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PAPER

Azobenzene-containing molecularly imprinted polymer microspheres with photoresponsive template binding properties†

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The first successful preparation of azobenzene (azo)-containing molecularly imprinted polymer (MIP) microspheres with photoresponsive template binding properties is described. A methacrylate azo functional monomer with a pyridine group was used for this purpose, and its good solubility in acetonitrile allowed the implementation of molecular imprinting *via* precipitation polymerization, leading to azo-containing MIP microspheres (number-average diameter = 1.33 μm, polydispersity index = 1.15) with obvious molecular imprinting effects towards the template 2,4-dichlorophenoxyacetic acid (2,4-D), rather fast template rebinding kinetics, and appreciable selectivity over structurally related compounds. The binding association constant K_a and apparent maximum number N_{max} for high-affinity sites of the imprinted polymer in the dark environment were determined by Scatchard analysis to be 2.3×10^4 M^{-1} and 10.0 μmol g^{-1}, respectively. Most importantly, the binding affinity of the imprinted sites in azo-containing MIP microspheres was found to be photoresponsive towards the template, which decreased upon UV light irradiation (as revealed by the resulting lower K_a value for high-affinity sites and reduced specific bindings), whereas it could be recovered during the subsequent thermal (or visible light-induced) back-isomerization. Furthermore, this photoregulation process proved to be highly repeatable under photoswitching conditions.

Introduction

Molecular imprinting is a facile and versatile approach for preparing artificial receptors with tailor-made molecular recognition binding sites.[1–7] A typical molecular imprinting process usually involves the formation of a complex between a template molecule and a functional monomer *via* either non-covalent interactions (such as hydrogen bonds, ionic interactions, hydrophobic interactions, and metal-ion chelating interactions) or reversible covalent bonds (*e.g.*, boronate ester, ketal and acetal, or Schiff base), which is subsequently copolymerized with a crosslinking monomer in the presence of a suitable porogenic solvent. After polymerization, the template is removed from the obtained crosslinked polymer network, leading to a molecularly imprinted polymer (MIP) with binding cavities complementary to the shape, size and functionality of the template. The virtues of the MIPs include the outstanding affinity and selectivity towards the template molecules as well as their high mechanical stability and remarkable resistance to extreme pH conditions, organic solvents, metal ions, and autoclave treatment. Such highly appealing physical and chemical characteristics make MIPs very promising candidates for many applications, including separation, chemical sensors, antibody mimics, biomimetic catalysis, drug delivery, drug development, and so on.[1–7]

Despite the tremendous progress made in the molecular imprinting field, many challenges still remain to be addressed, especially in the design of advanced MIP materials mimicking the biological receptors.[8–10] It is well known that the biological receptors have many fascinating characteristics such as their high responsivity towards external stimuli (*e.g.*, the temperature). The presently developed MIPs, however, are normally difficult to deform for regulating their binding properties because a high fraction of a crosslinker is usually used in molecular imprinting to stabilize the binding sites. Recently, the rational use of certain special co-functional monomers (*e.g.*, *N*-isopropylacrylamide (NIPAAm), acrylic acid, and azobenzene (azo) monomers) in molecular imprinting, together with the appropriate choice of the amounts of the crosslinkers used, makes it possible to obtain responsive MIPs towards different stimuli such as temperature, pH, and light.[11]

Photoirradiation has become one of the most frequently adopted external stimuli for responsive materials because light is a clean energy and can be manipulated precisely, rapidly and remotely.[12,13] Numerous well-established photoresponsive molecular systems are now available.[14–22] Among them, azo-containing systems have attracted great attention because they

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can exhibit excellent photoinduced fast and reversible isomerization between the *trans* and *cis*-isomers of the azo moieties upon exposure to UV or visible light, which can trigger significant changes in the geometry and dipole moment of the azo chromophore.[14–22]

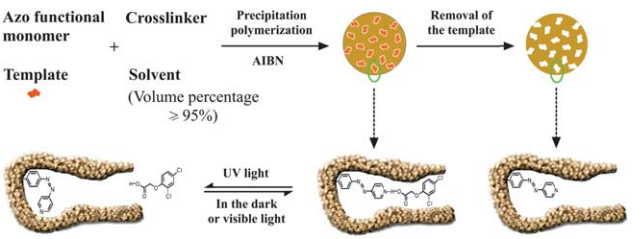
Recent years have witnessed considerable interest in photoresponsive MIP materials.[11,23–28] Such advanced functional MIP materials have been mainly prepared by introducing a functional azo moiety into the selective binding sites of the MIPs. The configuration of the azo groups can be regulated by light illumination, which brings about a large alteration of the spatial arrangement of the binding functionalities in the binding sites, thus leading to a significant change in the strength of the host–guest interactions. Minoura and coworkers described the first preparation of azo-containing MIP membranes with photoregulated template binding properties by using *p*-phenylazoacrylanilide as the functional monomer.[23,24] The groups of Lam and Yu reported the fabrication of a photoresponsive MIP for the photoregulated release and uptake of caffeine by using 4-((4-methacryloyloxy)phenylazo)benzoic acid as the functional monomer.[25] Later, Lam and coworkers also prepared photoresponsive molecularly imprinted hydrogels for the photoregulated release and uptake of pharmaceuticals in the aqueous media by using a water-soluble azo functional monomer 4-((4-methacryloyloxy)phenylazo)benzenesulfonic acid.[26] Schmitzer and coworkers developed a new azo monomer di-(ureidoethylenemethacrylate)azobenzene and successfully prepared a photoresponsive MIP by using it as both the functional monomer and crosslinker.[27] Takeuchi and coworkers synthesized a photoresponsive porphyrin-imprinted polymer by using a novel azo functional monomer bearing a diaminopyridine group.[28] While all the above reports have demonstrated that the photoresponsive azo-containing MIPs are of great potential in many applications such as smart separation, extraction, and assays as well as efficient drug delivery systems, their rather limited physical formats (*i.e.*, bulk polymer membranes,[23,24] bulk monoliths,[25,27,28] and bulk hydrogels[26]) have significantly influenced their applications. In particular, MIP particles with irregular shapes and relatively large sizes (normally tens of micrometers in diameter) are normally obtained after the time-consuming and laborious grinding and sieving of the bulk MIPs, which are inappropriate for such applications as binding assays and drug delivery systems because the best physical format for such applications is spherical beads.[29,30] Moreover, the binding sites inside the MIP particles with relatively large sizes are inaccessible, thereby significantly lowering the template loading capacities of the MIP particles. Therefore, the development of azo-containing MIP microspheres with photoresponsive template binding properties remains an important goal.

