Analytical Methods

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2013, 5, 124

Photonic and magnetic dual responsive molecularly imprinted polymers: preparation, recognition characteristics and properties as a novel sorbent for caffeine in complicated samples†

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We demonstrated the construction and characteristics of photonic and magnetic dual responsive molecularly imprinted polymers (DR-MIPs) prepared by combination of stimuli-responsive polymers and a molecular imprinting technique. The resultant DR-MIPs of Fe_3O_4@MIPs exhibited specific affinity for caffeine and photoisomerization induced reversible uptake and release of caffeine upon alternate UV and visible light irradiation. With irradiation at 365 nm, 62.5% of the receptor-bound caffeine was released from the DR-MIPs back into solution. Subsequent irradiation with visible light caused 93.6% of the released caffeine to be rebound by the DR-MIPs. The novel DR-MIPs were used as a sorbent for the enrichment of caffeine from real water and beverage samples. Recoveries ranging from 89.5–117.6% were achieved. The magnetic property of DR-MIPs provided fast and simple separation while the photonic responsive property offered simple template elution with the assistance of UV-Vis irradiation. The simple, rapid and reliable DR-MIPs based method proved potentially applicable for trace caffeine analysis in complicated samples.

Received 17th August 2012
Accepted 16th November 2012

DOI: 10.1039/c2ay25922b

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1 Introduction

Stimulant or abused-drug identification and measurement are mandatory in various fields including forensic sciences, the pharmaceutical industry, competitive sports and environmental health.[1–4] Amongst stimulants, caffeine is famous for its physiological effects such as gastric acid secretion, diuresis and stimulation of the central nervous and cardiovascular systems. It is considered a risk factor for cardiovascular diseases and may also cause depression and hyperactivity.[5,6] Many methods, such as spectrophotometry,[7] electrochemistry,[8] chromatography[9] and bioassays,[10] have been developed for caffeine determination. During these studies of caffeine, a promising material, molecularly imprinted polymers (MIPs), has been increasingly used. For instance, the selective extraction of caffeine by molecularly imprinted solid phase extraction,[11] a specific caffeine assay based on molecularly imprinted photonic hydrogels,[12] and the voltammetric detection of caffeine using a MIPs-embedded carbon paste electrode[13] have been reported.

MIPs afford the creation of specific recognition sites in synthetic polymers by a process that involves co-polymerization of functional monomers and cross-linkers around template molecules.[14] The molecules are removed from the polymers, rendering complementary binding sites capable of subsequent template molecule recognition.[15] MIPs have aroused extensive interest and been widely applied in many fields, such as extraction and separation[16–19] and chemo-/bio-sensors,[15,20] owing to their desirable selectivity, physical robustness, thermal stability, as well as low cost and easy preparation.

Another promising material, stimuli-responsive polymers, has also received widespread interest. These polymers are able to respond to specific external stimuli with considerable changes such as molecular chain structure, solubility, and so on.[21] Many stimuli signals are available including pH, temperature, ionic strength, magnetism and light.[21–24] Recently, a new strategy that combines the concept of molecular imprinting with stimuli-responsive polymers has attracted great interest, such as pH,[25] temperature,[26] photonic[27] and magnetic[28] characteristic intelligent and functionalized MIPs.

Light is considered an ideal manipulation tool due to its superior clean, precise and remote controllable properties. Thereby, the ultraviolet visible (UV-Vis) photoinduced *trans–cis* isomerization of azobenzene and its derivatives has become a

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/c2ay25922b

research hotspot. A number of azobenzene-based functional monomers have been synthesized, including 4-[(4-methacryloyloxy)phenylazo]benzoic acid,[29] 4-[(4-methacryloyloxy) phenylazo]benzenesulfonic acid,[30] fluorine-substituted azobenzene chromophore (4-methacryloyloxy)nonafluoro azobenzene,[31] acetonitrile-soluble azo functional monomer with a pyridine group 4-[(4-methacryloyloxy)phenylazo]pyridine,[32] 5-(3,5-dioctyloxyphenyl)-10,15,20-tri-4-carboxyphenylporphyrin,[33] and so on. Consequently, different morphological photoresponsive MIPs have been attained, such as bulk monoliths,[29] bulk hydrogels[30] and microspheres.[32] Excitingly, photoresponsive MIPs microspheres simultaneously with thermo-responsive characteristics,[34] or simultaneously with thermo- and pH-responsive features,[35] have been reported lately, demonstrating ideal template binding properties in aqueous media. However, to the best of our knowledge, no photonic and magnetic dual responsive MIPs have been reported.

In this work, we describe for the first time the successful preparation of photonic and magnetic dual responsive MIPs (DR-MIPs) by suspension polymerization and their application to the extraction of caffeine from real samples. The purpose of introducing the magnetic property is that magnetic particles enable simple, rapid and efficient separation of analytes from matrices. Herein, the prepared DR-MIPs retained the photoisomerization properties of azobenzene chromophore and were responsive to an external magnetic field. Molecular imprinting effects and photoregulated uptake and release of trace caffeine were systematically investigated. The achieved DR-MIPs were successfully applied to the extraction of caffeine in water and beverage samples, indicating great potential for the analysis/removal of the stimulant in complicated samples.

2 Experimental

2.1 Reagents

$FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, ammonia and oleic acid for preparation of magnetic nanoparticles were purchased from Shanghai Chemical Reagents Company (Shanghai, China). *para*-Aminobenzoic acid, sodium nitrite, phenol, methacrylic acid anhydride, 4-(dimethylamino)pyridine and triethylamine for synthesis of photoresponsive functional monomer were purchased from Tianjin Reagent Plant (Tianjin, China) or J&K Technology Limited (Beijing, China). Azobisisobutyronitrile (AIBN), trimethylolpropane trimethacrylate (TRIM), caffeine, styrene, polyvinylpyrrolidone (PVP) for MIPs preparation were purchased from Sigma-Aldrich (Shanghai, China). Tetrahydrofuran (THF, Tianjin Jiangtian Chemicals, Tianjing, China) was refluxed over sodium and then distilled. High performance liquid chromatography (HPLC) grade methanol and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). HPLC water was doubly purified deionized water (18.2 MΩ cm specific resistance) obtained with a Pall Cascada laboratory water system (USA). All other reagents such as dimethyl sulfoxide (DMSO) and ethanol were supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China) and used without a further purification step.

The morphological evaluation was performed by scanning electron microscopy (SEM, Hitachi S-4800 FE-SEM, operating at 5 kV). UV-Vis spectra were recorded using a Thermo Scientific NanoDrop 2000/2000c spectrophotometer (Thermo, USA). Nuclear magnetic resonance (NMR) spectra were measured with a Bruker AVIII-500 spectrometer (Germany). For the photoregulated uptake and release studies, a spectrofluorometer (WFH-203B, China) was employed. The amounts of analytes were determined by HPLC-UV (Skyray Instrument Inc., China). A C_{18} column of 250 mm × 4.6 mm i.d. (Arcus EP-C_{18}, 5 μm, Waters, USA) was used as the analytical column. HPLC-UV conditions employed for caffeine were as follows: mobile phase, methanol/water (40 : 60, v/v); flow rate, 1.0 mL min^{-1}; room temperature; UV detection, 275 nm; injection volume, 10 μL.

