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ORIGINAL PAPER

A study of the precipitation polymerization of bisphenol A-imprinted polymer microspheres and their application in solid-phase extraction

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Abstract Bisphenol A (BPA) imprinted polymer microspheres were prepared by precipitation polymerization and utilized as sorbents for packing common solid-phase extraction columns. In order to find out the influence of solvent on the size and structure of the microspheres, different ratios of toluene to acetonitrile (0/100, 15/85, 25/75, 30/70, v/v) were used as solvents in the precursor and mainly discussed. Particle size, surface area, and pore volume of BPA-imprinted particles increased with the ratios of toluene in the precursor. The selectivity of the BPA-imprinted particles was evaluated toward structure analogs such as diethylstilbestrol and phenol. Microspheres as prepared were then utilized as sorbents for solid-phase extraction of BPA from urine, with recoveries of 94.63 ± 2.85%.

Keywords Precipitation polymerization · Solvent · Molecular imprinting · Solid-phase extraction · Bisphenol A

Introduction

Molecular imprinting involves preparing synthetic polymers with predetermined molecular recognition properties and is attracting widespread interest [1–4], especially in solid-phase extraction (SPE) [5–9], chromatographic separations [10, 11], biomimetic sensors [2], and catalysis [12].

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The simplest methods for MIP production should be free-radical solution polymerization, wherein a monolith of highly cross-linked forms upon copolymerization of functional monomer(s) with cross-linkers. For SPE applications, grinding and sieving of the monolith is normally necessary. However, MIP particles of irregular size and shape are obtained from grinding processes in low yields, and drawbacks associated with handling and applications of such heterogeneous products are obvious. Synthetic strategies without the need of boring grinding, and sieving like suspension [13, 14], dispersion [5], and seeded polymerization [15, 16] have been evolved. Despite of undoubted value, optimization of reliable experimental protocols can be lengthy, the general applicability is questionable in some cases, and residual emulsifier or stabilizer, which can remain absorbed on the surfaces of the particles, potentially compromises selective rebinding of molecules to the imprinted material.

As an alternative approach, precipitation polymerization has emerged as a method for producing high-quality imprinted spherical particles. However, it has proved difficult to produce larger imprinted monodisperse microspheres that are suitable for direct application in SPE and HPLC by precipitation polymerization [17]. Matching the solubility parameter of the developing polymer network to that of the porogenic solvent(s) is particularly important, especially when simultaneous control of polymer morphology is also desirable [18]. These criteria are usually met for copolymerization of divinylbenzene (DVB) in mixtures of acetonitrile and toluene [17–22]. The group of Stöver has done extensive research on precipitation polymerization of DVB in acetonitrile or acetonitrile mixed with toluene [19–22], the size of the obtained particle could be tuned upward 10 μm by careful optimization [22]. Factors that might affect the size and structure of the obtained particles were studied in detail, like solvent, initiator, and temperature etc., and solvent was mainly discussed. Other groups applied these research results in molecular imprinting field recently [23, 24]. Uniform molecularly imprinted microspheres and nanoparticles were prepared by precipitation polymerization by Yoshimatsu et al. [23] through utilizing different ratios of DVB to ethylene glycol dimethacrylate, varying the composition of the cross-linking monomer allowed the particle size of the MIP beads to be altered in the range of 130 nm to 2.4 μm. Narrow particle size distribution (9.5 ± 0.5 μm) MIP was obtained using an antiepileptic drug, carbamazepine as template molecule, and DVB-80 as crosslinker by Beltran et al. [24]. Through these articles, we found that only particles made by DVB alone as crosslinking agent in a near theta point solvent through precipitation polymerization can be altered to around 10 μm in size, which could be appropriate for common SPE application.

Although there are substantial studies on application of MIP to SPE (MISPE) [5–9], only few of them were by utilizing microspheres prepared by precipitation polymerization [24, 25]. Nearly 10 μm and nano-sized microspheres were used by Beltran et al. [24] and Xu et al. [25], respectively. However, thorough study of the influence of solvent on the size, structure, and imprinting effect of the imprinted microspheres is not found to our knowledge. In this study, bisphenol A (BPA) imprinted polymer microspheres were prepared by precipitation polymerization and the procedure was optimized. The morphology and structure of the microspheres

were also well studied by scanning electron microscopy (SEM) and BET physisorption investigation. Then they were utilized as sorbents for common SPE columns coupled with high performance liquid chromatograph (HPLC) and applied in extracting BPA from acetonitrile and urine. The method has been successfully applied to the analysis of BPA in urine showing the applicability of BPA-imprinted polymer microspheres for analysis of real samples.

Materials and methods

Materials

All the reagents were used as received. Azobisisobutyronitrile (AIBN) and acrylamide (AA) were purchased from Experimental Reagents, Ltd. (Shanghai, China). DVB-80 was purchased from Alfa-Aesar (MA, USA). Phenol was purchased from Lingfeng Reagents Co. Ltd. (Shanghai, China). Toluene was purchased from Qiangshun Reagents Co. Ltd. (Shanghai, China). Diethystilbestrol (DES) and BPA were kindly provided by School of Public Health from Southeast University.

