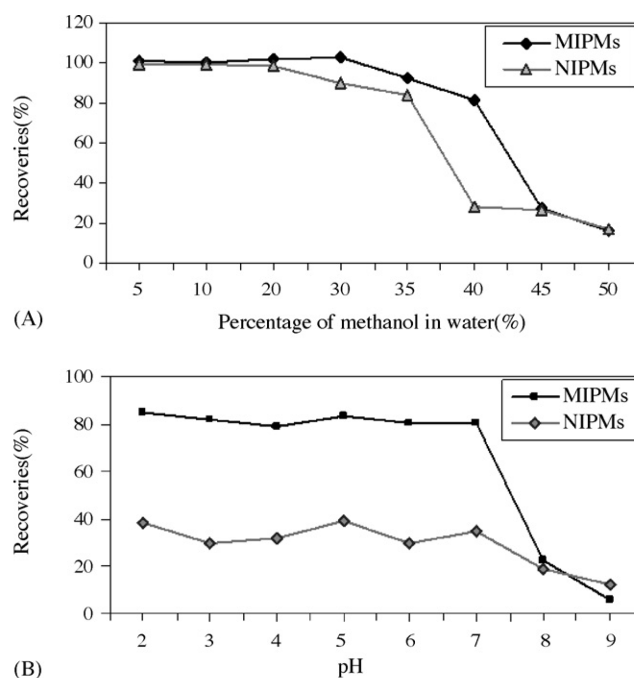

Fig. 5. Recoveries of CAP on MIPMs and NIPMs cartridges under different washing solutions. (A) The different percentages of methanol in water as the washing solution; (B) CAP recoveries by using Britton-Robinson buffers at different pH values in 40% methanol as the washing solution. The concentration of each CAP loading solution was 0.1 μmol mL^{-1}. The volume of loading and washing solution were 1 and 3 mL, respectively.

tridges, and also on the NIPMs cartridges. To optimize the washing solutions, the pH values of washing conditions were further screened. The Britton-Robinson buffers at the pH values from 2.0 to 9.0 were used for washing. The results (Fig. 5B) indicated that the recoveries on the MIPMs and NIPMs cartridges were only slightly changed in the pH ranges of 2 to 7. The recoveries on the MIPMs cartridges after washing with 40% methanol at pH values of 8.0 and 9.0 were largely decreased, compared with those of the NIPMs cartridges. Thus, the pH values of loading and washing solution were appropriate under either neutral or acidic conditions. In addition, lower CAP recoveries of the MIPMs at higher pH values indicated that this may be attributed to the negative charge repulse between CAP and the functional base in the polymers.

3.3. Specificity evaluation of the MIPMs

To evaluate the selectivity of the MIPMs, analogues of CAP, TAP and FF were selected. Standard solutions of each of the two analogues with 2.0 μg in 1 mL PBS buffer (pH 7.0, 0.05 mol L^{-1}) were individually percolated through the MIPMs and NIPMs cartridges, and then washed with 3 × 1 mL 40% methanol in PBS (pH 7.0, 0.05 mol L^{-1}) buffer, eluted with 2 mL methanol, and finally analyzed by HPLC-UV. The results demonstrated (Fig. 6) that this washing solution could severely reduce the non-specific interactions between the CAP and the analogues.

The recovery was only 29.6% (RSD, ±6.1%) for TAP and 37.4% (RSD, ±3.1%) for FF on the MIPMs cartridges, and 5.1% (RSD, ±4.2%) recovery for TAP and 27.4% (RSD, ±5.2%) for FF on the NIPMs cartridges. Thus, the specific affinity of the MIPMs to TAP and FF were 24.5% and 10.0%, respectively. Although the affinity of TAP was slightly lower than that of FF, TAP exhibited stronger specific affinity to the MIPMs than FF, compared to the recoveries on the NIPMs cartridges. These results demonstrated that the MIPMs could specifically recognize CAP with moderate cross-reactivity effect.