In this paper, we describe for the first time the successful preparation of azo-containing MIP microspheres with photoresponsive template binding properties *via* precipitation polymerization by using an acetonitrile-soluble azo functional monomer with a pyridine group (*i.e.*, 4-((4-methacryloyloxy)-phenylazo)pyridine, Schemes 1 and 2). The chemical structures, morphologies, particle sizes and size distributions, template rebinding properties and substrate selectivity of the obtained azo-containing MIP microspheres as well as their binding properties under photoirradiation conditions were characterized in detail.

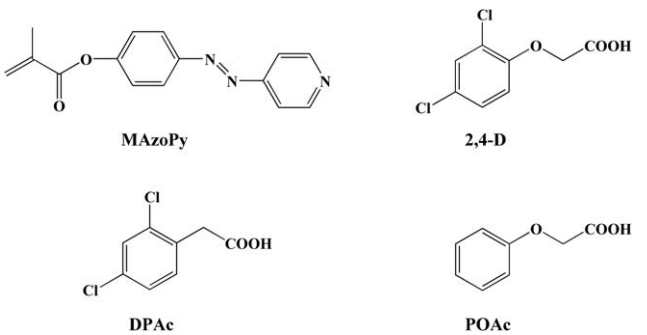
Experimental

Materials

Ethylene glycol dimethacrylate (EGDMA, Alfa Aesar, 98%) was purified by distillation under vacuum. Acetonitrile (Tianjin Jiangtian Chemicals, China, analytical grade (AR)) was refluxed over calcium hydride (CaH_2) and then distilled. Tetrahydrofuran (THF, Tianjin Jiangtian Chemicals, 99%) was refluxed over sodium and then distilled. *N*,*N*-Dimethylformamide (DMF, Tianjin Jiangtian Chemicals, 99.5%) was dried with anhydrous magnesium sulfate ($MgSO_4$) and then distilled under vacuum. Methanol (Tianjin Jiangtian Chemicals, AR) was distilled prior to use. Triethylamine (TEA, Tianjin Jiangtian Chemicals, 99%) was dried with anhydrous sodium sulfate (Na_2SO_4) and then distilled. Azobisisobutyronitrile (AIBN, Tianjin Jiangtian Chemicals, Chemical purity (CP)) was recrystallized from ethanol and stored at −18 °C before use. Thionyl chloride (Tianjin Jiangtian Chemicals, 99.5%) was purified by distillation just prior to use. Methacrylic chloride was obtained by the reaction between methacrylic acid and thionyl chloride. Methacrylic anhydride was prepared by reacting methacrylic acid and methacrylic chloride in the presence of an aqueous solution of sodium hydroxide, following a literature procedure.[31] 4-(4-Hydroxyphenylazo)pyridine was prepared following a previously reported procedure (Scheme S1 in the supporting information†).[32] 4-Aminopyridine (Shanghai Jingchun Chemical Company, China, AR), 4-dimethylaminopyridine (DMAP, Merck, AR), 2,4-dichlorophenoxyacetic acid (2,4-D, Alfa Aesar,



Scheme 1 Schematic illustration for the preparation of azo-containing MIP microspheres with photoresponsive binding sites.



Scheme 2 The chemical structures of the azo functional monomer MAzoPy, the template molecule 2,4-D, and the related test compounds of the template (*i.e.*, DPAc and POAc).

98%), 2,4-dichlorophenylacetic acid (DPAc, Acros, 99%), phenoxyacetic acid (POAc, Acros, 98 + %), and all the other reagents were commercially available and used as received. The chemical structures of 2,4-D, DPAc and POAc are shown in Scheme 2.

Preparation of the methacrylate azo functional monomer with a pyridine group 4-((4-methacryloyloxy)phenylazo)pyridine (MAzoPy) (Scheme 2 and Scheme S1 in the supporting information†)

To a solution of 4-(4-hydroxyphenylazo)pyridine (2.3901 g, 12 mmol) in dried THF (150 mL), methacrylic anhydride (3.57 mL, 24 mmol), 4-dimethylaminopyridine (DMAP, 0.1320 g, 1.2 mmol), and triethylamine (TEA, 1.67 mL, 12 mmol) were added successively. After being stirred at 40 °C for 24 h, the reaction mixture was poured into a large amount of water (300 mL) and extracted with chloroform. The obtained organic phase was dried over Na_2SO_4 and the chloroform removed using a rotary evaporator, leading to a brown solid product (3.12 g, yield: 95%). m.p. 144–145 °C (determined by polarizing optical microscope (POM) with a heating rate of 10 °C min^{-1}); UV-vis (acetonitrile): λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1}) = 318 (18800), around 432 (700); ^1H NMR ($CDCl_3$): δ (ppm) = 8.80 (d, J = 4.8 Hz, 2H, Ar-H), 8.01 (d, J = 8.8 Hz, 2H, Ar-H), 7.69 (d, J = 4.8 Hz, 2H, Ar-H), 7.31 (d, J = 8.8 Hz, 2H, Ar-H), 6.39 (s, 1H, CH_2 = C-), 5.81 (s, 1H, CH_2 = C-), 2.08 (s, 3H, -CH_3).

Preparation of azo-containing MIP and non-imprinted polymer (NIP) microspheres

The azo-containing MIP microspheres were prepared *via* precipitation polymerization using MAzoPy, 2,4-D, EGDMA, AIBN, and acetonitrile as the functional monomer, template, crosslinker, initiator, and porogenic solvent, respectively, according to the following procedure: MAzoPy (0.4005 g, 1.5 mmol), 2,4-D (0.3316 g, 1.5 mmol), and dried acetonitrile (75 mL) were added into a one-neck round-bottom flask (100 mL) successively; a clear homogeneous solution was obtained after the mixture was stirred at ambient temperature for 3 h in the dark (the azo monomer was kept in the *trans*-rich state, Scheme 1). EGDMA (0.85 mL, 4.5 mmol) and AIBN (0.0086 g, 0.0525 mmol) were then added into the above system. The reaction mixture was bubbled with argon for 20 min in an ice bath and the flask then sealed and immersed in a thermostated oil bath at 60 °C. After 48 h of polymerization at 60 °C in the dark, the resulting polymer particles were collected by centrifugation of the reaction mixture, which were subsequently purified through Soxhlet extraction with methanol–acetic acid (9 : 1 v/v, 48 h) and acetonitrile (48 h) successively until no template could be detected in the extraction solution. After being dried at 40 °C under vacuum for 24 h, khaki colored azo-containing MIP microspheres were obtained in a yield of 66%.