Acetonitrile and methanol for HPLC were purchased from Fisher Scientific (New Jersey, USA).Water for HPLC was obtained from a Heal Force ultrapure water unit (Shanghai, China).

Microsphere preparation

Typically, 0.791 mmol BPA (180.9 mg) was mixed with 4.75 mmol AA (0.3375 g) in a 50 mL round-bottomed flask with 25 mL solvent toluene/acetonitrile = 0/100, 15/85, 25/75, 30/70, v/v). Afterward, 1 mL DVB and 0.0252 g AIBN were added to the above solution. After ultrasonic treatment and oxygen removal by nitrogen purging 5 min, respectively, the solution was sealed and heated on a digital rotary evaporator for 2 h at 50 °C and then 24 h at 60 °C. The rotary evaporator rotated at about 5 rpm. Non-imprinted polymers (NIP) were prepared the same except the addition of BPA. The micro-spheres were collected by ultrafiltration and washed by acetone, toluene, and acetyl alcohol successively. Templates removal was realized by washing the microspheres intensely by methanol/acetic acid (9/1, v/v) for 48 h in a Soxhlet extractor. Then they were dried in a vacuum oven at 60 °C over night. Conditions for preparation of various BPA-imprinted (PPM) and control (PPN) polymer microspheres are listed in Table 1.

Characterization of PPM/PPN

The morphology of the particles was scanned using a ZEISS ULTRA plus system. The particles in ethanol solution were dripped onto the aluminum foil until the solvent evaporated and were observed using an SEM. The number of particles measured in scanning electron micrographs is about 100. The size distribution of the microspheres can be drawn by the number of particles in each region.

Table 1 Conditions for preparation of BPA-imprinted (PPM) and control (PPN) polymer microspheres

Name	AA/mg	DVB/mL	Toluene Con.[a] (vol%)	BPA/mg	Porogen amount/mL	*T*/°C
PPM 0 T %	202.5	0.6	0	109.5	20	60
PPN 0 T %	202.5	0.6	0	0	20	60
PPM 15 T %	202.5	0.6	15	109.5	20	60
PPN 15 T %	202.5	0.6	15	0	20	60
PPM 25 T %	202.5	0.6	25	109.5	20	60
PPN 25 T %	202.5	0.6	25	0	20	60
PPM6 25 T %	303.8	0.6	25	164.3	20	60
PPN6 25 T %	303.8	0.6	25	0	20	60
PPM18 25 T %	101.3	0.6	25	54.8	20	60
PPN18 25 T %	101.3	0.6	25	0	20	60
PPM 30 T %	202.5	0.6	30	109.5	20	60
PPN 30 T %	202.5	0.6	30	0	20	60

[a] Solvent for preparation of polymer grafted microspheres was mixtures of toluene and acetonitrile (0/100, 15/85, 25/75, 30/70, v/v)

Surface area, pore volume, and pore size distribution of the particles were measured with an ASAP2020 M + C automated gas adsorption system using nitrogen at 77 K as an adsorbate.

Functional groups of MIP/NIP were characterized by Fourier transform infrared spectroscopy (FTIR) (Nicolet 6700).

SPE was carried out on a VacMaster (Uppsala, Sweden) vacuum manifold, which was equipped with a vacuum pump and a 10-well platform.

All chromatographic evaluations were performed using a Shimadzu LC-20A (Kyoto, Japan) instrument equipped with a quaternary pump, a manual sampler, a diode array detector, and an LC solution workstation.

Application of the microspheres to solid-phase extraction (MISPE)

Typically, 100 mg dry particles prepared above were packed between two frits in 1 mL SPE columns. Acetonitrile solution containing 0.2 mM phenol, 0.1 mM BPA, and 0.1 mM DES was prepared for loading. After activated by 5 mL methanol and 1 mL acetonitrile, each column was loaded 1 mL solution mentioned above. The columns were washed by 0, 1, or 2 mL acetonitrile before eluted by 0.5 × 2 mL methanol and analyzed by HPLC directly. The flow rate of the solvent/solution was maintained at about 1 mL min^{-1}.

Urine from healthy volunteer was first treated by ultrafiltration. Definite amounts of phenol and DES mixed with BPA were dissolved together to make up 4 nmol mL^{-1} phenol, 2 nmol mL^{-1} BPA, and 2 nmol mL^{-1} DES real sample. The additions of phenol and DES were for comparison. Before samples loading, the columns were activated by 5 mL methanol and 5 mL ultrapure water. Afterward the samples were loaded, and then washed by 5 mL ultrapure water and 1 mL

acetonitrile. Finally, targets retained on the columns were eluted by 0.5 × 2 mL methanol and analyzed by HPLC directly.

