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Yun-Kai Lv*, Yan-Dong He, Xue Xiong, Jin-Zhi Wang, Hai-Yan Wang, Ya-Meng Han

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ARTICLE

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A novel restricted access media - molecularly imprinted magnetic microsphere (RAM-MIMM) was prepared through layer by layer modification. The magnetic nanoparticles was coated with silica gel, modified with amino group, coated by BPA-imprinted sol-gel film and grafted with epoxy silane coupling agent. The RAM-MIMMs were characterized by SEM, TEM, FT-IR and adsorption experiments. The RAM-MIMMs were demonstrated with the average diameters around 300 nm and the coating thickness in the range of 10-15 nm, and exhibited high selectivity (2.54) of the imprinted cavities and hydrophilicity of the external surface with water compatibility and exclusion biomacromolecules. The RAM-MIMMs were used for magnetic dispersion microextraction of BPA from milk samples. The average recoveries were obtained in the range of 85.2%-98.6% with precision 2.2-4.6%. The limits of detection and quantitation of the proposed method were in the range of 4.70–10.51 μg kg^{-1} and 15.65-35.02 μg kg^{-1}, respectively.

Introduction

Bisphenol A (BPA) is an important chemical widely used in water pipes, infant bottles, food packaging, tableware and DVDs. Nevertheless, BPA is consider as an endocrine disruptor, and has been linked to all sorts of health concerns, including heart disease, cancers and developmental problems.[1] Hence, monitoring of BPA has been giving rise to international concern.[2-4] In 2002, a provisional tolerable daily intake for BPA at 0.01 mg/kg body weight per day was established,[3] and the European Commission (EC) established a specific migration limit (SML) for BPA of 0.6 mg/kg of food or food stimulant.[4] Therefore, the determination of BPA has already become the worldwide hot subject. Many methods have been described for determination of BPA in environmental or biological samples including HPLC,[5] GC-MS,[6] LC-MS[7] and LC-ESI-MS/MS.[8] As a complex matrix sample, sample pretreatment is mandatory for the determination of BPA. To date, the most widely used sample preparation methods for the analysis of BPA are solid-phase extraction (SPE),[9] liquid membrane extraction (LME),[10] pressurized liquid extraction (PLE),[11] solid-phase microextraction (SPME),[12] matrix solid-phase dispersion (MSPD)[13] and dispersive liquid-liquid microextraction (DLLME).[14]

So far, SPE is the most widely used sample pretreatment techniques. Adsorbent is a key factor in solid-phase extraction, and the molecular imprinting technology is an effective method for the preparation of selective adsorbent. So molecularly imprinted solid-phase extraction (MISPE) obtained rapid development due to their selective preconcentration in comparision with SPE using the commonly sorbents.[15] In the past few years, magnetic MISPE, also called molecularly imprinted polymer - magnetic dispersion extraction (MIP-MDE),[16] has received increasing attention because of their unique magnetic response. Compared to ordinary MISPE techniques, the MIP-MDE has solved problems like tedious column packing procedure, low flow rate, and poor repeatability. The advantages of the MIP-MDE are obvious, such as its simplicity, short extraction time, low consumption of organic solvents, and easy separation of magnetic particles from complex samples. Therefore, magnetic molecularly imprinted polymers have attracted considerable attention in sample preparation and applied in the separation of BPA in environmental water and food samples.[17-21]

The previously developed MIPs are normally only compatible with organic solvents, and they mostly fail to show specific template binding in aqueous solutions, which significantly limits their practical application in environmental and biological samples.[22] On the other hand, In the process of sample pretreatment, biomacromolecules, such as proteins and lipids, in complex samples are accumulated on the particle surface and blocked the imprinting sites, which are still the bottleneck for the analytical efficiency. In order to obtain better purification and selective enrichment in complex matrix samples, restricted access media-molecularly imprinted polymer (RAM-MIP) has aroused great attention.[23-26]

Restricted access materials (RAMs) are one kind of the most effective sorbents for purification of biomacromolecules in sample pretreatment. RAMs generally possess dual surface configurations.[24] The inner layer is accessible only to small molecules and has the ability for their retention and separation. The outer surface employs both size exclusion and hydrophilic interactions to prevent large biomolecules from accessing the inner surface. The application of RAM to direct analysis of drugs in biological fluids has become well established.[25]

In this study, a facile and highly efficient approach to prepare restricted access media-molecularly imprinted magnetic microsphere (RAM-MIMM) is based on layer-by-layer fabrication on the surface of monodispersed magnetic microsphere for selective enrichment, purification and fast separation of trace BPA from the milk sample. Firstly, silica gel was coated on the surface of magnetic microsphere (Fe_3O_4@SiO_2) and the amino group (-NH_2) was grafted onto the surface of Fe_3O_4@SiO_2. Subsequently, the molecularly imprinted magnetic microsphere (MIMMs) was prepared by surface imprinting sol-gel technique using BPA, phenyltrimethoxysilane (PTMS) and tetraethoxysilane (TEOS). Finally, the hydrophilic surface molecularly imprinted magnetic microsphere was synthesized by grafting epoxy silane coupling agent and ring-opening reaction. The RAM-MIMMs were employed as sorbent for magnetic dispersion microextraction (MDME) of trace BPA from milk samples.