2.2 Synthesis of photoswitchable functional monomer of 4-[(4-methacryloyloxy)phenylazo]benzoic acid

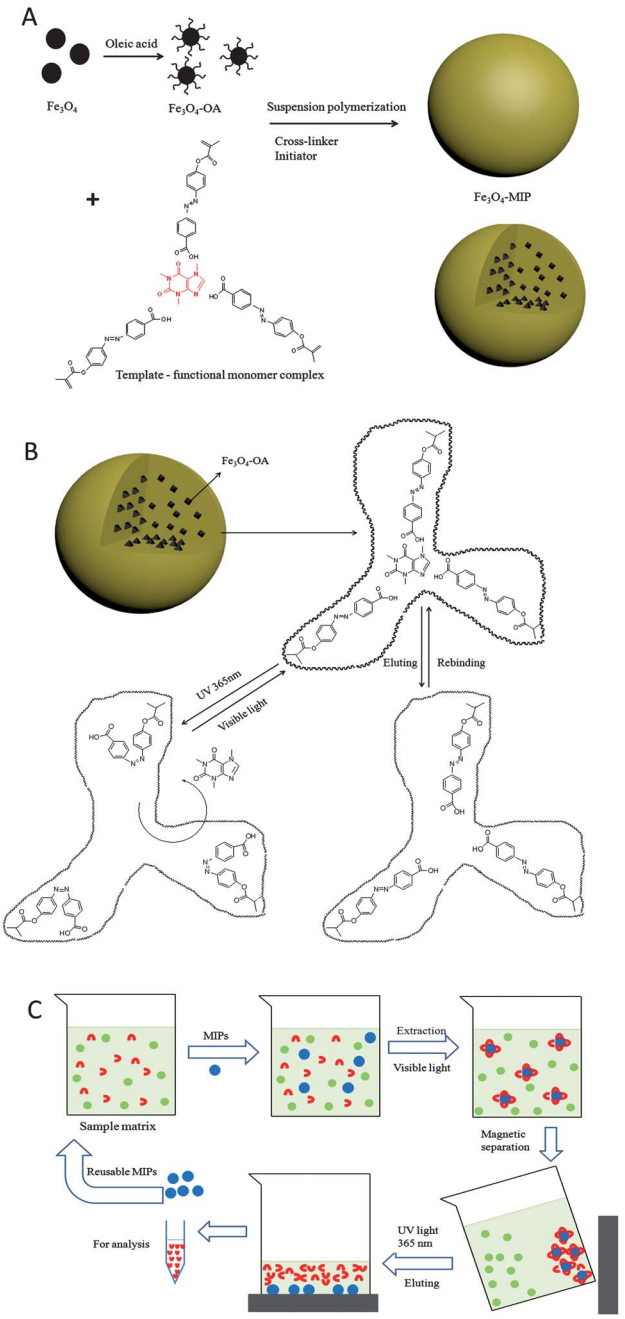
In this work, 4-[(4-methacryloyloxy)phenylazo]benzoic acid (MPABA) was chosen as the photoswitchable functional monomer. MPABA was synthesized according to the reported method[29] with minor modification. The preparation and structure confirmation are shown in Scheme S1 and Fig. S1 (ESI†).

2.3 Synthesis of hydrophobic magnetic Fe_3O_4 nanoparticles

Fe_3O_4 nanoparticles capped with oleic acid were synthesized by a chemical coprecipitation method following the process described.[36] Typically, 4.0 g of $FeCl_3 \cdot 6H_2O$ and 1.80 g of $FeCl_2 \cdot 4H_2O$ (molar ratio of $FeCl_2$ to $FeCl_3$ is 2 : 1) were dissolved in 75 mL purified water in a 250 mL three necked flask with vigorous stirring at 80 °C under a nitrogen atmosphere. Then ammonia (25 wt%) was added to the solution to regulate the pH to 11. Subsequently, 6 mL oleic acid was added dropwise. After reaction for 1 h, the resultant hydrophobic Fe_3O_4 nanoparticles were washed with water/methanol repeatedly with the aid of an external magnetic field.

2.4 Preparation of photonic and magnetic DR-MIPs

The photonic and magnetic DR-MIPs were prepared by suspension polymerization as reported[37] with necessary modifications. Illustration of the preparation procedure is shown in Scheme 1(A). Prior to polymerization, pre-assembly solutions were prepared by using template (caffeine, 0.2 mmol, 39.0 mg) and photoresponsive functional monomer (MPABA, 1.0 mmol, 0.31 g) dissolved in DMSO–ACN (5 mL/2 mL). MPABA is not soluble in ACN, chloroform or methanol, but is soluble in DMF and DMSO. Thus a mixture of ACN–DMSO was employed as porogen for the MIPs fabrication. Stored in the dark for 12 h, the pre-assembly solutions, oleic acid modified Fe_3O_4 nanoparticles (100 mg dispersed in 2 mL toluene), dispersing solution (styrene, 20 mmol, 2.08 g), crosslinker (TRIM, 6.0 mmol, 2.02 g), and initiator (AIBN, 30 mg) were mixed for preparation of the polymerization precursor. PVP (0.2 g) was dissolved in 100 mL of ethanol/water (9 : 1, v/v) in a three necked round bottomed flask. The mixture was stirred vigorously and purged with nitrogen to displace oxygen while the temperature increased to 70 °C. Then the polymerization precursor was



Scheme 1 Schematic illustrations of (A) preparation process of DR-MIPs of Fe_3O_4@MIPs by suspension polymerization, (B) possible mechanism of photoregulated uptake and release of template molecules from the DR-MIPs, and (C) the whole procedure for extraction of caffeine from real samples using the DR-MIPs.

added into the flask, and the reaction was allowed to proceed at 70 °C for 24 h in the dark. After polymerization, the polymer was separated by an external ferromagnet. The template in the polymer was removed by Soxhlet extraction with methanol/acetic acid (9 : 1, v/v) for 24 h and methanol for 8 h in the dark, respectively. The resultant MIPs were dried to a constant weight under vacuum at 40 °C.

As a control, dual responsive non-imprinted polymers (DR-NIPs) were prepared in exactly the same way, except the template was not used in the polymerization procedure. For comparison, single photonic responsive MIPs (SR-MIPs) were prepared by bulk polymerization as reported.[29] Traditional magnetic MIPs (mag-MIPs) were prepared by suspension polymerization using methacrylic acid (MAA) as the functional monomer as reported.[37]

2.5 Spectroscopic characterization

Spectroscopic characterization of the functional monomer and the subsequent MIPs and control materials was carried out in DMSO. All photoisomerization studies were performed with 1.0 mg of MIPs or NIPs in 3.0 mL of DMSO.

2.6 Binding assay

Binding properties of the MIPs and NIPs were studied in DMSO–ACN in the dark. Typically, 20 mg polymer particles were dispersed in a 5 mL flask containing 2.0 mL DMSO–ACN solutions of various concentrations of caffeine. After shaking for 24 h at room temperature in the dark, the samples were centrifuged and the supernatant solutions were collected, the concentrations of which were determined using HPLC-UV. The binding amount of caffeine was determined by subtracting the residual amount of caffeine in solution from the total amount of caffeine. Meanwhile, the binding kinetics were tested by monitoring the temporal amount of caffeine in the solutions. Selectivity experiments were carried out using theophylline as a structural analogue.