C18 reversed phase SPE columns were used the same as above for comparison. The recovery of a target was calculated by Eq. 1:

$$\text{Recovery } (\%) = (\text{Target eluted}/\text{Target loaded}) \times 100. \quad (1)$$

Selectivity (α) of a MIP was calculated by Eq. 2:

$$\alpha = \text{Recovery of a MIP}/\text{Recovery of the corresponding NIP}. \quad (2)$$

HPLC analysis

HPLC was performed to determine the concentrations of the three kinds of compounds. The solution of methanol/water (70/30, v/v) was used as the eluent at a flow rate of 1 mL min^{-1}, and the analytes were detected by UV adsorption at 230 nm. The sample volume was 20 μL, and the temperature of C-18 reversed phase column (Kromasil, 4.6 × 250 mm) was kept at 25 °C.

Results and discussions

Preparation of micro-beads of BPA molecularly imprinted polymer

As is known to all, the size and porosity of microspheres influence the back pressure of SPE columns. In order to get microspheres of appropriate size and porosity, BPA molecularly imprinted polymers were prepared in a near theta solvent [17–22]. It has been found that microspheres crosslinked by DVB in mixtures of acetonitrile and toluene can be tuned to size around 10 μm and big surface area [24] which could be valid for SPE application. In this study, acetonitrile mixed with toluene and DVB were chosen as solvent and crosslinker, respectively, for synthesizing micro-beads of BPA molecularly imprinted polymer. Meanwhile, the addition of toluene would contribute to the formation of porous microspheres [21]. This is important for adsorption and molecular recognition.

Characterization of the particles

Morphology of the obtained particles characterized by SEM

Figure 1 illustrates the overall images of imprinted and NIP microspheres. When the concentration of toluene in the solvent was low (0 or 15 vol%) during preparation, the sizes of imprinted and control polymer microspheres obtained are smaller compared to the ones prepared in solvent with high concentration of toluene (25 or 30 vol%), which is attributed to the solubility parameter of toluene which is near to that of DVB. When the concentration of toluene in the solvent was 30 vol%, the imprinted polymers obtained were irregular particles instead of monodisperse microspheres, while control polymers obtained were regular monodisperse microspheres. When the concentration of toluene in the solvent was higher than 35 vol%,

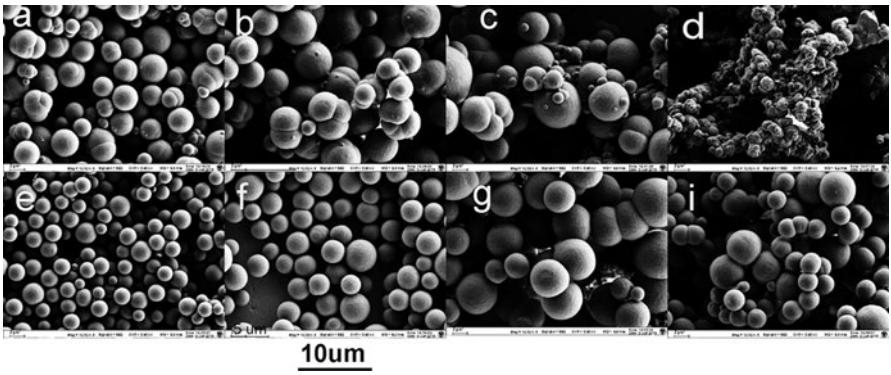

Fig. 1 Representative SEM image of particles prepared in different solvents. **a** PPM 0 T %, **b** PPM 15 T %, **c** PPM 25 T %, **d** PPM 30 T %, **e** PPN 0 T %, **f** PPN 15 T %, **g** PPN 25 T %, and **h** PPN 30 T %

the imprinted polymers and control polymers obtained were coagulum actually (results are not shown).

Size distributions of the obtained particles

Figure 2 demonstrates the size distributions of imprinted and NIP microspheres. In general, the size of the obtained particles increases with the amount of toluene added in the solvent during the preparation process unless coagulum or gel was produced. When the concentration of toluene in the solvent was 25 vol%, the sizes of imprinted polymers and control polymers particles obtained were about 5 μm.

Pore structures of the obtained particles

The influence of solvent on the pore structures of obtained microspheres was also studied. From Fig. 3, surface areas of the imprinted and control particles are very large when prepared in different solvents. The pore sizes of imprinted and control particles are smallest when the concentration of toluene in the solvent was 15 vol%, while the pore volume and surface area of the control particles prepared in this condition are the largest and decrease when the concentration of toluene in the solvent increases. The only difference between precursors of MIP and NIP is the addition of BPA, thus the solubility of solvent is changed toward DVB and AA. The pore size distributions of PPM/PPN prepared in different solvents are demonstrated in detail (Fig. 4), which is in accordance with the facts described in Fig. 3.