TAP possesses two hydroxyl groups with much more similar structure to CAP except that the $-SO_2CH_3$ substitutes for $-NO_2$, while FF has only one hydroxyl group and another hydroxyl group is replaced by −F atom compared to the structure of TAP. We hypothesize that the spatial effects, hydrogen bonding and ionic interactions dominate the specific affinity, and the specific recognition sites of imprinted polymers are mainly complementary to the template functional groups. The reciprocal interactions are responsible for the selective affinity of the materials. Thereby, the MIPMs with high selectivity produced with the protocol designed in this study could be used for clean-up and enrichment of CAP.

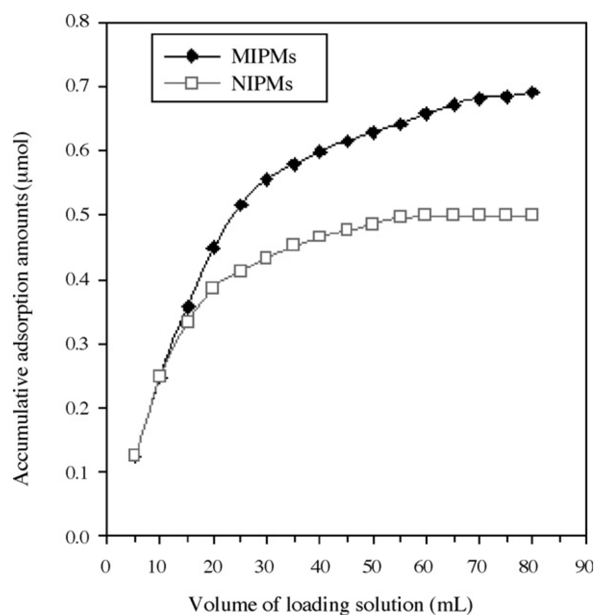

Fig. 7. Adsorption curves of CAP on MIPMs and NIPMs cartridges. The concentration of CAP was 0.025 μmol mL^{-1} in continuous loading process. Each 5 mL was collected for UV-spectrophotometer detection.

3.4. Measurement of adsorption capacity

To estimate the adsorption capacity of CAP on the MIPMs and NIPMs cartridges, an accumulative adsorption experiment was carried out using 80 mL solutions containing 0.025 μmol mL^{-1} of CAP continuously percolated through the MIPMs and NIPMs cartridges.

The accumulative adsorption capacities of CAP by the MIPMs and NIPMs are shown in Fig. 7. For NIPMs, when

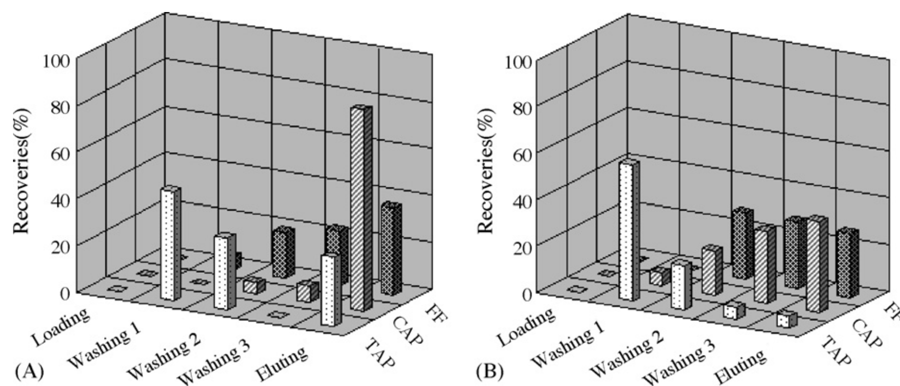

Fig. 6. Recoveries obtained by individually percolating CAP, FF and TAP on MIPMs and NIPMs cartridges: (A) the recoveries on the MIPMs; (B) the recoveries on the NIPMs. The washing solution was 3 × 1 mL 40% methanol in 0.05 mol L^{-1} PBS (pH 7.0).