Experimental

Materials and chemicals

Ferric chloride ($FeCl_3 \cdot 6H_2O$), anhydrous sodium acetate and trichloroacetic acid (TCA) were purchased from Tianjing Tianda Chemicals Corporation (Tianjing, China). Glycol, bisphenol A (BPA), 3,3′, 5, 5′-tetrabromobisphenol A (TBBPA) and hydroquinone (HQ) were purchased from Tianjing Huadong Chemical Reagent Co. (Tianjing, China). Tetraethoxysilane (TEOS), 3-aminopropyltriethoxysilane (APTES), 3-(2, 3-epoxypropoxy) propytrimethoxysilane (KH-560) and phenyltrimethoxysilane (PTMS) were purchased from Nanjing Lianye Chemical Co., Ltd. (Shanghai, China). All the chemicals used were of the analytical or the HPLC grade. Ultrapure water is used throughout the experiments. Samples for HPLC analysis were filtered through a 0.22 μm membrane filter.

Instrumentation and HPLC analysis

HPLC analysis was performed using a SHIMADZU LC-20AT pump and a SPD-20A UV detector set at 278 nm. All separations were carried out on a Venusil XBP C18 column (250×4.6 mm, 5 μm) with a flow rate was 1.0 mL min^{-1} at 25 ^oC. The mobile phase was composed of methanol and ultrapure water (70:30, v/v) and the aliquots of 10 μL were injected into the column and the chromatograms were recorded. The concentrations of the BPA before and after the adsorption were recorded by a T6 UV-VIS spectrophotometer (Purkinje General, China).

Preparation of RAM-MIMMs

The procedure for the synthesis of RAM-MIMMs is illustrated in Fig. 1. A representative preparation procedure is as follows.

Synthesis of magnetic Fe_3O_4 nanoparticles. The magnetic Fe_3O_4 nanoparticles were synthesized by hydrothermal method. $FeCl_3 \cdot 6H_2O$ (2.7 g) was dispersed in glycol (80 mL) with vigorous mechanical stirring. When the solution was entirely transparent, anhydrous sodium acetate (7.2 g) was added into the solution with vigorous stirring. The solution was sealed in a Teflon-lined autoclave and placed in an oven to be heated at 200 ^oC for 10 h. The magnetic Fe_3O_4 nanoparticles were separated by a magnet and the supernatant was decanted. The black precipitate was washed with ethanol and ultrapure water three times to remove the solvent effectively, and then the product was dried under vacuum at 60 ^oC for 12 h.

Synthesis of the Fe_3O_4@SiO_2 microspheres. The silica coated magnetic Fe_3O_4 microspheres were prepared with a sol-gel approach. Fe_3O_4 magnetic nanoparticles (1.0 g) were dispersed in a mixture of ethanol (90 mL), ultrapure water (30 mL) and concentrated ammonia (2.5 mL) under ultrasonication for 5 min. Then, a mixture of 1 mL of TEOS and 30 mL of ethanol were added to the above solution drop by drop under mechanical stirring. The reaction was carried out at room temperature under stirring for 12 h. Finally, the synthesized Fe_3O_4@SiO_2 nanoparticles were obtained by the magnetic separation, washed with ultrapure water for 4 times thoroughly, and dried in the vacuum at 60 ^oC for 12 h.

Synthesis of amino-modified Fe_3O_4@SiO_2

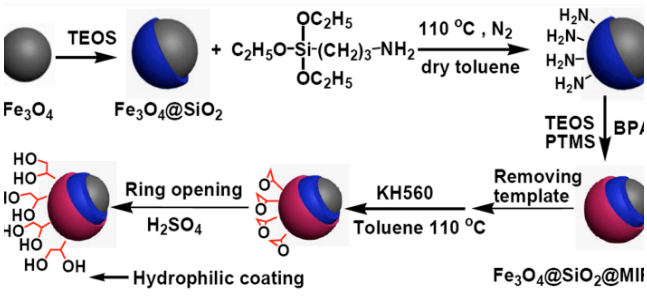

Fig. 1 Preparation protocol of the RAM-MIMMs.

MICROSPHERES. To prepare the amino-modified magnetic microspheres, 1.0 g of Fe_3O_4@SiO_2 microspheres dispersed in 50 mL of anhydrous toluene under ultrasonication for 5 min, and 5 mL of APTES was injected into the above mixture with vigorous stirring under the protection of nitrogen. The reaction was stirred and refluxed for 12 h. The product was collected, washed with ethanol several times, and then dried under vacuum at 40 ^oC for 12 h.

SYNTHESIS OF MIMMS. The molecularly imprinted magnetic microspheres (MIMMs) were prepared via a surface imprinting sol-gel technique. 0.5 g of BPA was added to 50 mL of methanol under stirring until the BPA fully dissolved, and 1.0 g of amino-modified Fe_3O_4@SiO_2 microspheres was added into the mixture. Then the mixture was stirred and refluxed for 30 min. Subsequently, a mixture solution of 2 mL of TEOS, 2 mL of PTMS and 1 mL of 1.0 mol L^{-1} HAc were added to the reaction system. The reaction was allowed to proceed for 24 h at room temperature to obtain particles with a high cross-linking structure. The product was collected, rinsed with ultrapure water for 4 times, and dried in the vacuum at 60 ^oC for 12 h.