Functional groups of the obtained particles

In order to find whether or not AA was well incorporated into the particles during polymerization, these microspheres were characterized by FTIR. The particles prepared in different solvents displayed bands of carbonylic groups of different strength at 1682 cm^{-1}, which is characteristic of functional monomer of AA, as indicated with arrows in Fig. 5. This shows that AA was incorporated into the

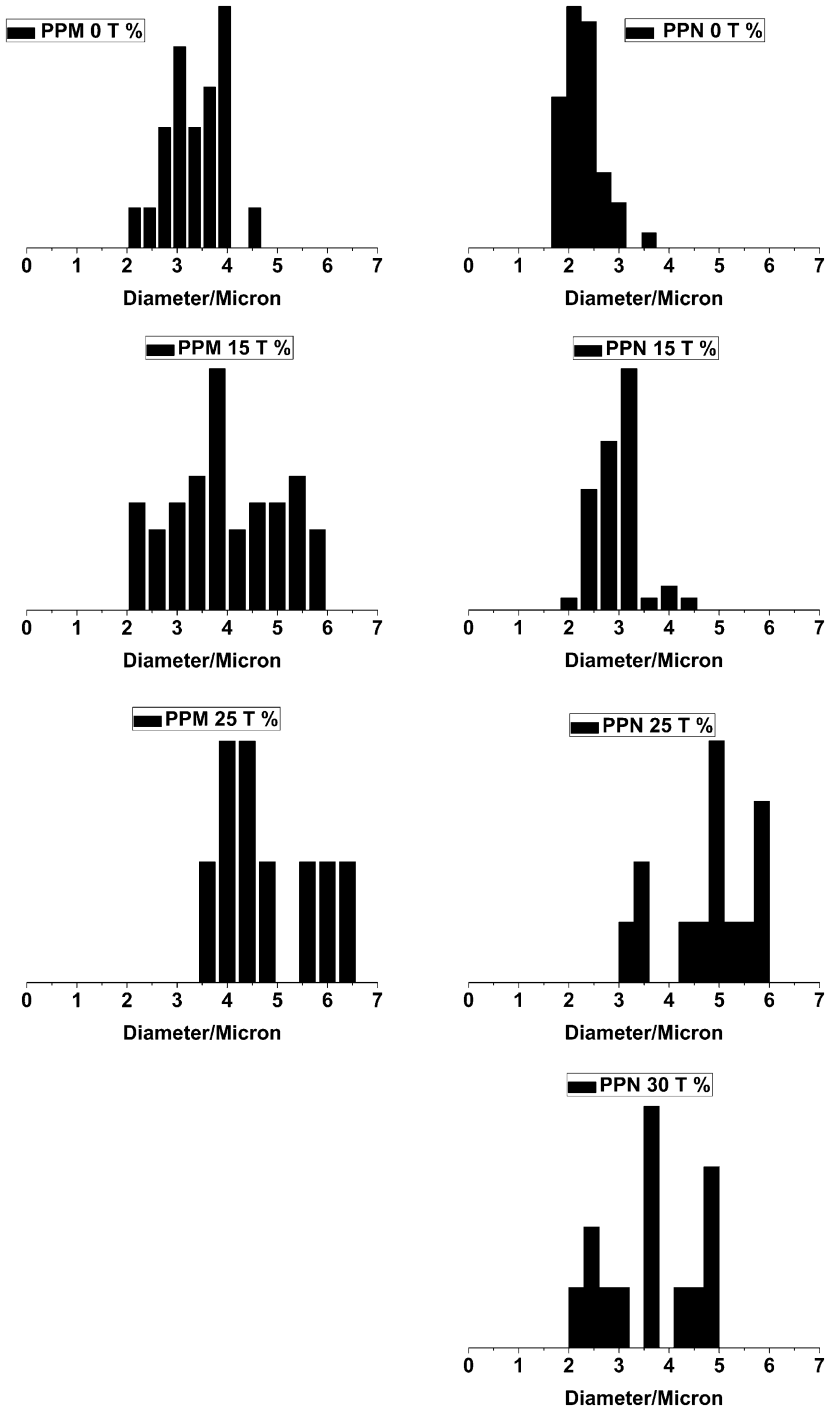

Fig. 2 Particle size distributions of particles prepared in different solvents

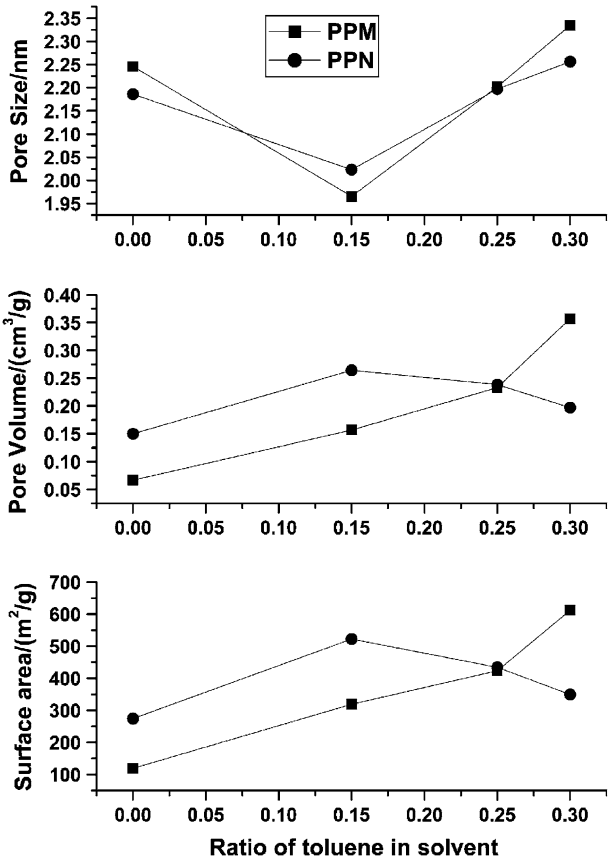

Fig. 3 Mean pore size, pore volume, and surface area of different particles

crosslinked polymers during polymerization process to different extent. Solvent not only influences the pore structures of particles prepared in precipitation polymerization, but also the composites are affected greatly.

MISPE

Before analysis of real samples, the SPE process was optimized. Imprinting effects of the imprinted polymer microspheres were assessed by SPE of mixtures of 0.2 mM phenol, 0.1 mM BPA, and 0.1 mM DES from acetonitrile. DES and phenol were structure analogs of BPA and used for comparison.

Influence of amount of packed microspheres on the recoveries and selectivity (α) of targets

In order to access the influence of amount(s) of packed microspheres on the recoveries of targets, 50, 75, and 100 mg dry PPM 25 T % and control particles