The corresponding azo-containing NIP microspheres (khaki color) were also prepared and purified under identical conditions except that the template was omitted (yield: 65%).

Characterization

^1H NMR spectra were recorded on a Varian Unity plus-400 spectrometer (400 MHz).

Fourier transform infrared (FT-IR) spectra of the azo-containing MIP/NIP microspheres were obtained using a Nicolet Magna-560 FT-IR spectrometer.

The elemental analyses of the MIP/NIP microspheres were carried out to determine their chemical compositions and crosslinking densities by using Elementar Vario EL (Germany). By assuming that the obtained MIP/NIP microspheres contain x moles of the bound MAzoPy unit (molecular formula: $C_{15}H_{13}N_3O_2$) and y moles of the bound EGDMA unit (molecular formula: $C_{10}H_{14}O_4$), the following equations can be derived for the weight fractions of carbon (C_C) and nitrogen (C_N):

$$C_C = (15x+10y)M_C/\left(xM_{MAzoPy} + yM_{EGDMA}\right);$$
$$C_N = 3xM_N/\left(xM_{MAzoPy} + yM_{EGDMA}\right)$$

where M_C is the atomic weight of carbon, M_N the atomic weight of nitrogen, M_{MAzoPy} the molecular weight of MAzoPy, and M_{EGDMA} the molecular weight of EGDMA. The molar fractions of the bound EGDMA unit in the MIP/NIP microspheres (*i.e.*, $y/(x + y)$, which can also be utilized to express the crosslinking densities of the MIP/NIP microspheres) can thus be obtained by introducing C_C and C_N values (determined by elemental analysis) into the above two equations.

The morphologies, particle sizes, and size distributions of the azo-containing MIP/NIP microspheres were determined with a scanning electron microscope (SEM, Shimadzu SS-550). All of the SEM size data reflected an average of about 100 particles each, which were calculated by using the following formulas:

$$D_n = \sum_{i=1}^{k} n_i D_i / \sum_{i=1}^{k} n_i;\ D_w = \sum_{i=1}^{k} n_i D_i^4 / \sum_{i=1}^{k} n_i D_i^3;\ U = D_w/D_n$$

where D_n is the number-average diameter, D_w the weight-average diameter, U the size distribution index, k the total number of the measured particles, D_i the particle diameter of the determined microspheres, and n_i the particle number of the microspheres with a diameter D_i.

A UV-vis scanning spectrophotometer (TU1900, Beijing Purkinje General Instrument Co., Ltd) was utilized to obtain the UV-vis spectra of the samples at 25 °C. The photochemical isomerization of the MAzoPy solution was investigated by irradiating them firstly with a 365 nm UV lamp (12 W) and then with a 450 nm visible light lamp (18 W, wavelength range: 400–550 nm, λ_{max} = 450 nm). The thermal back-isomerization of the MAzoPy solution was studied by its exposure to 365 nm UV light (12 W) for 210 s, and its subsequent time spent in the dark at 25 °C, with the UV-vis spectra of the MAzoPy solution measured at different times.

The concentrations of the template molecule 2,4-D and its related compounds were quantified with a high-performance liquid chromatograph (HPLC, Scientific System Inc., USA) equipped with a UV-vis detector. The wavelength used for the determination of 2,4-D, DPAc, and POAc was 284, 272, and 270 nm, respectively. A mixture of methanol and 0.5% aqueous solution of acetic acid (4/1 v/v) was used as the mobile phase at a flow rate of 1 mL min^{-1}.

The binding kinetics of the template molecule 2,4-D with the azo-containing MIP/NIP microspheres was evaluated by batch adsorption experiments, where 5 mg of MIP/NIP microspheres were incubated with a solution of 2,4-D in acetonitrile (0.5 mL,

0.05 mM) at 25 °C in the dark for different times. After centrifugation, the amounts of the template remaining in the supernatants (expressed as F) were determined by HPLC and those bound to the MIP/NIP microspheres (B) could thus be obtained by subtracting F from the initial template concentration.

Equilibrium binding experiments were performed by incubating a 2,4-D solution in acetonitrile (0.5 mL, 0.05 mM) with different amounts of azo-containing MIP/NIP microspheres at 25 °C for 6 h either in the dark or under the irradiation of 365 nm UV light (16 W). The amounts of the template bound to the MIP/NIP microspheres were then quantified with HPLC.

The binding isotherm of the azo-containing MIP microspheres was studied with Scatchard analysis:[33] MIP microspheres (5 mg) were incubated with a series of 2,4-D solutions in acetonitrile (C = 0.04–1.0 mM, 0.5 mL) at 25 °C for 6 h either in the dark or under the irradiation of 365 nm UV light (16 W). After centrifugation, the amounts of the template bound to the MIP microspheres (B) were determined by HPLC. The Scatchard equation used is $B/F = (N_{max} - B)K_a$, where K_a and N_{max} represent the binding association constant and apparent maximum number of the binding sites, respectively.

The binding selectivity of the azo-containing MIP/NIP microspheres was evaluated by measuring their rebinding capacities towards 2,4-D and its structurally related compounds (DPAc and POAc, Scheme 2): 5 mg of MIP/NIP microspheres were incubated with 0.5 mL of a mixed solution of 2,4-D, DPAc, and POAc in acetonitrile ($C_{2,4\text{-D, DPAc or POAc}}$ = 0.05 mM) at 25 °C for 6 h in the dark and the amounts of 2,4-D and the related compounds bound to the MIP/NIP microspheres were quantified by HPLC.