2.7 Photoregulated uptake and release studies

The studies on the photoregulated uptake and release of caffeine or theophylline by the DR-MIPs were performed by alternately switching on and off the UV light irradiation on the mixtures of MIPs and analytes, as shown in Scheme 1(B). A series of samples with DR-MIPs or DR-NIPs (1.0 mg) in DMSO–ACN (3 mL) were prepared. Initially, the samples were incubated at room temperature in the dark for 12 h, and then the amount of analytes bound by the MIPs was determined by HPLC-UV. For the photoregulated release of caffeine, the mixture was irradiated at 365 nm by the excitation beam from the spectrofluorometer. The UV-Vis spectra of the suspension solution were measured every 10–20 min. After 2 h of UV light irradiation at 365 nm, the UV light was switched off and the binding amounts of the analytes were determined. For the photoregulated uptake of caffeine, irradiation with visible light was adopted. Besides this, all procedures were similar to those in the release study. The studies on the photoregulated release and uptake of the template or its analogue by DR-NIPs were carried out in a similar manner. During the measurements, samples were shaken continuously using a shaker thereby assuring they were dispersed uniformly.

2.8 Analysis of water and beverage samples

The obtained DR-MIPs were applied to three kinds of real sample. A tap water sample was collected from our laboratory after flowing for about 10 min, and then filtered with a 0.45 μm

filter membrane prior to use. Cola beverage and tea samples were purchased from a local supermarket, and prepared according to the reported procedure[38] with slight modification. Briefly, 200 μL of cola beverage was diluted 100 times with water and then applied to the extraction procedure. In order to measure caffeine in a tea water sample, 1.0 g dried tea leaves were dissolved in 250 mL of boiling water for 30 min. When the solution was cooled, it was degassed in an ultrasonic bath for 10 min, and then 1 mL of the sample was diluted 100 times with water and the resulting solution was applied to the extraction procedure.

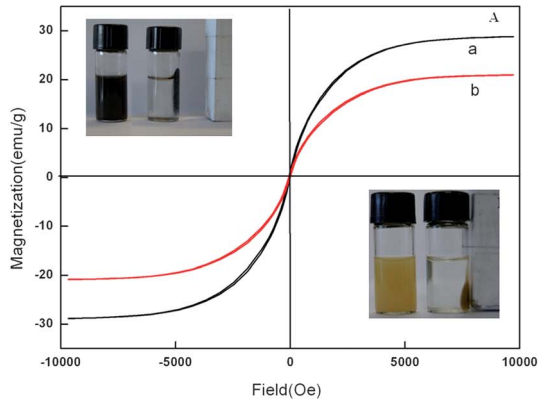
The whole extraction procedure was as follows. The DR-MIPs (100 mg) were added into a beaker, and were conditioned in sequence with 5.0 mL methanol and 3.0 mL water. Then the polymers were separated with an external magnetic field and the supernatant was discarded. After conditioning, 20 mL sample solutions were added. The mixture was sonicated for 30 min. After the extraction was complete, the polymers were separated from the sample matrix and washed with 2.0 mL methanol. Finally, the template caffeine was eluted from the MIPs by 1.0 mL of DMSO–methanol solution containing 0.5% acetic acid twice with the help of UV irradiation for 10 min. The eluate was evaporated under nitrogen at 40 °C, and the residue was redissolved in 1.0 mL DMSO for further analysis. Scheme 1(C) shows the whole extraction procedure.

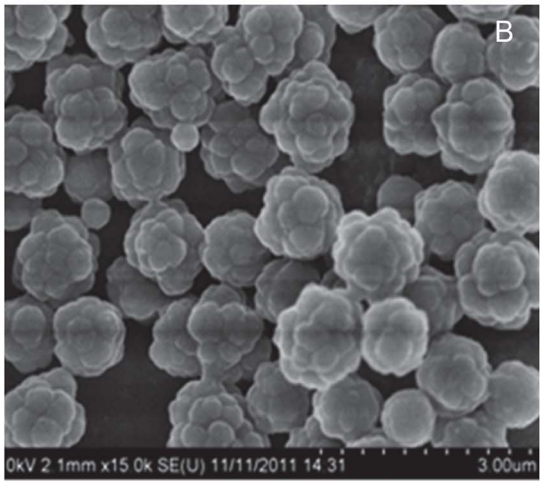
3 Results and discussion

3.1 Preparation and characterization of the DR-MIPs

To date, there have been three main methods developed for preparing magnetic MIPs, illustrated in Scheme S2 (ESI†), as follows: (1) Fe_3O_4@SiO_2 nanoparticles were prepared first, and then Fe_3O_4@SiO_2@MIPs were prepared by a sol–gel process using silyl reagents;[39] (2) Fe_3O_4@SiO_2 nanoparticles were prepared first, and then vinyl double bond[36] or reversible addition–fragmentation chain transfer agents[40] were introduced, and magnetic MIPs were finally prepared by precipitation polymerization dependent on a free radical polymerization mechanism; (3) oleic acid modified Fe_3O_4 was prepared first, and then Fe_3O_4@MIPs were prepared by suspension polymerization dependent on free radical polymerization mechanism.[41,42] Compared to the two former methods usually involving complicated preparation processes and long times, suspension polymerization is simpler for preparing magnetic MIPs. So, herein, suspension polymerization was adopted to prepare DR-MIPs (Scheme 1(A)). Two mature methods have been developed to synthesize magnetic Fe_3O_4 nanoparticles. One is a coprecipitation method,[37] and the other is a solvothermal reduction method.[43] Because magnetic Fe_3O_4 nanoparticles prepared by a solvothermal reduction method are hydrophilic, they are unfavorable for suspension polymerization. Magnetic Fe_3O_4 nanoparticles prepared by a coprecipitation method can be easily transformed to be hydrophobic by modification with oleic acid. Thus, a coprecipitation method was employed to prepare magnetic Fe_3O_4 nanoparticles.

Fig. 1(A) shows the magnetic hysteresis loops analysis of the Fe_3O_4 nanoparticles and DR-MIPs, and the insets illustrate the dispersion and agglomeration process of the Fe_3O_4 nanoparticles and DR-MIPs. The homogeneously dispersed magnetic microspheres could go straight towards the magnet and adhere to the inner side wall of the vials when the external magnetic field was applied, and the turbid solution became clear and transparent. It is seen that there is a similar general shape to the





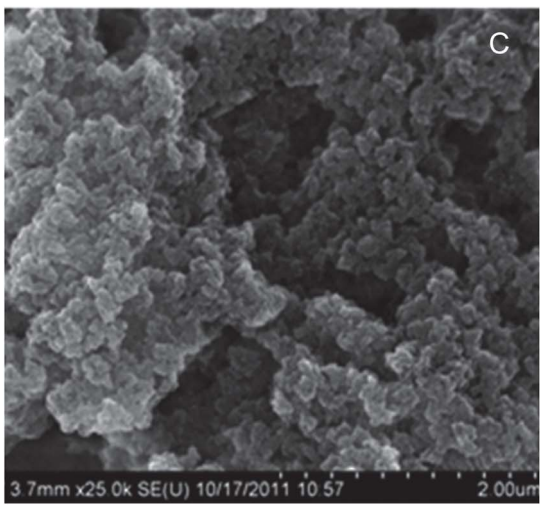


Fig. 1 (A) Magnetic hysteresis loops of Fe_3O_4 nanoparticles (a) and DR-MIPs (b), and the insets show the dispersion and agglomeration process of the Fe_3O_4 nanoparticles (upper) and DR-MIPs (below). (B) SEM image of DR-MIPs prepared by suspension polymerization. (C) SEM image of SR-MIPs prepared by bulk polymerization.

two curves, while the saturation magnetization value of DR-MIPs is little lower than that of the Fe_3O_4 nanoparticles. The results suggested that the prepared Fe_3O_4@MIPs were magnetically responsive. Fig. 1(B) and (C) show the SEM images of DR-MIPs and SR-MIPs, respectively. Unlike the irregular SR-MIPs particles prepared by bulk polymerization, DR-MIPs exhibited regular spherical shapes with diameters around 1 μm, which was favourable for mass transfer.