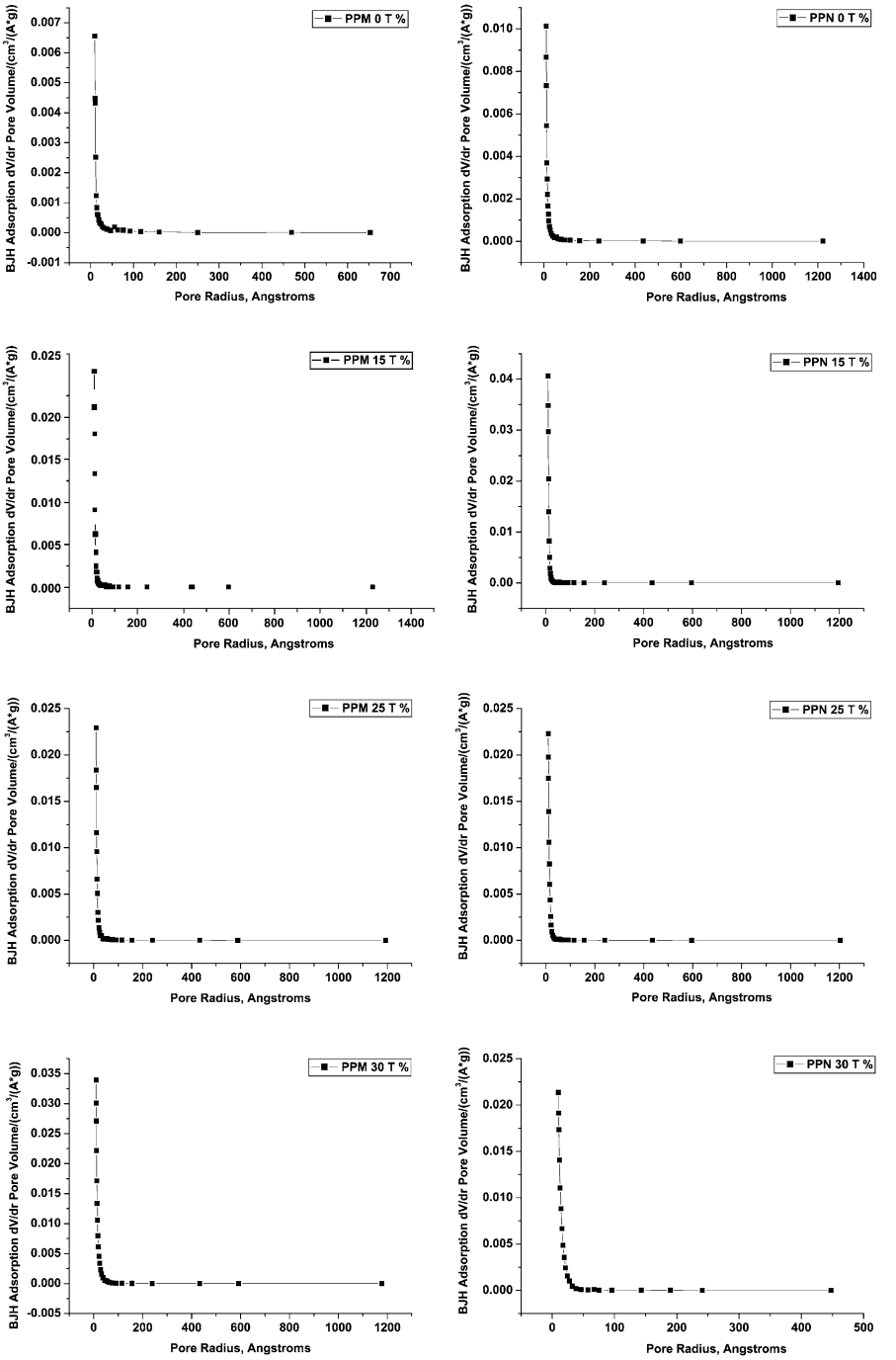

Fig. 4 Pore size distributions of different particles

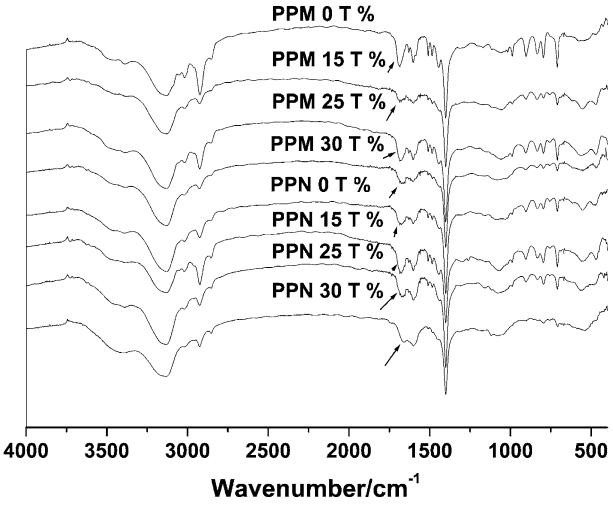

Fig. 5 FT-IR spectra of particles studied in this study

prepared above were packed between two frits in 1 mL SPE columns. Figure 6 clearly demonstrates the effect of different amount of packed microspheres on the recoveries of targets. Obviously, recoveries of BPA increases when amount of packed microspheres of PPM 25 T % and control particles increases, but the extents of increases for them are different. Selectivity of PPM 25 T % will decrease if the amount of packed microspheres of PPM 25 T % and control particles increase. α of PPM 25 T % is 2.81 when amount of packed microspheres is 50 mg, while α is 1.04 when amount of packed microspheres is 100 mg. This is attributed to the fact that the interaction between PPM 25 T % and BPA is more specific and larger than the interaction between PPN 25 T % and BPA. This difference is obvious when amount of packed microspheres is 50 mg. However, this difference is shaded when amount of packed microspheres is 100 mg, the nonspecific interactions were dominated in this case.

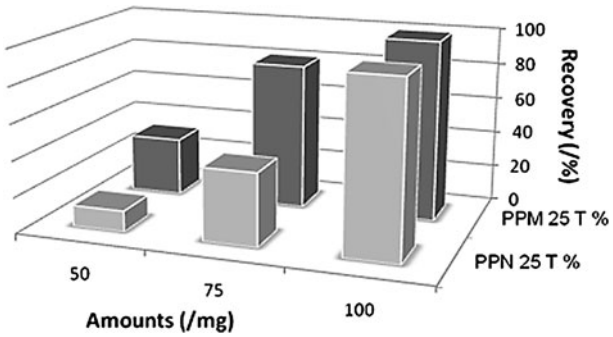

Fig. 6 Influence of amount of packed microspheres on the recoveries of targets without a washing step. Sample: 0.1 mM BPA in acetonitrile, 1 mL

Influence of volume of washing solvent on the recoveries and selectivity (α) of targets

In order to access the influence of different volumes of washing solvent on the recoveries of targets, 0, 1, and 2 mL acetonitrile were utilized as washing solvent for PPM 25 T % and control SPE columns before targets were eluted. Amount of packed microspheres is 100 mg. Figure 7 clearly demonstrates the effect of different volumes of washing solvent on the recoveries of targets. Obviously, recoveries of BPA decreases when volume of washing solvent of PPM 25 T % and control particles increases, but the extents of decreases for them are different. Selectivity of PPM 25 T % increases if the volume of washing solvent of PPM 25 T % and control particles increase. α of PPM 25 T % is 6.75 when volume of washing solvent is 2 mL, while α of PPM 25 T % is 1.04 when volume of washing solvent is 0 mL. This is attributed to the fact that the interaction between PPM 25 T % and BPA is more specific and larger than the interaction between PPN 25 T % and BPA. This difference is obvious when volume of washing solvent is 2 mL, but it is shaded when volume of washing solvent is 0 mL and the nonspecific interactions were dominated in this case.