To remove BPA, the polymers were eluted with 25 mL of mixture of methanol and 1 mol L^{-1} HCl (1:1, v/v) under stirring for 5 h. Then the product was collected by an external magnetic field, washed with 50 mL of mixture of methanol and 6 mol L^{-1} HCl (1:1, v/v), and neutralized with 0.1 mol L^{-1} KOH and washed by ultrapure water. Finally, the obtained MIMMs were finally dried under vacuum at 60 ^oC for 12 h.

SYNTHESIS OF GLYCOL GROUP-MODIFIED BPA-IMPRINTED MAGNETIC MICROSPHERES. The restricted access media-molecularly imprinted magnetic microspheres (RAM-MIMMs) were prepared by Grafted glycol group on the external surface of the microspheres. 0.5 g of BPA-imprinted magnetic microspheres was dispersed in 50 mL of anhydrous toluene by ultrasonic vibration and 5 mL KH-560 was added. The reaction mixture was stirred vigorously and refluxed for 12 h. Finally, the product was obtained by the magnetic separation, washed with ethanol several times and dried in the vacuum at 40 ^oC for 12 h.

0.5 g of epoxy-modified magnetic microspheres was added to 50 mL of a 0.1 mol L^{-1} sulfuric acid solution. The mixture was then stirred for 12 h at 60 ^oC. At the end of the reaction, the particles were collected, washed with ultrapure water and dried under vacuum overnight at 40 ^oC. The restricted access media - non-imprinted magnetic microspheres (NIPs) were also prepared using an identical procedure without BPA.

Rebinding test and selectivity evaluation

50 mg of the magnetic microspheres (RAM-MIMMs or NIPs) were respectively dispersed into 10 mL of various concentrations (0.05 - 1.0 mg mL^{-1}) of BPA solutions. All of the mixtures were properly sealed and incubated under agitation in a horizontal shaker for 12 h at room temperature. Afterwards, the magnetic microspheres were isolated from the mixture by an external magnetic field and the equilibrium concentration of BPA in the supernatant was determined by HPLC analysis. The equilibrium adsorption capacity (Q) was calculated by using the following equation: $Q=V(Co-Ce)/m$, where V is the volume of solution (mL); m is the quality of the magnetic microspheres; Co and Ce represent the initial and equilibrium concentrations of BPA in solution, respectively.

The selectivity of RAM-MIMMs was investigated with BPA and structurally analogous compounds TBBPA and HQ at the 100 mg L^{-1} level. The same procedure was performed for the NIPs.

The enrichment of the RAM-MIMMs

10 mg and 50 mg of RAM-MIMMs were respectively applied to 5 μg L^{-1} and 100 μg L^{-1} of 20 mL mixture standard solution (BPA, TBBPA and HQ) that had been adjusted to pH 5.0. The mixture was agitated for 30 min with a mechanic stirrer. Subsequently, the magnetic microspheres were isolated from the mixture by an external magnetic field. The analytes were desorbed from the isolated particles with 1 mL methanol (containing 1.5% acetic acid, V/V) under sonication for 2 min. Finally, the eluate was evaporated at 50 ^oC, and then the residue was dissolved in 1 mL of mobile phase.

The magnetic dispersion extraction of BPA from milk samples

All milk samples were pretreated before analysis by the following procedure.[21] 20 g of milk sample was added into a conical flask, and 5.0 mL of TCA (2.5%, V/V) was also added to remove protein from the matrix. After being shaken for 1 min and centrifuged at 5000 rpm for 10 min. The supernatant was collected. The precipitate was extracted twice with 2 mL of methanol. The supernatant and eluate were merged, and evaporated to dryness at mild temperature under a stream of nitrogen. Subsequently, the residues were redissolved in methanol, and the solution was adjusted the pH value to 5.0. 10 mg of RAM-MIMMs was put in a test tube and activated in turn by 3.0 mL of water and 3.0 mL of methanol. The RAM-MIMMs were separated with a magnet, and then the above processed sample was added in this test tube. After the mixture was agitated for 30 min with a mechanic stirrer at room temperature, the supernatant was decanted. Subsequently, the RAM-MIMMs were washed with 2.0 mL of water. Finally, BPA was eluted from the RAM-MIMMs with 1.0 mL of methanol with 1.5% acetic acid and then evaporated to dryness at 50 ^oC under nitrogen. The residues were redissolved in 0.5 mL of mobile phase for HPLC analysis. The milk was spiked with BPA at three different levels with 50, 100 and 200 μg kg^{-1}, and each experiment was carried out in triplicate.

Results and discussion

Characterization of RAM-MIMMs

MORPHOLOGICAL CHARACTERISTICS. (1) SEM images of magnetic Fe_3O_4 nanoparticles. The magnetic Fe_3O_4 nanoparticles were synthesized by hydrothermal method as reported by Huang *et al*.[27] It is important that the sorbents should possess super paramagnetic properties to realize rapid