Table 2
The CAP recoveries obtained using different loading volumes

Volume (mL)	Average recovery (%)[a]	RSD (%)
5 (0.1 μg)	97.9	4.8
25 (0.1 μg)	100.2	7.1
50 (0.1 μg)	101.0	3.3
100 (0.1 μg)	96.1	3.5
100 (0.01 μg)	101.3	4.6

[a] $n = 3$.

the volume of loading solution reached 60 mL, the adsorption capacity was close to saturation. When the volume of loading solution reached 80 mL, the maximum adsorption capacity of MIPMs was 0.69 μmol (222 μg) with the difference of about 0.19 μmol, in comparison to the NIPMs under the same condition.

In this experiment, the aqueous solution including 30% methanol was used as the loading solution. In order to maintain higher recovery of CAP, the solution including 30% methanol was selected as washing solution in the next clean-up and enrichment of spiked samples.

3.5. Enrichment from aqueous loading solutions

The volume of CAP loading solutions, ranging from 5 to 100 mL, was evaluated by percolating through the MISPE cartridges, with an elution volume of 2 mL methanol. The total amounts of CAP loading CAP were 0.1 μg in 5, 25, 50 and 100 mL, plus 0.01 μg in 100 mL. The results (Table 2) indicated that the CAP could be almost entirely absorbed by MISPE and eluted. The estimated recovery from 100 mL solutions containing 0.01 μg of CAP was 101.3 ± 4.6% in three replicates. The results demonstrated that there were almost no breakthrough phenomena when the percolated volumes of samples ranged from 5 to 100 mL.

3.6. Application for spiked samples

The ultimate objective of this work was to use the MIPMs as off-line SPE sorbents, attaining the goal of quick and direct purification and enrichment of CAP from the aqueous samples. Matrix interferences were evaluated using MISPE for clean-up and preconcentration of CAP from practical samples of milk and shrimp. The samples were homogenized and spiked with three levels of CAP, followed by the extraction procedure described above. First, the spiked shrimp homogenates were centrifuged to remove most matrices; subsequently, trichloroacetic acid was selected for protein precipitation. After filtration, the supernatants were directly percolated through the MISPE cartridges.

The chromatographic diagrams of spiked milk and shrimp samples presented in Fig. 8 indicated that the 5 μg kg^{-1} of CAP could not be detected by conventional HPLC-UV method due to the complex matrix interferences. Also, previous reports indicated that the sensitivity of common HPLC-UV analysis for CAP in in aquaculture tissue was about 10 μg kg^{-1} [7,35]. However, after the MISPE treatment, washed with 30% methanol in PBS (pH 7.0, 0.05 mol L^{-1}), the interferences were mostly removed and the CAP of 5 μg kg^{-1} was also detectable by HPLC-UV analysis. Nevertheless, the CAP of the spiked sample treated with NISPE had not been detected yet, without improved sensitivity (Fig. 8C and D). Moreover, the recoveries and reproducibility of CAP in the tested samples were highly satisfactory