Influence of ratio of crosslinker to the total monomers in the precursor on the recoveries and selectivity (α) of targets

Ratio of crosslinker to the total monomers is a important factor that influences the recoveries and selectivity (α) of obtained particles for targets. Figure 8 shows that the recovery of BPA on PPM 25 T is 67.37% when the ratio of crosslinker is 50%. However, the recoveries of BPA on PPM 25 T are almost the same (32.38 and 32.94%) when the ratio of crosslinker is 60 and 70%. This indicates that ratio of crosslinker have little influence on the recoveries of BPA on PPM 25 T and control when the ratio is above 60%. When the ratio of crosslinker is low (<50%), functional groups of AA plays a more important role in imprinting and retaining BPA, since the interaction between AA and BPA should be stronger than that

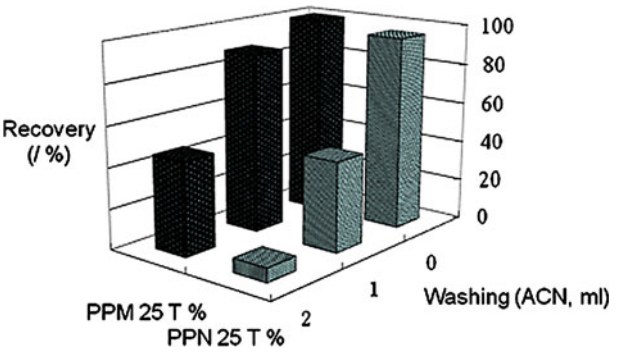

Fig. 7 Influence of volume of washing solvent on the recoveries of targets. Mass of packed microspheres: 100 mg; Sample: 0.1 mM BPA in acetonitrile, 1 mL

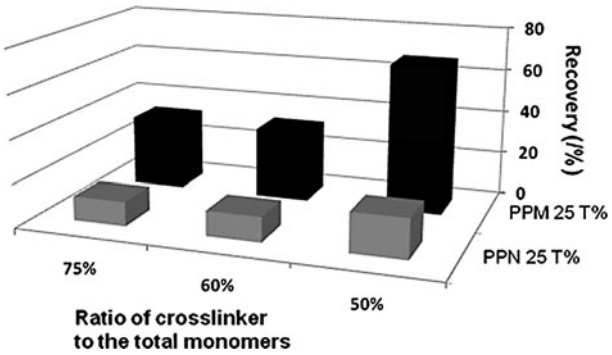

Fig. 8 Influence of ratio of crosslinker to the total monomers in the precursor on the recoveries of targets without a washing step. Mass of packed microspheres: 50 mg; Sample: 0.1 mM BPA in acetonitrile, 1 mL

between DVB and BPA. The change extent of selectivity for PPM 25 T % is not so strong as the change extent of recovery when ratio of crosslinker changes. α values of PPM 25 T % are 3.73 and 2.95, respectively, when ratios of crosslinker are 50 and 75%.

Influence of solvent in the precursor on the recoveries and selectivity (α) of imprinted particles

From Fig. 9, recoveries of BPA increases on imprinted and control particles packed columns when ratio of toluene increases in the precursor. Selectivity of imprinted particles also increases when ratio of toluene increases in the precursor. This is attributed to the fact that pore size, surface area, and pore volumes of imprinted

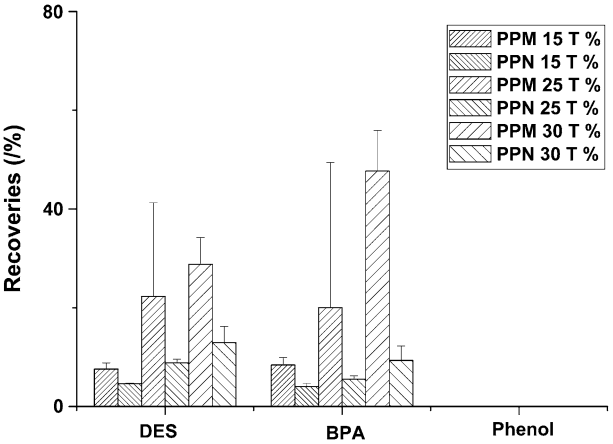

Fig. 9 Extraction recoveries for DES, BPA, and phenol on different particles packed columns using a washing step 2 mL acetonitrile. Mass of packed microspheres: 100 mg; Sample: 0.1 mM BPA, 0.1 mM DES, and 0.2 mM phenol in acetonitrile, 1 mL

particles increase when ratio of toluene in the precursor increases (Fig. 3), this is in favor of target retaining. Although pore size of control particles increase when ratio of toluene in the precursor increase from 15 to 30%, surface area and pore volumes of control particles decrease when ratio of toluene in the precursor increases from 15 to 30%. This leads to the extent of increase of recovery of control particles is smaller than the extent of increase of recovery of imprinted particles. This difference is obvious when amount of packed microspheres is 50 mg. So PPM 30 T % was chosen for real sample analysis.

Recoveries and selectivities (α) of imprinted particles for structure analogs

In Fig. 9, we can also compare recoveries and selectivities (α) of imprinted particles for structure analogs. PPM 30 T % shows a clear imprinting effect toward BPA and α for BPA is 5.10, while phenol was not found in the elution solution. Also a clear imprinting effect toward DES is found and α of PPM 30 T % for DES is 2.22. Since the molecular structure and size of DES are similar to that of BPA, it's very common for BPA-imprinted particles to specifically retain DES. Although phenol is a structure analog of BPA, its molecular size is smaller than BPA and DES, the imprinting sites of BPA-imprinted polymer are not fit for phenol molecules.