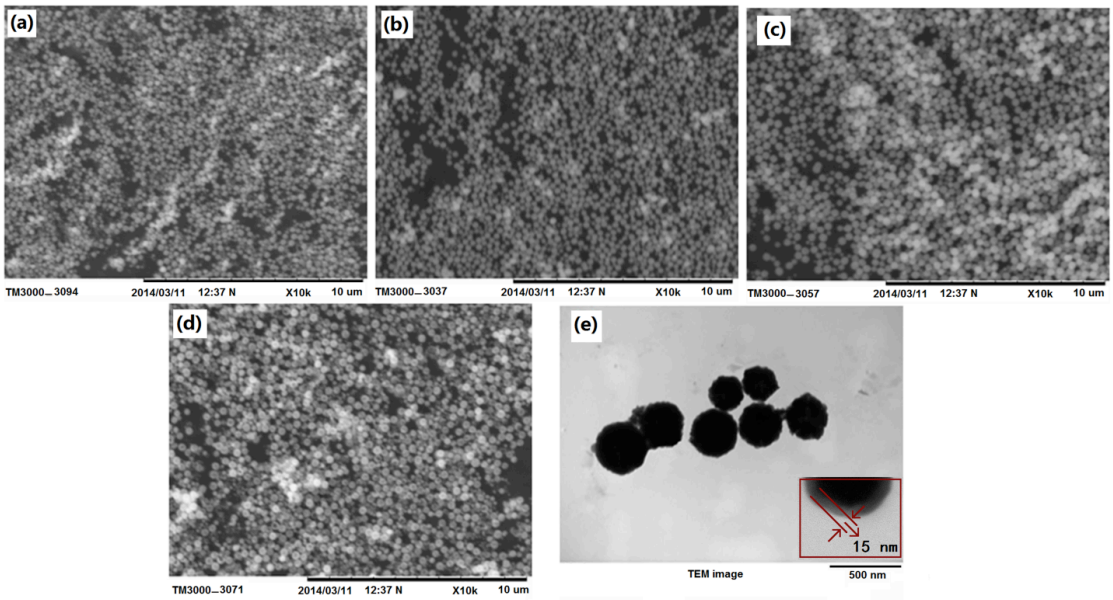

Fig. 2 SEM images of (a) Fe3O4, (b) Fe3O4@SiO2, (c) MIMMs, (d) RAM-MIMMs and (e) TEM image of RAM-MIMMs.

separation in a magnetic field, so the Fe_3O_4 nanopaticles were selected as magnetic core. Fig. 2a showed the SEM image of the naked Fe_3O_4 nanopaticles. It is obvious that these particles remained a uniform size distribution with a mean diameter of about 300 nm.

(2) SEM images of Fe_3O_4@SiO_2. In order to further grow multi-layer core-shell structures and graft modification, it is crucial that the magnetic silica nanoparticles prepared were monodisperse and stable. In the process of the coated silica gel, we made some improvement, comparing with previous research[27], by adding TEOS to the solution dropwise, the magnetic silica nanoparticles showed narrow size distribution and no agglomeration (Fig. 2b). Subsequently, the amino group was grafted successfully onto the surface of the magnetic silica nanoparticles by modification with APTES.

(3) SEM images of molecularly imprinted magnetic microspheres (MIMMs). For the synthesis of BPA-imprinting microspheres, the non-covalent interactions and the sol-gel technique were employed in preparation. In this study, the surface hydrophobicity of the BPA-imprinted magnetic microspheres (Fig. 2c) was synthesized through sol-gel method with TEOS and PTMS as cross-linking agent and HAc as a catalyst.

(4) SEM and TEM images of RAM-MIMMs. The hydrophilicity of the external surface was modified by silicification with KH-560. The results showed that a satisfactory RAM-MIMM was obtained. From the SEM and TEM image of RAM-MIMMs (Fig. 2d and 2e), it was obvious that RAM-MIMMs were regular spheres with a mean diameter of 300 nm and the surface of synthesized RAM-MIMMs was rough. Fig. 2e shows the distinct core-shell structure of the hydrophilic imprinted film-coated silica nanoparticles with 15 nm thin layer. This image suggests that core-shell nanoparticles with more regular morphological features were prepared through a step-by-step grafting procedure. This image also reveals that the grafting reaction process did not significantly result in the agglomeration and change in size of particles, which can be attributed to the fact that the reaction occurred only on the particle surface.

Characteristic of the FT-IR spectra. To ascertain the presence of the RAM-MIMMs, FT-IR spectra were obtained from Fe_3O_4, Fe_3O_4@SiO_2, amino-modified Fe_3O_4@SiO_2 nanoparticles, MIMMs and RAM-MIMMs. As shown in Fig. 3a, the observed features around 570.01 cm^{-1} attributed to the Fe_3O_4 characteristic peak. Fig. 3b showed the bands around 1088.90 cm^{-1} resulted from Si-O-Si and Si-O-H stretching vibrations. In Fig. 3c, the adsorption observed around 1556.91 cm^{-1} and 2937.77 cm^{-1} indicated the existence of amino-groups in the modified silica nanoparticles. For MIMMs, as shown in Fig. 3d, the adsorption observed around 1442.45, 1518.76 and 1606.51 cm^{-1} attributed to the stretching vibration peaks of cyclobenzene skeleton. In addition, compared with MIMMs, the aliphatic hydroxyl adsorption peak at 3432.49 cm^{-1} of the RAM-MIMMs was obviously increased in Fig. 3e.