3.2 Photoisomerization of the DR-MIPs

MPABA is a favourable functional monomer for the fabrication of photoresponsive MIPs, as it possesses a photoresponsive azobenzene chromophore, a benzoic acid functionality for substrate interaction and a polymerizable methacryloyl group. So in this work, MPABA was chosen as the photoresponsive functional monomer. As can be seen from Fig. 2(A), the UV-Vis spectra of MPABA in DMSO exhibited one strong absorption band around 335 nm and another weak one around 439 nm, which are typical for the azo compounds and can be ascribed to the π–π* and *n*–π* electron transition of the N=N bond, respectively. Upon irradiation at 365 nm, the intensity of the π–π* transition decreased rapidly, while that of the *n*–π* transition showed a slight increase, which could be attributed to the *trans–cis* isomerization of the azobenzene chromophore (Fig. 2(A)). Irradiating the thermodynamic *cis*-MPABA by visible light, the intensity of the π–π* transition increased rapidly, as shown in Fig. 2(B). The results indicated that the photoinduced isomerization was reversible. The spectroscopic responses of the resultant MIPs towards irradiation at 365 nm (Fig. 2(C)) and visible light (Fig. 2(D)) are quite similar to those of the MPABA functional monomer, respectively, indicating that the azobenzene chromophore of the monomer still possessed its photo-switching properties after incorporation into the cross-linked polymer network. Affected by the rigid environment of the three dimensional MIPs, the rate constants of the *trans–cis* and *cis–trans* isomerization of azobenzene chromophores were somewhat smaller than that of the MPABA. Therefore, the magnetic responsive Fe_3O_4@MIPs possessed photoresponsive properties. That is, the prepared Fe_3O_4@MIPs were magnetic and photonic dual responsive MIPs.

3.3 Binding properties of the DR-MIPs

Binding properties of the DR-MIPs, including binding kinetics, static adsorption equilibrium and binding selectivity were evaluated by batch adsorption experiments. For this purpose, the effect of dispersion medium on binding capacity was first investigated using the batch procedure. As seen from Fig. S2 (ESI†), when the dispersion medium changed from DMSO to ACN, the binding capacity first increased and then decreased. This can be explained as follows. During the batch adsorption experiment, the obtained MIPs had a lower binding capacity when pure DMSO was used as the dispersion medium because the hydrogen bonding interaction was interrupted by the polar solvent. The binding capacity was also lower when pure ACN was used as the dispersion solvent because the functional monomer MPABA was insoluble in ACN, and therefore the conformation could not change freely. Meanwhile, *trans–cis* isomerization of MPABA was very difficult in ACN solvent. For the DMSO–ACN mixture, DMSO could provide the necessary solubility and ACN could increase the hydrogen bonding

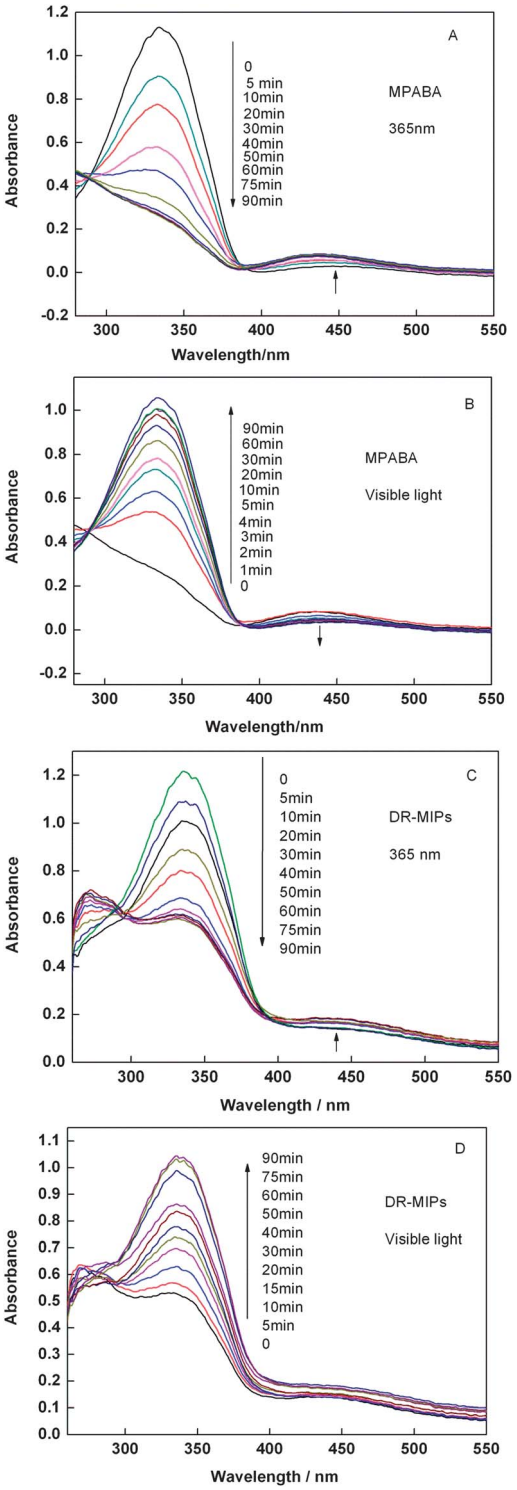


Fig. 2 UV-Vis spectra of the photoisomerization of functional monomer MPABA (50 μM in DMSO): (A) *trans–cis* isomerization upon irradiation at 365 nm and (B) subsequent *cis–trans* isomerization by visible light irradiation; and MPABA based MIPs (1.0 mg in 3.0 mL DMSO): irradiation at (C) 365 nm and (D) visible light.

strength. Therefore, the optimal dispersion medium of DMSO–ACN (1 : 2, v/v) was used in the following experiments.

The results of the binding kinetics experiments were shown in Fig. 3(A). It can be clearly seen that DR-MIPs had a faster template rebinding process than that of the bulk SR-MIPs, which could be attributed to the fact that for those DR-MIPs with a small particle size, the imprinted templates are situated at or in the proximity of the material's surface. For the irregular bulk polymers, most recognition sites are located in the interior area of the bulk materials, resulting in incomplete template removal, small binding capacity and slow mass transfer.

Static adsorption equilibrium experiments were performed to study the template rebinding properties of the DR-MIPs. Fig. 3(B) shows that the DR-MIPs bound more caffeine than the control SR-MIPs and the corresponding DR-NIPs. That the increase of binding capacity of the DR-MIPs compared with bulk MIPs is not significant might be because the DR-MIPs were prepared by suspension polymerization, in which water was used as the dispersion medium. Water can weaken the non-covalent interactions between template molecules and functional monomers, so MIPs prepared by suspension polymerization did not display remarkably improved binding ability.