The studies on the photoregulated release and uptake of the template 2,4-D and its analogues by the azo-containing MIP microspheres were performed by alternately switching on and turning off the UV light irradiation on the mixtures of MIP microspheres and a solution of analytes: A series of samples were prepared by adding 5.0 mg of azo-containing MIP microspheres and a mixed solution of 2,4-D, DPAc, and POAc in acetonitrile (0.5 mL, $C_{2,4\text{-D, DPAc or POAc}}$ = 0.05 mM) into plastic Eppendorf tubes (2 mL), respectively, which were subsequently sealed and put into an incubator equipped with a 365 nm UV lamp (16 W). After their incubation at 25 °C in the dark for 6 h, one sample was taken out from the incubator and centrifuged to determine the amounts of the analytes bound by the MIP microspheres (by HPLC). The UV light was then switched on in the incubator to irradiate the remaining samples immediately after the first sample was taken out. After 3 h of UV light irradiation with incubation for the samples at 25 °C, the UV light was switched off and the second sample was taken out for the determination of the binding of the analytes. The remaining samples were then incubated at 25 °C in the dark for another 18 h, and the third sample was taken out for the determination of the binding of the analytes. The UV light was then switched on again to irradiate the remaining samples immediately at 25 °C after the third sample was taken out. The above photoswitching procedures (*i.e.*, UV light on for 3 h and UV light off for 18 h alternately) were repeated until all of the other samples were measured.

The studies on the photoregulated release and uptake of the template 2,4-D and its analogues by the azo-containing NIP microspheres were carried out similarly.

Results and discussion

Over the past few years, we have been working in the fields of molecular imprinting and photoresponsive azo-containing functional materials.[5,8,17,34–38] In this paper, we combine the above two fields together and aim to develop azo-containing MIP microspheres with photoresponsive template binding properties. The spherical physical format and rather small sizes of such photoresponsive azo-containing MIP microspheres make them highly promising in many potential applications such as smart separation, extraction, and assays, efficient drug delivery systems, and intelligent chemical carriers (which can release chemical reagents according to the requirements under light irradiation). To the best of our knowledge, there has been no report to date on the azo-containing spherical MIP particles with photoresponsive template binding properties.

Precipitation polymerization has proven very versatile for preparing MIP micro-/sub-microspheres because of its easy operation and lack of need for any surfactant or stabilizer.[30,35,36,39–41] Spherical MIP particles with a diameter ranging from 100 nm to several micrometers can be readily obtained by tuning the polymerization conditions, including the kind of solvents used, the amounts of the initiator and solvent used, and the polymerization temperature and time, which are highly useful for different application purposes. In particular, it has been well established that the choice of an appropriate solvent is pivotal for the successful preparation of MIP microspheres *via* precipitation polymerization, and acetonitrile has proven to be the most commonly utilized solvent for this purpose.[30,35,39,41] Therefore, it can be anticipated that the development of an acetonitrile-soluble azo functional monomer should make it possible to prepare spherical azo-containing MIP particles *via* precipitation polymerization. In this context, it is worth mentioning that the groups of Lam and Yu also attempted to prepare photoresponsive azo-containing MIP particles *via* precipitation polymerization, but only a bulk MIP monolith was obtained due to the use of an inappropriate solvent (in their case, a mixture of DMF and acetonitrile was used as the polymerization solvent instead of pure acetonitrile because their azo functional monomer was not soluble in acetonitrile itself).[25]

It is also known that 4-vinylpyridine is a widely used functional monomer in the molecular imprinting field, which can form noncovalent interactions with many template molecules bearing various functional groups such as phenol,[35,42] hydroxyl,[43] or carboxylic acid.[35,36,44] Therefore, a methacrylate azo functional monomer with a pyridine unit (*i.e.*, MAzoPy, Scheme 2) was designed for our purpose, which was readily prepared by reacting methacrylic anhydride with 4-(4-hydroxyphenylazo)pyridine in the presence of a very effective acylation catalyst DMAP in quite good yields (Scheme S1 in the supporting information†), and its purity was satisfactory, as determined *via* both thin layer chromatography and ^{1}H NMR spectroscopy. To our delight, this azo functional monomer proved to be fully soluble in acetonitrile and could thus be directly utilized to prepare azo-containing MIP microspheres *via* precipitation polymerization for a wide range of templates.

The UV-vis spectrum of the solution of MAzoPy in acetonitrile exhibited one strong absorption band around 318 nm and another very weak one around 432 nm, which are typical for the

azo compounds and can be ascribed to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electron transitions of the N=N bond, respectively (Fig. 1).[45] By irradiation with 365 nm UV light the studied azo monomer solution underwent significant *trans* to *cis* photoisomerization (Fig. 1a). The intensity of the $\pi \rightarrow \pi^*$ transition band around 318 nm decreased rapidly, whereas that of the $n \rightarrow \pi^*$ transition band around 432 nm slightly increased. The existence of isobestic points at 271 and 366 nm was characteristic for the existence of two distinct absorbing species in equilibrium with each other and at the same time proved that no side reaction took place during the photoisomerization process in the range studied.[45] Irradiating the thermodynamically less stable *cis*-MAzoPy with visible light led to the *cis* to *trans* back-isomerization of MAzoPy in solution (Fig. 1b). However, the finally recovered absorbance of *trans*-MAzoPy was lower than that before UV irradiation with the recovery of the *trans*-isomer being 83%. Similar phenomena were also observed previously by others.[46,47] Its real cause is not totally clear yet, and further investigation is ongoing to provide a reasonable explanation. Nevertheless, the photochemical isomerization became completely reversible upon subsequent cycles of UV and visible light irradiation (Fig. S1 in the supporting information†). In the absence of visible light (*i.e.*, in the dark), the thermal back-isomerization of the MAzoPy solution was slow, but near to complete recovery could be reached after a rather long time (Fig. 1c).

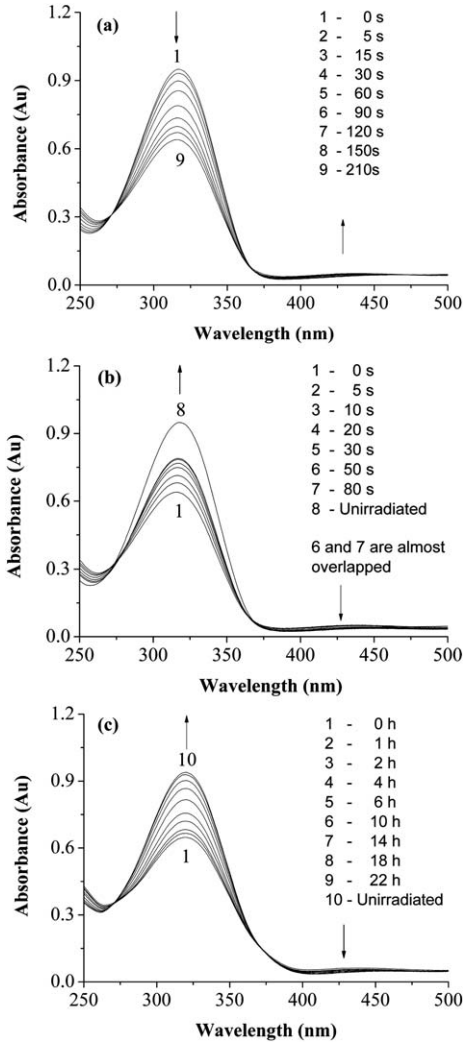


Fig. 1 UV-vis spectral changes in dependence of time for the solution of MAzoPy in acetonitrile ($C = 0.05$ mM) at 25 °C: (a) upon irradiation with 365 nm UV light; (b) upon irradiating the solution (which has been irradiated with 365 nm UV light for 210 s) with visible light; (c) the thermal back-isomerization of the azo monomer solution after its being irradiated for 210 s.