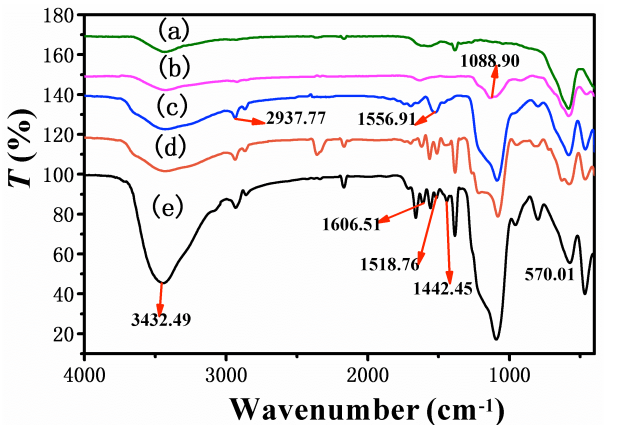

Fig. 3 FI-IR spectra of (a) Fe_3O_4, (b) Fe_3O_4@SiO_2, (c) amino-modified Fe_3O_4@SiO_2, (d) MIMMs and (e) RAM-MIMMs.

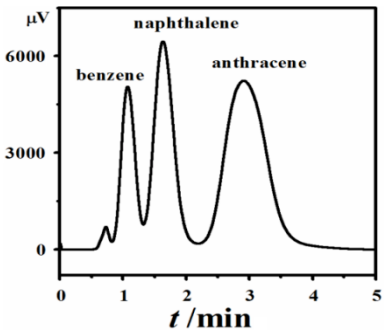

Fig. 4 Chromatographic separation profiles of benzene, naphthalene and anthracene on the RAM-MIMM columns. HPLC column size was 50 mm × 4.6 mm. The mobile phase was water/acetonitrile (60/40, v/v). The flow rate was 0.5 mL·min−1. The analytes were detected at 254 nm.

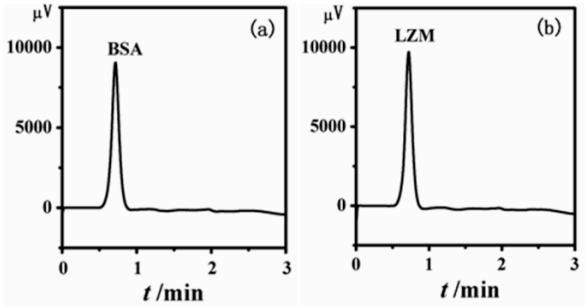

Fig. 5 Chromatographic separation profiles of BSA and LZM on the RAM-MIMM columns. HPLC column size was 50 mm × 4.6 mm. The mobile phase was 0.05 M Na2HPO4 / acetonitrile (60/40, v/v). The flow rate was 1.0 mL·min−1. The analytes were detected at 280 nm.

HYDROPHOBIC AND HYDROPHILIC PROPERTIES. To verify the reverse characteristic of microspheres, the hydrophobicity changes of the microspheres before and after grafting of the epoxy group have been investigated. The results indicated that superficial static state contact angle of the MIMMs is about 116.58 degree; however the contact angle of the RAM-MIMMs is about 29.90 degree. It verifies that the hydrophilicity enhances using above epoxy modified method. To investigate further, stainless steel columns (55×4.6 mm) were packed with the microspheres using a slurry packing procedure. Under the pressure of 2.1 MPa, this column is no leakage by detecting its effluxions with ultraviolet absorption. The hydrophobicity tests of the RAM-MIMMs, expressed by the relative retention factors of various types of apolar solutes to anthracene in water/acetonitrile (60:40, v/v),[26] are shown in Fig. 4. As it could be observed in Fig. 4, order of the peak in HPLC curve confirmed reversed-phase chromatographic property of the RAM-MIMMs, as expected by their identical inner phase structure. The hydrophobic property in inner layer which have a large number of structures of benzene is accessible only to small molecules due to a hydrophobic effect on BPA, and is important for the adsorption of BPA.

The ability of exclusion protein from the above prepared RAM-MIMM column was evaluated by injecting 40 mg mL^{-1} solutions of bovine serum albumin (BSA) and lysozyme (LZM) respectively. The mobile phase system was acetonitrile: 0.05 M disodium hydrogen phosphate (40:60, v/v). From Fig. 5, despite the difference in molecule weight of two proteins, their chromatographic retention time was roughly the same. Thus, this indicates that macromolecules are excluded and interact only with the outer surface of the RAM-MIMMs, which minimizes the adsorption of matrix proteins. Meanwhile, having injecting the mixture of BPA and BSA, we found that this led to be partially overlapping chromatographic peaks between protein and BPA on account of low column efficiency of the stainless steel column packed.

Optimization of magnetic dispersion extraction conditions

EFFECT OF SAMPLE PH. The effect of sample pH on the adsorption of BPA was studied over the pH range 2.0-7.0 using 20 mg of magnetic microspheres in 10 mL of the solution containing 0.05 mg mL^{-1} of BPA. The results were shown in Fig. 6a. The best results were achieved in the pH 5.0. This is because the primary driving forces for the rebinding process, such as hydrophobic interactions, was strongly related to the sample pH. The state of the BPA in the sample was influenced by the pH of sample. Under mild conditions, most of the BPA was in a molecular state, enhancing their adsorption by the RAM-MIMM sorbent. Hydrogen bonding also contributed to the molecular recognition process, and such bonding was suppressed in strongly acidic or basic solutions due to the ionic state of BPA.[21] Thereby, a sample pH of 5.0 was selected as the optimum enrichment condition for subsequent experiments.