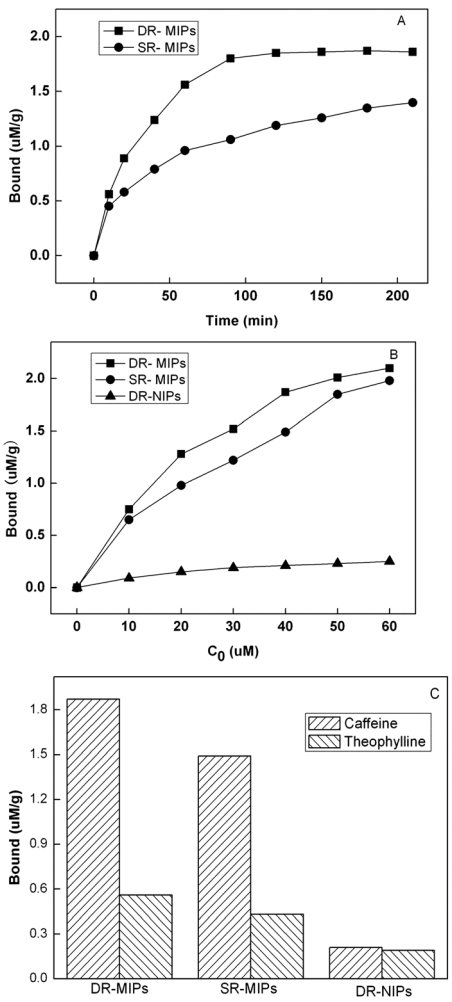


Fig. 3 (A) Kinetic binding curves of DR-MIPs and SR-MIPs. MIPs, 30 mg; dispersion solvent, 3 mL; $C_{caffeine}$, 40 μM; 25 °C in the dark. (B) Static isotherms of DR-MIPs, SR-MIPs and DR-NIPs. MIPs or NIPs, 30 mg; dispersion solvent, 3 mL; 25 °C in the dark for 12 h. (C) Binding selectivity of DR-MIPs, SR-MIPs and DR-NIPs for caffeine and theophylline. MIPs or NIPs, 30 mg; $C_{caffeine}$ or $C_{theophylline}$, 40 μM; dispersion solvent, 3 mL.

Various mathematical models, including Scatchard analysis, Freundlich isotherm (FI) and the Langmuir isotherm[44–47] were employed to further evaluate the molecular binding properties of DR-MIPs.

The Scatchard equation can be expressed as

$$\frac{Q}{C_e} = \frac{Q_{max}}{K_d} - \frac{Q}{K_d}$$

where Q stands for the binding capacity (mol g^{-1}) of MIPs, K_d represents the equilibrium dissociation constant (mol L^{-1}), Q_{max} (mol g^{-1}) is the theoretical maximum adsorption amount of template molecule on polymers, and C_e (mol L^{-1}) is the equilibrium concentration in the solution. According to the slope and intercept of the Scatchard plot, namely the relationship between Q/C_e and Q, K_d and Q_{max} can be calculated.

The Freundlich isotherm describes Q as a power function of C, according to:

$$Q = aC^m$$

where a is a Freundlich parameter related to the binding affinity; m is the heterogeneity index, ranging between 0 and 1. The value of m becomes closer to 1 as heterogeneity decreases, with $m = 1$ being a homogeneous system.

Another simple and frequently used model in adsorption studies is the Langmuir, which can be expressed as:

$$Q = \frac{NKC}{1 + KC}$$

where Q and C are the amount of sorbed template and the concentration of free template in solution, respectively. N represents the binding site density or the monolayer saturation capacity and K is the adsorption constant.

As for the adsorption models, the constants for adsorption of caffeine onto DR-MIPs and SR-MIPs are listed in Table 1. The density and binding strength of the imprinted receptor sites in DR-MIPs were found to be 4.02 μmol g^{-1} for Q_{max} and 0.025 mol L^{-1} for K_d in DMSO. The relatively low binding strength (compared to other MIPs systems with hydrogen-bonding interactions) is likely to be caused by the interruption of the hydrogen-bonding interactions by the solvent media. As for the sorption isotherm models, from Table 1, it can be observed that the

Table 1 Constants for adsorption models

Isotherm model	Constant	DR-MIPs	SR-MIPs
Scatchard	Q_{max} (μmol g^{-1})	4.02	2.30
	K_d (mol L^{-1})	0.025	0.134
Freundlich	R^2	0.976	0.991
	A	0.136	0.252
	M	0.656	0.528
Langmuir	R^2	0.994	0.991
	N (μmol g^{-1})	3.004	2014
	K (L μmol^{-1})	0.026	0.00007

Langmuir isotherm model yielded a better fit than those by the Freundlich model, for correlation coefficients (R^2) above 0.99.

The binding selectivity of MIPs is often determined by comparing the binding amounts of the template with those of its analogues, which affords an indication of the cross-reactivity of the MIPs towards selected molecules. As can be seen from Fig. 3(C), both DR-MIPs and SR-MIPs showed certain binding capacity towards theophylline, suggesting the existence of cross-binding reactivity. Nevertheless, the MIPs adsorbed more caffeine than theophylline, and thus demonstrated the high selectivity of the MIPs towards templates. In the meanwhile, the adsorption capacity of NIPs was very close for the two compounds, since there were no selective recognition sites in NIPs and the adsorption of those compounds was non-selective.

3.4 Photoregulated release and uptake of caffeine

On the basis of the above results, it can be concluded that DR-MIPs showed obvious molecular imprinting effects towards the template, while the functional monomer still retained the photonic responsive characteristics. In the following experiments, photoresponsive template binding properties were further examined.

Reversibility of the photoisomerization of azobenzene chromophores in the DR-MIPs was studied by alternate irradiation at 365 nm and visible light. As shown in Fig. 4(A), the photoisomerization was reversible, and there was no apparent loss of photoreversibility after repetitive photo-switching. It is noted that the absorbances of the azobenzene chromophores in DR-MIPs gradually increased under 440 nm light with increasing irradiation time. This may well be because the *cis–trans* conversion rate is higher than that of the *trans–cis*, as reported in the literature.[29] Fig. 4(B) shows the changes in the amounts of bound caffeine in the presence of the DR-MIPs, control SR-MIPs and DR-NIPs under alternate irradiation at 365 nm and visible light. After the rebinding equilibrium was achieved, 1.87 μM g^{-1} MIPs caffeine was bound into the DR-MIPs. With the irradiation at 365 nm for 2 h, 62% of the caffeine was released from the material back into the solution. After the photoregulated release of caffeine, the DR-MIPs were irradiated by visible light for another 2 h. 93.6% of the released caffeine rebound into the DR-MIPs. Repeating the 365 nm and visible light irradiation cycle resulted in the release and uptake of caffeine in nearly identical amounts to the previous cycle. For the control SR-MIPs, after rebinding equilibrium, only 48% of bound caffeine was released back into solution after irradiation at 365 nm for 2 h, and only 58.3% was rebound into the SR-MIPs after irradiation with visible light. The results discussed above can find considerable explanation in the kinetic binding curves (Fig. 3(A)).