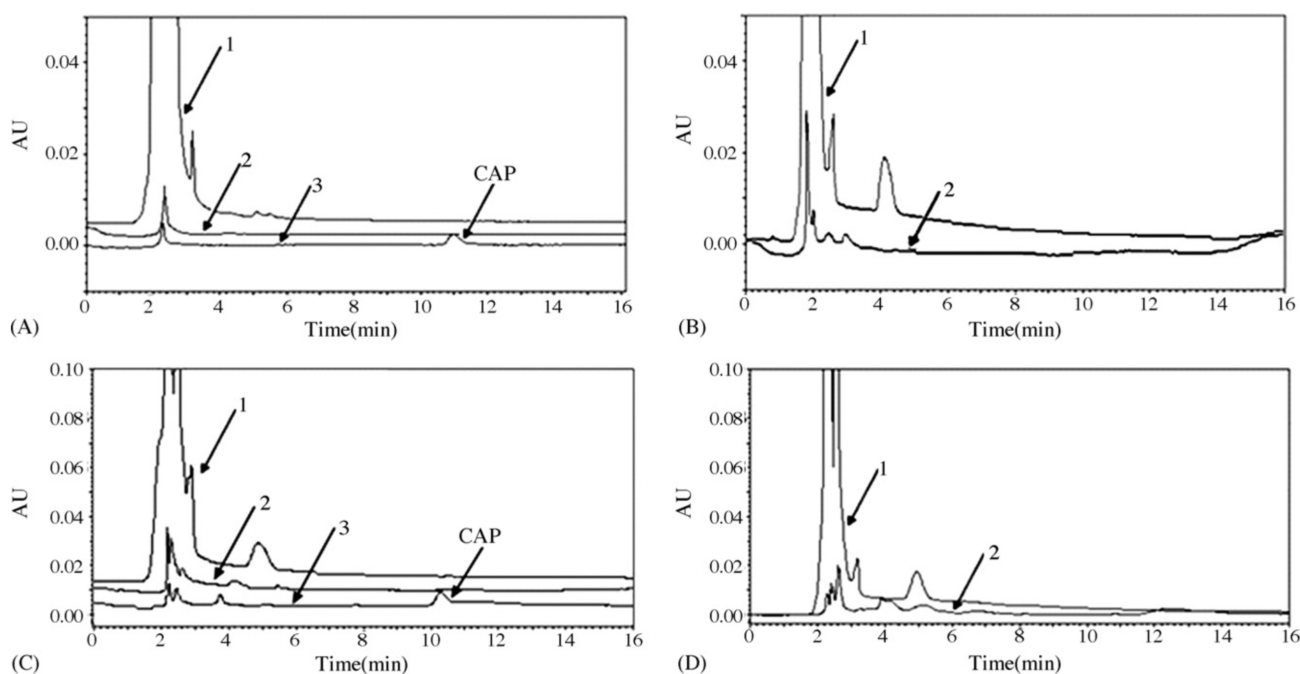

Fig. 8. The chromatograms obtained at 268 nm from the analysis of an extract of the spiked sample at 5 μg kg^{-1} without and with MISPE. A: milk; B: shrimp; 1: spiked sample without MISPE; 2: blank sample with MISPE; 3: spiked sample with MISPE. C: milk; D: shrimp; 1: spiked sample without NISPE; 2: spiked sample with NISPE.

Table 3
Analysis of CAP in spiked milk and shrimp samples on MISPE cartridges

Test Sample	Spiked level ($\mu g\,kg^{-1}$)	Recovery (%)	RSD (%)[a]
	100	90.2	3.9
milk	10	99.9	7.4
	5	92.7	2.7
	100	86.0	4.4
shrimp	10	89.0	7.5
	5	84.9	3.4

[a] $n = 3$.

(Table 3). The recoveries of CAP for milk and shrimp were both above 90% and 80%, respectively. In addition, matrix interferences seemed not to affect the affinity and specificity of the MIPMs.

Most significantly, the chromatograms of CAP obtained before and after MISPE treatment of spiked samples, obviously showed that the matrix interferences could be removed during the washing step, achieving a novel purification and enrichment of CAP from the spiked samples.

4. Conclusions

CAP-MIPMs were prepared by a simple production process of aqueous suspension polymerization and applied to the MISPE, which is highly desirable for large volume production in manufacturing. The MISPE can be successfully applied to clean-up and preconcentration of CAP with simple procedures and high efficiency, and simultaneously greatly increase sensitivity of conventional chromatographic methods. There, we propose that these MIPMs may be used in enrichment, purification and determination of trace CAP from complex food samples.