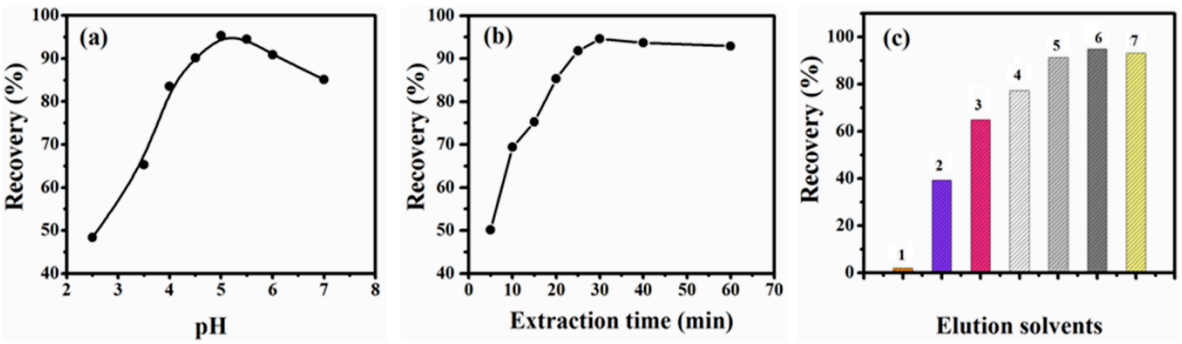

Fig. 6 The effects of (a) sample pH, (b) extraction time and (c) elution condition on the extraction efficiencies were studied. The elution solvents: 1. Water; 2. methanol/Water (1:4, v/v); 3. methanol; 4. 0.5% acetic acid methanol; 5. 1.0% acetic acid methanol; 6. 1.5% acetic acid methanol; 7. 2.0%

Effect of extraction time. The extraction procedure includes three steps: adsorption, washing and elution. The total time required for extraction is a key factor in the efficiency of the assay. As described for the magnetic dispersion extraction procedure, the interaction between the BPA and the sorbent was promoted by agitation. The effect of the adsorption time was studied by varying the stirring time (0-60 min). Fig. 6b indicated that 30 min was sufficient to achieve complete recovery. After the adsorption stage, the washing and elution steps took about 5 min. The whole extraction procedure could be accomplished within 35 min, which was superior to conventional SPE,[9] solid-phase microextraction[12] and stir bar sorptive extraction.[2] However, the extraction time of RAM-MIMMs was sightly less than MIMMs, and it may be due to stereo-hindrance effect.

Effect of elution condition on the recovery. In order to enhance the selectivity of RAM-MIMMs and decrease the matrix interference, the washing conditions were optimized. It is well known that compounds could be retained on the imprinted sorbents due to both specific and non-specific interactions. Thus, a washing solution with moderate elution strength was used to damage the nonspecific interactions and to let the target analyte be retained by specific interactions.[15] As seen from Fig. 6c, when purified water was used for eluent, the recovery reaches its lowest point. So, purified water was selected as the washing solvent. When acetic acid was added to methanol solution at concentrations from 0.5% to 1.5%, the recovery gradually increased. This was probably because the hydrogen bonds between the hydroxyl of BPA and amine groups of the RAM-MIMMs was destroyed by the addition of acetic acid. The recovery barely changed as the proportion of acetic acid was increased further. The different volumes of elution solvent were investigated (from 1.0 to 5.0 mL), and 1.0 mL was found to be the optimum volume. Thus, 1.0 mL of methanol containing 1.5% acetic acid with sonication for 2 min was selected for the elution stage.

Rebinding test and selectivity evaluation

The adsorption capacity was an important factor, because it determined how much imprinted sorbent was required to quantitatively concentrate the analytes from a given solution. As can be seen in Fig. 7, the adsorption capacity increased as initial BPA concentration was increased until the stable values were obtained. The adsorption capacities of RAM-MIMMs and NIPs for BPA were calculated as 40.2 and 24.1 mg g^{-1}, respectively. The adsorption capacity of RAM-MIMMs was about 1.6 times than NIPs. In general, the RAM-MIMMs possesses both specific and nonspecific binding sites, while the NIP only has nonspecific binding sites, which enables the RAM-MIMMs to take up more BPA than the NIPs. The template molecules are first adsorbed mainly to the nonspecific sites rather than the specific sites. After most of the nonspecific sites have been occupied, the specific sites began to get occupied. This is why the adsorption capacities of the RAM-MIMMs and NIP are similar at low concentrations. This obtained results are consistent with previous reported

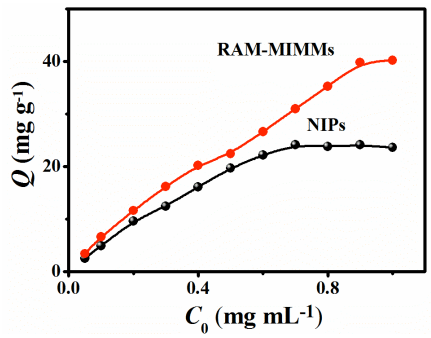

Fig. 7 Binding isotherm of the binding of BPA onto RAM-MIMMs and NIPs.

MIMMs.[21] In our experiments, it was found that adsorption capacity of MIMMs slightly higher than the RAM-MIMMs, which may be because the grafting hydrophilic group affected the template molecules into the imprinting sites.