The possible mechanism for photoregulated release and uptake of caffeine is shown in Scheme 1(B), owing to the photoisomerization of the DR-MIPs; that is, the conformational change has a crucial effect on the blocking of template molecules. It is well known that recognition cavities in MIPs are complementary to template molecules in shape, size and chemical functionality. When the DR-MIPs were irradiated by 365 nm light, the azobenzene chromophores of functional monomer MPABA in DR-MIPs underwent photoinduced *trans–cis* isomerization, leading to a change in the recognition cavities (in the receptor geometry) as seen in Scheme 1(B); as a result, they were not complementary to the template any more. Thereby, the host–guest interaction between recognition cavities and template molecules were significantly weakened, so the bound template molecules of caffeine were released back into solution. On the contrary, the azobenzene chromophores underwent *cis–trans* isomerization upon irradiation with visible light, and the geometry of the receptor sites returned to that of the original, which were complementary to the template molecule in shape, size and chemical functionality again, as shown in Scheme 1(B). Therefore, the caffeine molecules in solutions were taken up by the DR-MIPs.

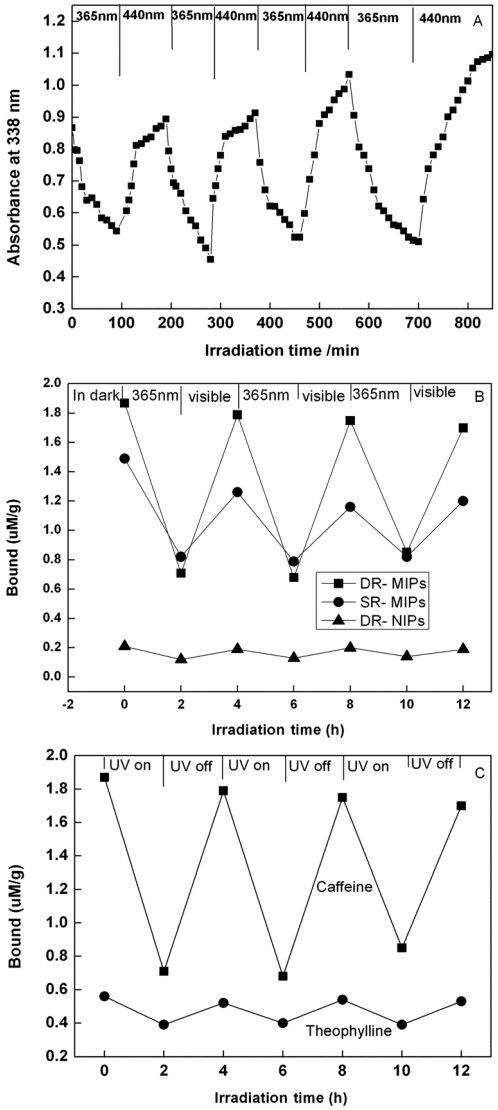


Fig. 4 (A) Reversibility of the photoisomerization process of the azobenzene chromophore in the DR-MIPs matrix (1.0 mg in 3.0 mL DMSO); (B) photoregulated release and uptake of caffeine by DR-MIPs, SR-MIPs and DR-NIPs under photo-switching conditions; and (C) photoregulated release and uptake of caffeine and theophylline by DR-MIPs (*Experimental conditions*: the mass of the MIPs used was 5.0 mg, and the initial concentration of the analytes in the solution was 40 μM).

In order to examine the substrate specificity of the imprinted materials, the photoregulated release and uptake of theophylline was studied. As can be seen from Fig. 4(C), irradiation of the DR-MIPs could also bring about the release and uptake of theophylline, but the amount involved was very low. In other words, theophylline was less sensitive to the change in receptor-site affinity brought about by the photoisomerization of the azobenzene chromophores.

3.5 Application of the DR-MIPs for extraction of caffeine from real samples

To evaluate the feasibility and practical applicability of the obtained DR-MIPs, they were applied to extract caffeine from real samples. In the meanwhile, traditional magnetic MIPs (mag-MIPs) for caffeine, without photoresponsive properties and using MAA as functional monomer, were used for comparison. The whole extraction procedure included conditioning, extraction, separation, washing and eluting, as shown in Scheme 1(C). In order to obtain the optimal extraction recoveries, various factors were studied and optimized, such as amount of magnetic MIPs, extraction time and elution solvent. When one parameter was changed, the other parameters were fixed at their optimized values. Different amounts of magnetic MIPs ranging from 50 to 300 mg were used to extract the analyte from 20 mL extraction solvent. The results indicated that recoveries were greater than 90% when 50 mg mag-MIPs or 100 mg DR-MIPs were used for extraction. Further increasing the amount of MIPs gave no improvement of recoveries, and on the contrary, more time was needed to separate those MIPs from the solvent. More DR-MIPs were used because of the relatively lower binding capacity compared with that of traditional imprinted ones (non photoresponsive MIPs, using MAA as functional monomers[48]). Extraction time was another important factor influencing the recovery. In the present work, extraction times from 10 to 60 min were investigated. The results suggested that, with the increase of extraction time from 10 to 30 min, the recovery increased from 39.5% to 95.2%. Further increasing the extraction time gave no further increase of recovery. In the meanwhile, sonication can reduce the time for extraction. Therefore, ultrasound-assisted extraction for 30 min was adopted in this work.

In order to obtain the optimal recoveries of caffeine, elution solutions, including methanol, ACN and acidified methanol were used for mag-MIPs; and DMSO, acidified ethanol, and acidified DMSO–methanol were used for DR-MIPs by virtue of UV-Vis irradiation, and the results were listed in Table 2. For the mag-MIPs, the best recovery was obtained using 5.0 mL methanol solution containing 0.5% acetic acid (1.0 mL each time). The addition of acetic acid was beneficial to the disruption of the hydrogen bonding interaction between the polymer and the template molecules. For the DR-MIPs, the monomer MPABA underwent *trans–cis* isomerization upon irradiation at 365 nm, which was beneficial for template elution. Therefore, 2 mL DMSO/methanol (1 : 1, v/v) containing 0.5% acetic acid (1.0 mL each time) was sufficient for template elution. The addition of DMSO facilitated the *trans–cis* isomerization of the monomer because of its good solubility. As seen from Table 2, with the assistance of light, the procedure for elution of the template and thereby extraction became simple and economic in terms of solvent.

Table 2 Recoveries of caffeine using different elution methods for DR-MIPs

	Method A[a]	Method B[b]	Combined A and B[c]
1st	32.6	41.5	61.2
2nd	61.8	66.5	95.9
3rd	77.5	79.7	97.2
4th	88.9	89.5	98.5
5th	94.5	96.7	99.5

[a] Using DMSO as solvent, 1 mL each time, and irradiation with 365 nm UV-Vis. [b] Using acidified methanol as eluting solvent without irradiation at 365 nm, 1 mL each time. [c] Using acidified DMSO–methanol as eluting solvent with irradiation at 365 nm, 1 mL each time.

Under the above optimized extraction conditions, the calibration curve for the detection of caffeine by using DR-MIPs was obtained by performing a linear regression analysis ranging from 50 nM to 30 μM. Good linearity was obtained with correlation coefficients of $R > 0.997$. The limit of detection (LOD) was 4.17 nM based on a signal-to-noise ratio of 3. Precision was calculated in terms of intraday repeatability ($n = 6$) and interday reproducibility (6 different days) at 5.0 μM. The intraday repeatability, evaluated as relative standard deviation (RSD), was 1.28%, and the interday reproducibility was 2.56%. The DR-MIPs based extraction with light irradiation coupled to HPLC-UV was demonstrated to be applicable for accurate quantitative determination of trace caffeine.