With this acetonitrile-soluble and highly photoresponsive azo functional monomer MAzoPy in hand, we started to test whether it could be utilized in molecular imprinting for the direct preparation of azo-containing MIP microspheres *via* precipitation polymerization. A herbicide 2,4-D (Scheme 2) was chosen here as the model template molecule because it could form hydrogen bonding with the pyridine group of MAzoPy in acetonitrile.[35] In addition, EGDMA, AIBN, and acetonitrile were used as the crosslinker, initiator, and porogenic solvent, respectively. The molar ratio of the reactant combination 2,4-D : MAzoPy : EGDMA : AIBN was 1 : 1 : 3 : 0.035 and the volume of the utilized acetonitrile was 97% relative to that of the whole reaction system. Note that the molar ratio of the template to MAzoPy is much higher than that used in a normal molecular imprinting system, where an excess of functional monomer is usually required in order to shift the equilibrium towards the complex formation. This could be attributed to the characteristics of precipitation polymerization, where a very large amount of porogenic solvent (typically ≥95% of the total reaction volume) is normally used, which leads to most of the template dissolving into the porogenic solvent instead of interacting with the functional monomer to form a prepolymerization complex.[30,35,39–41] Therefore, increasing the amount of template would be necessary to improve the formation of a prepolymerization complex as well as the imprinting efficiency.[48,49] As a reference, the corresponding azo-containing NIP particles were also prepared similarly by omitting the template in the reaction system. All the reactions were performed at 60 °C in an argon atmosphere in the dark (in order to keep the azo moieties in the *trans*-rich state, Scheme 1), and the resulting polymer particles were thoroughly purified through Soxhlet extraction, leading to khaki colored MIP and NIP particles with their yields being 66 and 65%, respectively. The khaki color of the MIP and NIP particles indicated that the azo functional moieties were successfully incorporated into the resulting polymers.

The morphologies, particle sizes, and size distributions of the obtained azo-containing MIP and NIP particles were firstly characterized *via* SEM (Fig. 2), and the results showed that spherical azo-containing MIP and NIP particles were successfully prepared *via* precipitation polymerization. The number-average diameters (D_n) of the MIP and NIP beads were 1.33 and 1.28 μm, respectively, and they had a polydispersity index (U) of 1.15 and 1.11, respectively. The rather similar diameters of the obtained azo-containing MIP and NIP microspheres revealed that the template molecule had little influence on the particle growth during the precipitation polymerization in this case, which is somewhat different from previous reports.[40,41]

Fig. 3 presents the FT-IR spectra of the obtained azo-containing MIP and NIP microspheres, from which it can be clearly seen that the MIP and NIP microspheres have rather similar chemical structures. The presence of three significant peaks

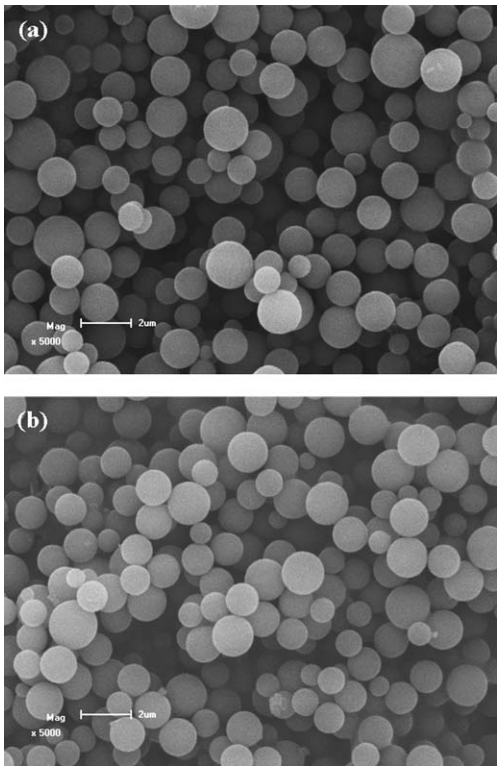


Fig. 2 Scanning electron micrographs of the azo-containing MIP (a) and NIP (b) microspheres.

around 1733 (C=O stretching), 1252 and 1144 cm^{-1} (C–O–C stretching) supported the existence of poly(EGDMA) in the obtained MIP and NIP microspheres. The characteristic peaks corresponding to the C=N stretching (1587 cm^{-1}) and C=C stretching (1460 cm^{-1}) in the pyridine rings could also be observed, revealing that poly(MAzoPy) was also present in the MIP and NIP microspheres. Furthermore, the presence of small peaks around 1637 cm^{-1} (corresponding to the C=C stretching mode) demonstrated that less than 100% of the bound EGDMA molecules were crosslinked in the MIP and NIP microspheres.[50]

The azo-containing MIP and NIP microspheres were further studied *via* elemental analysis to determine their chemical compositions, from which the crosslinking densities of the MIP and NIP microspheres were readily available as they could be simply expressed by the molar fractions of the bound EGDMA unit in the obtained polymers. The molar fractions of the bound EGDMA unit in both the MIP and NIP microspheres were determined to be 74%, which were in excellent consistency with that of EGDMA in the initial comonomer mixture (75%), suggesting that EGDMA had rather similar reactivity with MAzoPy in the studied reaction system. Moreover, the above results also demonstrated that the crosslinking densities of the azo-containing MIP and NIP microspheres were high enough for stabilizing the shapes of the imprinted cavities.

The binding kinetics of the template molecule 2,4-D with the azo-containing MIP and NIP microspheres was evaluated by batch adsorption experiments, which were carried out by incubating a certain amount of polymers in a dilute solution of 2,4-D in acetonitrile in the dark. Fig. 4 shows the adsorption uptake of the polymers *versus* the incubation time. It can be seen clearly that both the MIP and NIP microspheres reached their binding equilibriums at a time of about 1 h, indicating the occurrence of very fast template rebinding processes.