Analysis of urine samples

Urine samples were loaded on the imprinted and NIPs packed columns and treated according to the MISPE process optimized above. The chromatograms of urine samples without and after treating with C18 SPE columns, PPM 30 T % SPE columns, and PPN 30 T % SPE columns are demonstrated in Fig. 10. Before washed by 2 mL acetonitrile, all the three solid phases could fully retain BPA, DES, and phenol. However, phenol was fully erased after washed by 2 mL acetonitrile for all the three solid phases. PPM 30 T % SPE column had a selective absorption for BPA and DES, while C18 SPE column retained no BPA or DES and PPN 30 T % SPE column seemed retain DES better than BPA (Table 2). The reasons for this phenomenon are the same as discussed above. The peak shapes of BPA and DES in A, C, and D are different due to the solvent difference, and the solvent of urine sample was mostly water while the solvents for C and D were both methanol. To check the linear range, 1, 3, and 5 mL of urine samples were loaded. Good linearity for BPA was obtained with a determination coefficient (r^2) of 0.99879, while determination coefficient (r^2) of DES was 0.94648. The quantification limit (LOQ) and detection limit (LOD) of the method for BPA obtained was 1.68 and 1.00 nmol mL^{-1} determined by $S/N = 5$ and $S/N = 3$.

Conclusions

In this study, we describe the synthesis and characterization of BPA molecularly imprinted polymer microspheres by precipitation polymerization, and the solvent in preparation process influences the imprinting effect greatly and toluene of 30 vol%

Table 2 Determination of diethylstilbestrol (DES) and bisphenol A (BPA) in urine sample spiked with 4 nmol mL^{-1} phenol, 2 nmol mL^{-1} BPA, and 2 nmol mL^{-1} DES ($n = 2$)

Compound	Spiked (nmol mL^{-1})	Found (nmol mL^{-1})		Recovery (%)	
		PPM 30 T %	PPN 30 T %	PPM 30 T %	PPN 30 T %
BPA	2	1.8944 ± 0.0570	0.6754 ± 0.0944	94.63 ± 2.85	33.77 ± 4.72
DES	2	1.3342 ± 0.0706	1.0742 ± 0.0424	66.71 ± 3.53	53.71 ± 2.12
Phenol	4	nd	nd	nd	nd

nd none detected

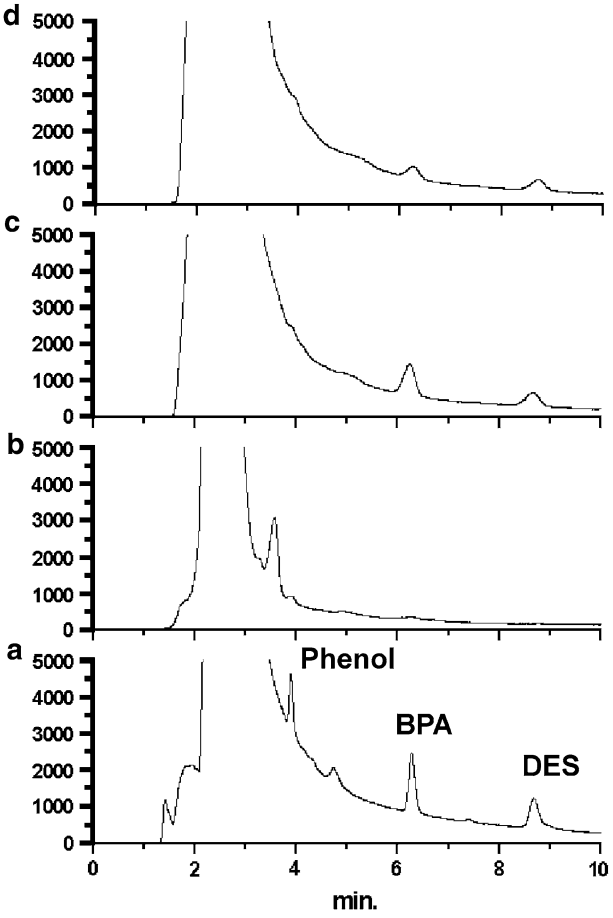

Fig. 10 Chromatography of urine sample spiked with 2 nmol mL^{-1} phenol, 1 nmol mL^{-1} BPA, and 1 nmol mL^{-1} DES without (**a**) and after treating with C18 SPE columns (**b**), PPM 30 T % SPE columns (**c**) and PPN 30 T % SPE columns (**d**). Mass of packed microspheres: 100 mg; Sample: spiked urine, 1 mL

showed a good result. The imprinted microspheres were successfully used as sorbent for selective extraction of BPA from urine samples. To our knowledge, this is the first time to study the solvent effect in preparation of BPA-MIP by precipitation polymerization and applied for SPE of BPA from urine samples.

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