The selectivity of RAM-MIMMs was investigated by the dynamic competitive adsorption experiments according to the previous extraction conditions for BPA and its structural analogues (TBBPA and HQ). Distribution coefficient (K_D), selectivity coefficient (k) and relative selectivity coefficient (k') were obtained according to the calculation formulas[28] and the results were listed in Table 1. $K_D = (C_0 - C_f)\ V/mC_f$, where C_0 and C_f (mg mL^{-1}) represented the initial and final concentration. The selectivity coefficient of the sorbent suggested the otherness of two substances adsorbed by one sorbent, $k = K_{D(BPA)}/K_{D(TBBPA)}$; the relative selectivity coefficient suggested the otherness of two sorbents, $k' = k_{MIP}/k_{NIP}$. As shown in Table 1, BPA, TBBPA and HQ had the similar K_D on the NIPs, but the RAM-MIMMs showed $K_{D(BPA)}$ was about two times greater than similar compounds. The k value of RAM-MIMMs was larger than that of the NIPs, which showed that the RAM-MIMMs had high selectivity for BPA over the analogues. The relative selectivity coefficient was 2.54 and 2.44 for TBBPA and HQ, respectively, which showed the high selectivity of the RAM-MIMMs than the NIPs. The superior rebinding ability proved that the target molecules were not simply adsorbed on the material surface, but selectively trapped in the imprinting cavities through hydrogen bonding and hydrophobic interactions.

Enrichment factor of MDE and MDME

The amount of adsorbent was an important factor which indicated the enrichment ability of RAM-MIMMs for the target analyte at a very low concentration. 50 mg of RAM-MIMMs

Table 1 Competitive adsorption of BPA, TBBPA and HQ by the RAM-MIMMs (A) and NIPs (B).

Analyte	C_0	C_f		K_D / mL g^{-1}		k		k'
		A	B	A	B	A	B	
BPA	100	89.1	92.1	40.6	28.6			
TBBPA	100	94.3	90.2	20.1	36.1	2.02	0.79	2.54
HQ	100	94.8	91.3	18.5	31.8	2.20	0.90	2.44

$K_D = (C_0 - C_f)\ V/mC_f$, where C_0 and C_f (mg mL^{-1}) represented the initial and final concentration; $k = K_{D(BPA)}/K_{D(TBBPA)}$; $k' = k_{MIP}/k_{NIP}$.

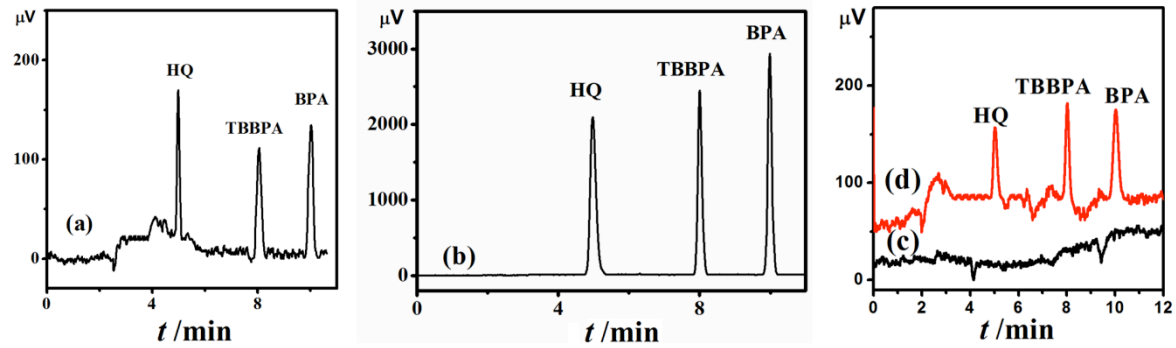

Fig. 8 Chromatograms of (a) the mixture standard solution (100 μg L^{-1}), (b) the eluate of 20 mL of the mixture standard solution through MDE with the 50 mg of RAM-MIMMs, (c) the mixture standard solution (5 μg L^{-1}) and (d) the eluate of 20 mL of the mixture standard solution through MDME with the 10 mg of RAM-MIMMs. The mobile phase was methanol/water solution (70/30, v/v). The flow rate was 1.0 mL·min^{-1}. The analytes were detected at 278 nm.

was applied to magnetic dispersion extraction (MDE) of 20 mL of the mixture standard solution (100 g L^{-1} of BPA, TBBPA and HQ). The extraction procedure has been described in experimental section. The chromatograms of the mixture standard solution and the mixture standard solution through MDE were respectively shown in Fig. 8a and Fig. 8b. The enrichment factors were 16.44, 17.52 and 18.34 for HQ, TBBPA, and BPA, respectively.

To evaluate the magnetic dispersion microextraction (MDME), 10 mg of the RAM-MIMMs was added to 20 mL of 5 μg L^{-1} mixture standard solution (BPA, TBBPA and HQ). Under the optimal conditions, the enrichment factors were obtained and the chromatograms were shown in Fig. 8c and Fig. 8d. When 5 μg L^{-1} of standard mixture solution was used to analysis directly, the peak signals of HQ, TBBPA and BPA were not present in the chromatogram (Fig. 8c). However, the peak signals of HQ, TBBPA and BPA in Fig. 8d were readily visible after enrichment. The enrichment factors were 15.01, 15.34 and 16.27 for HQ, TBBPA, and BPA, respectively. Therefore, the proposed microextraction method not only achieved the ideal enrichment effect, but also saved reagent in easy manipulation.