Equilibrium binding experiments were then performed to study the template rebinding properties of the azo-containing MIP and NIP microspheres by incubating a 2,4-D solution in acetonitrile with different amounts of polymers at ambient temperature for 6 h in the dark. Fig. 5 shows that the azo-containing MIP microspheres bound more 2,4-D than the NIP microspheres in a wide range of polymer concentrations. For example, in a dilute solution of 2,4-D in acetonitrile, while 16 mg of MIP microspheres bound 81% of 2,4-D, an equivalent amount of the corresponding control bound only 64%. This, together with the rather similar diameters of the MIP and NIP microspheres (which should lead to their similar specific surface areas and nonspecific template bindings), clearly reveals the successful generation of selective binding sites in the obtained MIP particles and thus the successful molecular imprinting process.

To get more insight into the binding characteristics of the azo-containing MIP microspheres, further studies were carried out using Scatchard analysis. The results showed that the Scatchard plot of the azo-containing MIP microspheres determined in the dark could be fitted into two straight lines (Fig. 6a), suggesting that the binding sites in the azo-containing MIP microspheres

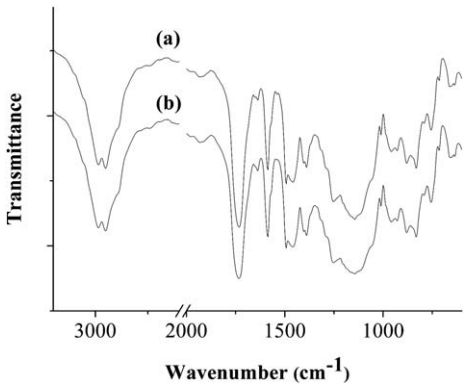


Fig. 3 FT-IR spectra of the azo-containing MIP (a) and NIP (b) microspheres.

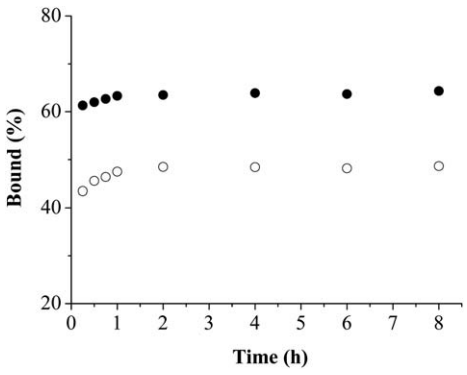


Fig. 4 Binding kinetics of 2,4-D on the azo-containing MIP (filled symbols) and NIP (open symbols) microspheres. 5 mg of MIP/NIP microspheres were incubated with a solution of 2,4-D in acetonitrile (0.5 mL, 0.05 mM) at 25 °C in the dark for different times.

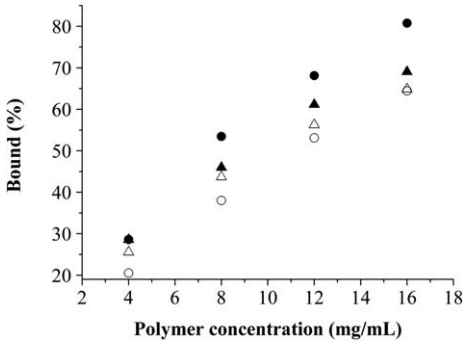


Fig. 5 Equilibrium bindings of 2,4-D (C = 0.05 mM) on different amounts of azo-containing MIP (filled symbols) and NIP (open symbols) microspheres in the dark (circle) and under UV light irradiation (triangle), respectively.

were heterogeneous and their affinities could be approximated by two binding association constants (K_a) corresponding to the high- and low-affinity sites, as usually observed for the MIPs prepared *via* the noncovalent molecular imprinting approach.[51] We are particularly interested in the high-affinity sites because they are mainly responsible for the specific binding of the MIP (*i.e.*, the binding difference between the MIP and its corresponding NIP[52]). In the present work, the K_a and N_{max} values of the high-affinity sites for the azo-containing MIP microspheres were evaluated from the Scatchard plot and found to be 2.3×10^4 M^{-1} and 10.0 μmol g^{-1}, respectively (Table 1). In addition, the K_a and N_{max} values of the low-affinity sites for the azo-containing MIP microspheres were also determined, which were 0.19×10^4 M^{-1} and 29.4 μmol g^{-1}, respectively.

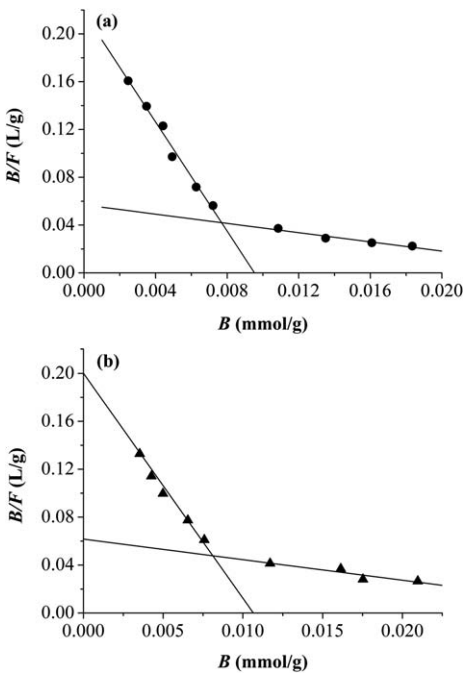


Fig. 6 Scatchard plots of the azo-containing MIP microspheres determined in the dark (a) and under the irradiation of UV light (b), respectively.

Table 1 The isothermal binding parameters of the azo-containing MIP microspheres determined in the dark and under the irradiation of UV light, respectively

	High-affinity binding sites [a]		Low-affinity binding sites [a]	
Sample	$k_a \times 10^{-4}$ M^{-1}	N_{max} /μmol g^{-1}	$k_a \times 10^{-4}$ M^{-1}	N_{max} /μmol g^{-1}
MIP in the dark	2.3	10.0	0.19	29.4
MIP under UV light irradiation	1.6	10.9	0.17	35.9

[a] K_a and N_{max} represent the binding association constant and apparent maximum number of the high- and low-affinity binding sites, respectively, which were obtained by Scatchard analysis.