In order to further evaluate the potential applications of DR-MIPs for selective preconcentration of caffeine in real samples, a 20 mL tap water sample, a cola sample diluted 100 times and a tea water sample, spiked with caffeine at 1.0, 2.0, and 5.0 μM, were preconcentrated by the DR-MIPs. Fig. 5 presents the chromatograms of caffeine from water, cola and tea water samples. Caffeine was remarkably concentrated by the DR-MIPs (Fig. 5(a)), which was attributed to the fact that the DR-MIPs had a much higher imprinting efficiency, so the matrix effects could be reduced and caffeine could be preconcentrated. In contrast, DR-NIPs gave poor detection and separation (Fig. 5(b)), so we can draw the conclusion that the extraction effect resulted from the specific binding of DR-MIPs. It was also extremely difficult to detect caffeine without performing the extraction preparation/enrichment process due to its low concentration (Fig. 5(c) and (d)).

The validation of the extraction method by the DR-MIPs was performed by examining the recoveries of spiked samples. The precision of the method was evaluated by calculating the RSD of the extraction at different concentration levels under the optimized conditions. The results were listed in Table 3. Satisfactory recoveries were obtained, such as 92.4–117.6% with precisions of 2.60–3.57% at 1.0 μM. This demonstrated the potential applicability of the DR-MIPs for simultaneous and highly efficient preconcentration, separation, and accurate quantification of caffeine in real samples. Moreover, the magnetic and

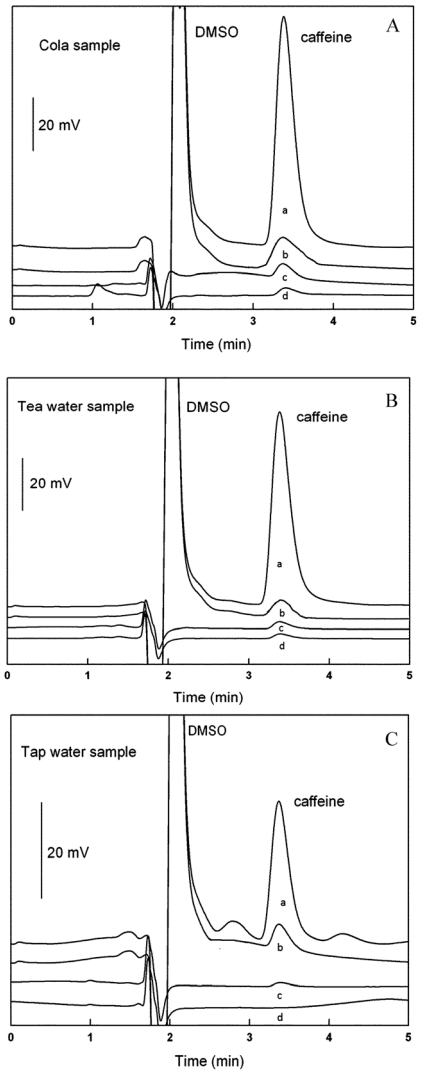


Fig. 5 HPLC-UV chromatograms of cola (A), tea water (B) and tap water (C) samples: (a) spiked sample extracted by DR-MIPs, (b) spiked sample extracted by DR-NIPs, (c) sample spiked with 1 μM caffeine without extraction, (d) sample without spiking/extraction. *Experimental conditions*: 20 mL spiked solutions; 100 mg DR-MIPs; wash solution, 3 mL methanol; elution solution, 2 mL acidified DMSO–methanol (5 : 5, v/v) with the help of UV-Vis; re-dissolution solution, 500 mL DMSO. HPLC conditions: mobile phase, methanol–water (50 : 50, v/v); flow rate, 1.0 mL min^{-1}; room temperature; UV detection, at 275 nm; injection volume, 20 μL.

photonic responsive properties offered a simple and robust method for the direct analysis of caffeine from real samples. When compared to the single photonic responsive MIPs, the magnetic properties of the DR-MIPs provided a simple and fast magnetic separation procedure. The extraction and cleanup were completed in one step, and the magnetic MIPs were easily separated from samples by an external magnetic field without centrifugation or filtration, and then were reusable, proving to be simple, rapid and eco-friendly. When compared to the single magnetic MIPs, the photonic responsive property of the DR-MIPs provided a simple template eluting method. With the assistance of UV-Vis, the template can be easily released from the recognition sites due to the *trans–cis* isomerization of the monomer. Therefore, as a novel sorbent, the DR-MIPs are ideal candidates for the uptake and release of trace caffeine in complicated matrices.

Table 3 Recoveries of caffeine determined by using DR-MIPs in different samples[a]

Sample	Level found (μM)	Level spiked (μM)	Recovery[b] (%)
Cola	4.18	1	92.4 ± 3.57
		2	99.5 ± 2.41
		5	90.6 ± 2.25
Tea water	2.83	1	95.2 ± 2.86
		2	91.7 ± 3.15
		5	93.6 ± 3.65
Tap water	ND[c]	1	117.6 ± 2.60
		2	89.5 ± 1.52
		5	92.1 ± 3.73

[a] Extraction conditions are as follows: 20 mL spiked solutions; 100 mg DR-MIPs; wash solution, 3 mL methanol; elution solution, 2 mL acidified DMSO–methanol (5 : 5, v/v) with the help of UV-Vis; re-dissolution solution, 1.0 mL DMSO. [b] Recovery (%) = [(total level of detected − level of endogenous)/level of spiked] × 100; data were expressed as the mean ± SD determined from triplicate independent experiments. [c] Not detected.

Conclusions

In summary, a type of photonic and magnetic DR-MIP, Fe_3O_4@MIPs, was prepared for the first time by combining the molecular imprinting technique with stimuli-responsive polymers. The resultant DR-MIPs retained the photoisomerization properties of the azobenzene chromophore and showed a response to an external magnetic field. The obtained DR-MIPs displayed remarkable molecular imprinting photo-responsive effects towards the template, fast rebinding kinetics, and high selectivity. The DR-MIPs were successfully applied to the preconcentration of trace caffeine from real samples. The idea of photonic and magnetic DR-MIPs presented here makes possible the photoregulated uptake and release of pollutants from environment samples, and the magnetic property allows for magnetic separation. The whole system of enrichment, separation, and release of pollutants can be recycled, so the system is convenient, cost-effective and environmentally friendly, which provides a new method for pollution abatement and analysis. The photonic and magnetic DR-MIPs could also be used in a drug controlled-release system. More work still needs to be done to improve the binding capacity and water compatibility of the dual responsive MIPs.

Acknowledgements

Financial support from the National Natural Science Foundation of China (21105117, 21275158, 21275068, 21171085), the Innovation Projects of the Chinese Academy of Sciences (KZCX2-EW-206), the Natural Science Foundation of Shandong Province of China (ZR2010BQ027, ZR2010BM027), and the 100 Talents Program of the Chinese Academy of Sciences, is gratefully acknowledged.