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References

[1] K. Vivekanandan, M.G. Swamy, S. Prasad, B. Mukherjee, Rapid. Commun. Mass. Spectrom. 19 (2005) 3025.
[2] P. Mottier, V. Parisod, E. Gremaud, P.A. Guy, R.H. Stadler, J. Chromatogr. A 994 (2003) 75.
[3] Th. Gude, A. Preiss, K. Rubsch, J. Chromatogr. B 673 (1995) 197.
[4] A. Gantverg, I. Shishani, M. Hoffman, Anal. Chim. Acta 483 (2003) 125.
[5] C.J. Singer, S.E. Katz, J. Assoc. Off. Anal. Chem. 68 (1985) 1037.
[6] S. Yamato, H. Sugihara, K. Shimada, Chem. Pharm. Bull (Tokyo) 38 (1990) 2290.
[7] H.Y. Shen, H.L. Jiang, Anal. Chim. Acta 535 (2005) 33.
[8] A.F. Forti, G. Campana, A. Simonella, M. Multari, G. Scortichini, Anal. Chim. Acta 529 (2005) 257.
[9] L. Santos, J. Barbosa, M. Concei, C. Castilho, F. Ramos, C.A.F. Ribeiro, M.I.N. Silveira, Anal. Chim. Acta 529 (2005) 249.
[10] I.S. Park, D.K. Kim, N. Adanyi, M. Varadi, N. Kim, Biosens. Bioelectron. 19 (2004) 667.
[11] P.A. Guy, D. Royer, P. Mottier, E. Gremaud, A. Perisset, R.H. Stadler, J. Chromatogr. A 1054 (2004) 365.
[12] Q.Z. Zhu, P. Degelmann, R. Niessner, D. Knopp, Environ. Sci. Technol. 36 (2002) 5411.
[13] X.L. Zhu, J. Yang, Q.D. Su, J.B. Cai, Y. Gao, J. Chromatogr. A 1092 (2005) 161.
[14] R.B. Richard, N.E. Thompson, Curr. Opin. Biotechnol. 13 (2002) 304.
[15] A. Pizzariello, M. Stred'ansky, S. Stred'anska, S. Miertus, Sens. Actuators B 76 (2001) 286.
[16] A. Blomgren, C. Berggren, A. Holmberg, F. Larsson, B. Sellergren, K. Ensing, J. Chromatogr. A 975 (2002) 157.
[17] C. Alexander, H.S. Andersson, L.I. Andersson, R.J. Ansell, N. Kirsch, I.A. Nicholls, J. Mahony, M.J. Whitcombe, J. Mol. Recognit. 19 (2006) 106.
[18] G. Theodoridis, P. Manesiotis, J. Chromatogr. A 948 (2002) 163.
[19] M.C. Hennion, J. Chromatogr. A 856 (1999) 3.
[20] E. Caro, R.M. Marcé, F. Borrull, R.A.G. Cormack, D.C. Sherrington, Trends. Anal. Chem. 25 (2006) 143.
[21] E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, J. Chromatogr. B 813 (2004) 137.
[22] M.L. Mena, L. Agüí, P.M. Ruiz, P.Y. Sedeño, A.J. Reviejo, J.M. Pingarrón, Anal. Bioanal. Chem. 376 (2003) 18.
[23] R. Levi, S.M. Niven, S.A. Piletsky, S.H. Cheong, K. Yano, I. Karube, Anal. Chem. 69 (1997) 2017.
[24] S.L. Moullec, A. Bégos, V. Pichon, B. Bellier, J. Chromatogr. A 1108 (2006) 7.
[25] A. Zurutuza, S. Bayoudh, P.A.G. Cormack, L. Dambies, J. Deere, R. Bischoff, D.C. Sherrington, Anal. Chim. Acta 542 (2005) 14.
[26] A. Molinelli, R. Weiss, B. Mizaikoff, J. Agric. Food. Chem. 50 (2002) 1804.
[27] S.G. Hu, L. Li, X.W. He, J. Chromatogr. A 1062 (2005) 31.
[28] M. Ramos, P. Muñoz, A. Aranda, I. Rodriguez, R. Diaz, J. Blanca, J. Chromatogr. B 791 (2003) 31.
[29] A. Flores, D. Cunliffe, M.J. Whitcombe, E.N. Vulfson, J. Appl. Polym. Sci. 77 (2000) 1841.
[30] R.J. Ansell, K. Mosbach, J. Chromatogr. A 787 (1997) 55.
[31] J.P. Lai, X.W. He, J.F. Chen, Anal. Biol. Chem. 375 (2003) 264.
[32] S.G. Hu, S.W. Wang, X.W. He, Analyst 128 (2003) 1485.
[33] A. Rachkov, N. Minoura, J. Chromatogr. A 889 (2000) 111.
[34] J. Matsui, K. Fujiwara, S. Ugata, T. Takeuchi, J. Chromatogr. A 889 (2000) 25.
[35] A.E. Tyrpenou, G.G. Rigos, F. Athanassopoulou, J. Liq. Chromatogr. Rel. Technol. 25 (2002) 65.