The binding selectivity of the MIPs is often determined by comparing the binding of the template with those of its analogues, which affords an indication of the cross-reactivity of the MIPs towards selected molecules. The bindings of the azo-containing MIP and NIP microspheres towards 2,4-D were thus compared to two structurally related compounds, DPAc and POAc (Scheme 2), which have the same functionality (*i.e.*, carboxyl group) but differ either in the distance between the functional group and the benzene ring or in the numbers of substituents on the benzene ring. As can be seen clearly from Fig. 7, besides binding 2,4-D, the MIP microspheres also adsorbed DPAc and POAc, suggesting the existence of certain cross-binding reactivity. Nevertheless, the MIP microspheres showed significantly lower binding capacities towards DPAc and POAc than towards 2,4-D, thus demonstrating the high selectivity of the MIP microspheres towards 2,4-D. It is worth mentioning here that while the presence of certain cross-binding reactivity in the MIPs might be undesirable for such applications as sensors, this could actually be an advantage in sample treatment because a class of template analogues could also be removed or enriched efficiently.

On the basis of the above results, it can be concluded that azo-containing MIP microspheres could indeed be readily prepared *via* precipitation polymerization by using an acetonitrile-soluble azo functional monomer with a pyridine group (*i.e.*, MAzoPy), which showed obvious molecular imprinting effects towards the template, rather fast template rebinding kinetics, and appreciable

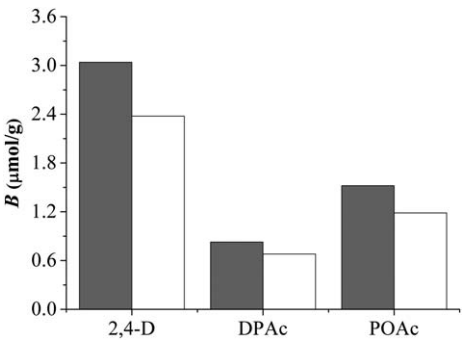


Fig. 7 Selective bindings of the azo-containing MIP (filled columns) and NIP (open columns) microspheres towards 2,4-D, DPAc and POAc in their mixed solution in acetonitrile ($C_{2,4\text{-D, DPAc or POAc}}$ = 0.05 mM) at 25 °C in the dark.

selectivity over structurally related compounds. In the following sections, a series of experiments were further carried out to check whether the obtained azo-containing MIP microspheres could show photoresponsive template binding properties.

The equilibrium binding experiments were performed by incubating a 2,4-D solution in acetonitrile with different amounts of polymers at ambient temperature for 6 h under the irradiation of UV light for the above purpose. As can be seen clearly from Fig. 5, the equilibrium binding capacities of the azo-containing MIP microspheres decreased upon exposure to 365 nm UV light in a wide range of polymer concentrations, suggesting that the template binding properties of the azo-containing MIP microspheres were indeed photoresponsive. It is worth mentioning here that in contrast with the azo-containing MIP microspheres, a certain increase in the equilibrium binding capacities was observed for the azo-containing NIP microspheres under the irradiation of UV light, in particular in the case of low polymer concentrations. This phenomenon might be stemmed from the dipole difference between the *trans*- and *cis*-azo isomers (4.4 Debye) and the slight polarity increase for the resulting *cis*-azo isomer under UV light irradiation.[15] This UV light-induced polarity increase for the azo groups on the surfaces of the NIP microspheres might promote an increase in the interactions between NIP particles and template molecules, thus leading to their increased nonspecific template binding capacities. Moreover, this effect should be amplified in the dilute solutions of NIP microspheres because UV light could penetrate more deeply into the dilute solutions and thus showed more influence on the binding properties of the NIP particles. Note that a similar increase in the nonspecific template bindings should also be present for the azo-containing MIP microspheres under the irradiation of UV light, which was, however, covered up by the more significant decrease of the template bindings due to the photoresponsive binding properties of the MIP microspheres. Therefore, to illustrate the influence of UV light irradiation on the binding properties of the azo-containing MIP microspheres more accurately, the specific bindings of the MIP microspheres under UV light irradiation were derived by subtracting the template bindings of the NIP microspheres from those of the MIP microspheres, and they were compared with those obtained in the dark (Fig. S2 in the supporting information†). The results clearly showed that the specific template bindings of the azo-containing MIP microspheres decreased greatly upon UV light irradiation. For instance, in a dilute solution of 2,4-D in acetonitrile, 16 mg of MIP microspheres showed a specific template binding of 17% (relative to the initial 2,4-D concentration) in the dark, while it decreased to 4% upon UV light irradiation. This might be ascribed to the *trans* to *cis* photoisomerization of the azo chromophores within the binding sites of the MIP microspheres, which could lead to the alteration of the spatial arrangement of their binding functionalities and thus the affinity change of the binding sites, as schematically illustrated in Scheme 1. In this context, it is worth mentioning that the use of *cis*-MAzoPy as the functional monomer in the above molecular imprinting system (*i.e.*, to perform the self-assembly of MAzoPy and the template as well as the precipitation polymerization under the irradiation of UV light) should also lead to azo-containing MIP microspheres with photoresponsive template binding properties.

The Scatchard analysis was also carried out by incubating a certain amount of azo-containing MIP microspheres with a series of template solutions with different concentrations under the irradiation of UV light. In an analogue with that determined in the dark (Fig. 6a), the Scatchard plot of the azo-containing MIP microspheres obtained under the irradiation of UV light could also be fitted into two straight lines (Fig. 6b), from which the K_a and N_{max} values of the high-affinity sites for the MIP microspheres were evaluated to be 1.6×10^4 M^{-1} and 10.9 μmol g^{-1}, respectively, while those of the low-affinity sites for the MIP microspheres were evaluated to be 0.17×10^4 M^{-1} and 35.9 μmol g^{-1}, respectively (Table 1). It can be seen clearly that the K_a value of the high-affinity sites for the azo-containing MIP microspheres obtained under the irradiation of UV light was obviously lower than that determined in the dark ($K_a = 2.3 \times 10^4$ M^{-1}), while their N_{max} values were rather similar (Table 1). These results strongly demonstrated that the affinity of the binding sites in the azo-containing MIP microspheres towards the template indeed decreased under the irradiation of UV light, which agreed well with their decreased specific template bindings under UV light irradiation. In addition, an increase in the N_{max} value of the low-affinity sites was also observed for the azo-containing MIP microspheres under the irradiation of UV light in comparison with that determined in the dark (Table 1). This, together with the almost similar K_a values of the low-affinity sites for the azo-containing MIP microspheres determined both in the dark and under UV light irradiation, revealed that the nonspecific template bindings of the MIP microspheres increased under UV light irradiation, which corresponded well with the increase in the nonspecific template binding capacities of the NIP microspheres under UV light irradiation (Fig. 5).