Notes and references

1 P. Van Eenoo, K. Deventer, K. Roels and F. T. Delbeke, *Forensic Sci. Int.*, 2006, **164**, 159–163.
2 A. J. Kueh, P. J. Marriott, P. M. Wynne and J. H. Vine, *J. Chromatogr., A*, 2003, **1000**, 109–124.
3 M. Thevis, A. Thomas, P. Delahaut, A. Bosseloir and W. Schanzer, *Anal. Chem.*, 2006, **78**, 1897–1903.
4 D. Kolpin, E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber and H. T. Buxton, *Environ. Sci. Technol.*, 2002, **36**, 1202–1211.
5 N. Spataru, B. V. Sarada, D. Tryk and A. Fujishima, *Electroanalysis*, 2002, **11**, 721–728.
6 S. Kerrigan and T. Lindsey, *Forensic Sci. Int.*, 2005, **53**, 67–69.
7 E. Dinc, G. Kokdil and F. J. Onur, *J. Pharm. Biomed. Anal.*, 2001, **26**, 769–778.
8 M. Aklilu, M. Tessema and M. R. Abshiro, *Talanta*, 2008, **76**, 742–746.
9 M. H. Spyridaki, P. Kiousi, A. Vonaparti, P. Valavani, V. Zonaras, M. Zahariou, E. Sianos, G. Tsoupras and C. Georgakopoulos, *Anal. Chim. Acta*, 2006, **242**, 573–574.
10 P. J. R. Roche, S. M. Ng, K. Page, N. Goddard and R. Narayanaswamy, *Sens. Actuators, B*, 2009, **139**(1), 97–103.
11 K. Farrington, E. Magner and F. Regan, *Anal. Chim. Acta*, 2006, **566**, 60–68.
12 X. B. Hu, G. T. Li, M. H. Li, J. Huang, Y. Li, Y. B. Gao and Y. H. Zhang, *Adv. Funct. Mater.*, 2008, **18**, 575–583.
13 T. Alizadeha, M. R. Ganjali, M. Zare and P. Norouzi, *Electrochim. Acta*, 2010, **55**, 1568–1574.
14 L. X. Chen, S. F. Xu and J. H. Li, *Chem. Soc. Rev.*, 2011, **40**, 2922–2942.
15 D. Cai, L. Ren, H. Zhao, C. Xu, L. Zhang, Y. Yu, H. Wang, Y. Lan, M. F. Robert, J. H. Chuang, M. J. Naughton, Z. Ren and T. C. Chiles, *Nat. Nanotechnol.*, 2010, **5**, 597–601.
16 S. F. Xu, J. H. Li and L. X. Chen, *Talanta*, 2011, **85**, 282–289.
17 S. F. Xu, J. H. Li and L. X. Chen, *J. Mater. Chem.*, 2011, **21**, 4346–4351.
18 X. L. Song, J. H. Li, J. T. Wang and L. X. Chen, *Talanta*, 2009, **80**, 694–702.
19 S. F. Xu, L. X. Chen, J. H. Li, W. Qin and J. P. Ma, *J. Mater. Chem.*, 2011, **21**, 12047–12053.
20 J. H. Li, Z. Zhang, S. F. Xu, L. X. Chen, N. Zhou, H. Xiong and H. L. Peng, *J. Mater. Chem.*, 2011, **21**, 19267–19274.
21 C. D. H. Alarcon, S. Pennadam and C. Alexander, *Chem. Soc. Rev.*, 2005, **34**, 276–285.
22 X. Li, J. Zuo, Y. Guo and X. Yuan, *Macromolecules*, 2004, **37**, 10042–10046.
23 M. Karg, I. Pastoriza-Santos, B. Rodriguez-González, R. Klitzing, S. Wellert and T. Hellweg, *Langmuir*, 2008, **24**, 6300–6306.
24 G. V. Martins, J. F. Mano and N. M. Alves, *Langmuir*, 2011, **27**, 8415–8423.
25 C. Wang, A. Javadi, M. Ghaffari and S. Gong, *Biomaterials*, 2010, **31**, 4944–4951.
26 X. Liu, T. Zhou, Z. Du, Z. Wei and J. Zhang, *Soft Matter*, 2011, **7**, 1986–1993.
27 N. Minoura, K. Idei, A. Rachkov, Y. Choi, M. Ogiso and K. Matsuda, *Macromolecules*, 2004, **37**, 9571–9576.
28 Y. Li, C. Dong, J. Chu, J. Qi and X. Li, *Nanoscale*, 2011, **3**, 280–287.
29 C. Gong, M. H. Lam and H. Yu, *Adv. Funct. Mater.*, 2006, **16**, 1759–1767.
30 C. Gong, K. Wong and M. H. Lam, *Chem. Mater.*, 2008, **20**, 1353–1358.
31 Q. Tang, C. Gong, M. H. W. Lam and X. Fu, *Sens. Actuators, B*, 2011, **156**, 100–107.
32 L. Fang, S. Chen, Y. Zhang and H. Zhang, *J. Mater. Chem.*, 2011, **21**, 2320–2329.
33 T. Takeuchi, K. Akeda, S. Murakami, H. Shinmori, S. Inoue, W. Lee and T. Hishiya, *Org. Biomol. Chem.*, 2007, **5**, 2368–2374.
34 L. Fang, S. Chen, X. Guo, Y. Zhang and H. Zhang, *Langmuir*, 2012, **28**, 9767–9777.
35 Y. Ma, Y. Zhang, M. Zhao, X. Guo and H. Zhang, *Chem. Commun.*, 2012, **48**, 6217–6219.
36 Q. Gao, D. Luo, J. Ding and Y. Feng, *J. Chromatogr., A*, 2010, **1217**, 5602–5609.
37 Y. Zhang, R. Liu, Y. Hu and G. Li, *Anal. Chem.*, 2009, **81**, 967–976.
38 G. Theodoridis and P. Manesiotis, *J. Chromatogr., A*, 2002, **948**, 163–169.
39 X. Wang, L. Wang, X. He, Y. Zhang and L. Chen, *Talanta*, 2009, **78**, 327–332.
40 Y. Li, C. Dong, J. Chu, J. Qi and X. Li, *Nanoscale*, 2011, **3**, 280–287.
41 L. Chen, J. Liu, Q. Zeng, H. Wang, A. Yu, H. Zhang and L. Ding, *J. Chromatogr., A*, 2009, **1216**, 3710–3719.
42 L. Chen, X. Zhang, L. Sun, Y. Xu, Q. Zeng, H. Wang, H. Xu, A. Yu, H. Zhang and L. Ding, *J. Agric. Food Chem.*, 2009, **57**, 10073–10080.
43 H. Deng, X. Li, Q. Peng, X. Wang, J. Chen and Y. Li, *Angew. Chem., Int. Ed.*, 2005, **44**, 2782–2785.
44 R. J. Umpleby, S. C. Baxter, M. Bode, J. K. Berch, R. N. Shah and K. D. Shimizu, *Anal. Chim. Acta*, 2001, **435**, 35–42.
45 E. Corton, J. A. García-Calzón and M. E. Díaz-García, *J. Non-Cryst. Solids*, 2007, **353**, 974–980.
46 J. A. García-Calzón and M. E. Díaz-García, *Sens. Actuators, B*, 2007, **123**, 1180–1194.
47 A. L. Medina-Castillo, G. Mistlberger, J. F. Fernandez-Sanchez, A. Segura-Carretero, I. Klimant and A. Fernandez-Gutierrez, *Macromolecules*, 2010, **43**, 55–61.
48 N. W. Turner, C. I. Holdsworth, S. W. Donne, A. McCluskey and M. C. Bowyer, *New J. Chem.*, 2010, **34**, 686–692.