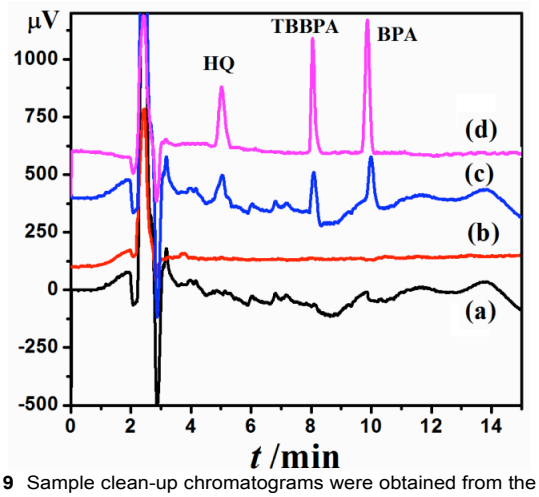

Fig. 9 Sample clean-up chromatograms were obtained from the milk samples. (a) Blank milk (non-spiked); (b) blank milk with a clean-up of the 10 mg of RAM-MIMMs; (c) a spiked milk (BPA 100 μg kg^{-1}); (d) spiked milk (BPA 50 μg kg^{-1}) with a clean-up of 10 mg of RAM-MIMMs. Chromatographic conditions as in Fig. 8.

Sample clean-up

In order to investigate the potential of the RAM-MIMMs for the selective entrapment of target analyte from complex milk samples, satisfactory sample clean-up was achieved by MDME. Fig. 9 revealed the chromatograms obtained for the blank milk sample (Fig. 9a), the blank milk after MDME (Fig. 9b), the spiked milk (Fig. 9c) and the spiked milk after MDME (Fig. 9d). Figure 9a and Figure 9b showed chromatograms of the blank milk by direct injection analysis and the blank milk after MDME with the 10 mg of RAM-MIMMs. The comparison of them indicated that the proposed method had obtained the good clean-up effect. Then, as shown in Fig. 9c, the milk sample spiked with BPA and its analogues (HQ and TBBPA) could be found in HPLC analysis by direct injection, but the quantification was difficult due to the very weak signal and the interference from the sample matrix components. When the spiked sample was treated with RAM-MIMMs and analyzed, the interfering peak of the sample matrix components reduced and BPA was concentrated (Fig. 9d). The results indicated that the method provided satisfactory clean-up of milk sample.

Determination of BPA in milk samples

Under the optimized conditions, the RAM-MIMMs were applied to magnetic dispersion microextraction of BPA from

Table 2 Average recoveries (R), relative standard deviations (RSDs, n = 3), limit of detection (LOD) and limit of quantitation (LOQ) of BPA and its analogues were obtained after MDE of the spiked milk samples (n = 3).

Analyte	Spiked level (μg kg^{-1})	Detected (μg kg^{-1})	R (%)	RSD (%)	LOD[a] (μg kg^{-1})	LOQ[b] (μg kg^{-1})
HQ	50	42.6	85.2	3.1	10.51	35.02
	100	91.9	91.9	4.6		
	200	185.2	92.6	4.1		
TBBPA	50	46.8	93.5	2.8	5.46	18.18
	100	98.6	98.6	4.4		
	200	189.0	94.5	2.2		
BPA	50	44.9	89.7	3.9	4.70	15.65
	100	94.2	94.2	3.7		
	200	192.2	96.1	3.7		

[a] LOD calculated as 3 times the signal-to-noise ratio ; [b] LOQ calculated as 10 times the signal-to-noise ratio.

milk sample. The accuracy of the method was estimated by determining milk sample spiked with BPA, TBBPA and HQ at three different concentration levels (50, 100 and 200 μg kg^{-1}). The results were shown in Table 2. The average recoveries of BPA, TBBPA and HQ from spiked milk sample were in the range of 85.2%-98.6% with relative standard deviations (RSDs) of 2.2-4.6%. The obtained results are consistent with previous reported results, [12, 21] which indicate that the developed method is reliable for determining BPA in milk samples. The limits of determination (LOD, *S/N* = 3) and the limits of quantitation (LOQ, S/N = 10) of the milk samples were 10.51 and 35.02 μg kg^{-1} for HQ, 5,46 and 18.18 μg kg^{-1} for TBBPA, 4.70 and 15.65 μg kg^{-1} for BPA. The LOQ could meet the requirement of specific migration limits determination.[4] The proposed method has a lower LOD and better clean-up effect than the SPME method[12] and the method of magnetic molecularly imprinted polymer,[21] respectively. So the present sample preparation procedure is simple and could be effective for the analysis of environmental, food and biological samples.

Conclusions

The successful preparation of restricted access media-molecularly imprinted magnetic silica microspheres demonstrated the feasibility of controlled layer by layer formation of hydrophilic molecularly imprinted magnetic microspheres with specific recognition and clean-up effect in complex samples. 10 mg of RAM-MIMMs were used as sorbent for magnetic dispersion microextraction of trace BPA from milk samples, which not only provided a convenient, economical, fast and highly efficient extraction, but it also successfully eliminated the sample matrix interferences and prevented protein in biological samples from accumulating on the microsphere surface and blocking the imprinting sites. This methodological study and application in the separation, enrichment and purification of chemical contaminants in food will be an important field.

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Notes and references

College of Chemistry and Environmental Science, Hebei University, Baoding 071002, China; Tel.: +86 312 5079795; E-mail: lvyunkai@hbu.edu.cn (Y.-K. Lv).

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