The photoresponsive template binding properties of the azo-containing MIP microspheres were further confirmed by their photoregulated release and uptake of the template molecule. Fig. 8a shows the change of the binding capacities of azo-containing MIP/NIP microspheres towards 2,4-D and its analogues (DPAc and POAc) under repetitive photoswitching conditions. The experiments were performed by firstly incubating a series of samples (which were composed of 5 mg of azo-containing MIP/NIP microspheres and 0.5 mL of a solution containing 2,4-D, DPAc, and POAc in acetonitrile ($C_{2,4\text{-D, DPAc or POAc}} = 0.05$ mM)) at 25 °C in the dark for 6 h until the equilibrium analyte bindings were reached. As expected, the azo-containing MIP microspheres showed significantly higher binding capacities towards 2,4-D than towards DPAc and POAc, revealing again the high selectivity of the MIP microspheres towards the template. The UV light irradiation on the above mixed solutions with equilibrium analyte bindings led to the obvious release of the template from the azo-containing MIP microspheres into the solutions, and the template bindings of the MIP microspheres decreased from 54 to 48% after 3 h of UV light irradiation. The subsequent thermal back-isomerization of the azo-containing MIP microspheres in the dark caused an increase in the template binding from 48 to 52% (our experimental results showed that the visible light-induced back-isomerization of the azo-containing MIP microspheres could also be used to cause the template uptake from the solutions). Repeating the photoswitching cycles resulted in the release and uptake of 2,4-D in quantities very similar to those of the previous cycles, which clearly demonstrated the

reversibility of the binding site configuration and substrate affinity in the course of photoswitching of azo chromophores. Note that although the azo-containing MIP microspheres were also able to bring about some degree of photoregulated release and uptake for the structural analogues of the template (*i.e.*, DPAc and POAc), their extents were significantly lower than that of 2,4-D under the similar experimental conditions. This also indicated that the binding sites in the azo-containing MIP microspheres possessed a specific affinity for the template 2,4-D. It is worth pointing out here that a slight photoregulated release and uptake of the analytes were also observed for the azo-containing NIP microspheres under repetitive photoswitching conditions, but they were in the opposite direction in comparison with the MIP microspheres. For example, the bindings of the azo-containing NIP microspheres towards 2,4-D increased from 41 to 43% upon UV light irradiation (just like that observed in Fig. 5), while it dropped back to 41% upon switching off the UV light. This could also be attributed to the polarity changes of the azo moieties on the surfaces of NIP microspheres under the photoswitching conditions. To exclude the influence of this photoinduced nonspecific binding changes, the dependence of the specific analyte bindings of the azo-containing MIP microspheres on photoswitching conditions were also presented (Fig. 8b), which demonstrated the photoregulated release and uptake of the template by the azo-containing MIP microspheres more clearly. Based on the above results, it can be concluded that the azo-containing MIP microspheres indeed show obvious photoresponsive template binding properties and the affinity of the binding sites in the MIP microspheres towards the template can be easily tuned by the simple photoswitching. To our knowledge, this is the first demonstration of azo-containing MIP microspheres with photoregulated release and uptake abilities towards the template molecule.

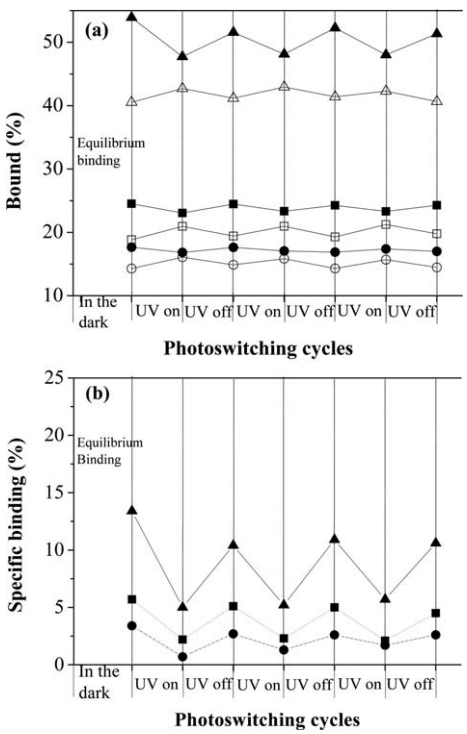


Fig. 8 Photoregulated release and uptake of 2,4-D (triangle) and its analogues [POAc (square), DPAc (circle)] by the azo-containing MIP (filled symbols) and NIP (open symbols) microspheres under photoswitching conditions: (a) change of the bound analyte amounts (percentage values) for both the MIP and NIP microspheres; (b) change of the specific bindings for the MIP microspheres. The mass of the MIP/NIP microspheres used was 5.0 mg, the initial concentration of all the analytes in the solution was 0.05 mM (0.5 mL), and the duration time for the UV-on and UV-off switching period was 3 and 18 h, respectively.

Conclusions

This paper demonstrates for the first time that azo-containing MIP microspheres with photoresponsive template binding properties can be easily prepared *via* precipitation polymerization by using an acetonitrile-soluble azo functional monomer with a pyridine group. The resulting azo-containing MIP microspheres had a number-average diameter of 1.33 μm and a polydispersity index of 1.15, and they showed obvious molecular imprinting effects towards the template, fast template rebinding kinetics, and an appreciable selectivity over structurally related compounds. Furthermore, they have also proven to exhibit obvious photoresponsive binding properties towards the template molecule. Considering the great versatility of precipitation polymerization in the preparation of uniform spherical MIP particles with different diameters (ranging from 100 nm to several micrometers) and the general applicability of MAzoPy in the preparation of MIPs for a wide range of templates (those with various functional groups such as phenol, hydroxyl, or carboxylic acid), we believe that the findings we report here represent a facile, general, and efficient way for fabricating advanced photoresponsive spherical azo-containing MIP beads with desired particle sizes. Furthermore, we also foresee that such spherical azo-containing MIP particles should be of tremendous potential in many applications such as smart separation, extraction, and assays, intelligent chemical carriers, and efficient drug delivery systems.

Acknowledgements

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