Photoresponsive Molecularly Imprinted Hydrogels for the Photoregulated Release and Uptake of Pharmaceuticals in the Aqueous Media

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Received July 21, 2007. Revised Manuscript Received November 8, 2007

A water-soluble azobenzene-containing functional monomer 4-[(4-methacryloyloxy)phenylazo] benzenesulfonic acid (**MAPASA**) was developed for the fabrication of a photoresponsive molecularly imprinted hydrogel material that can function in the biocompatible aqueous media. Cross-linking the functional monomer with various bisacrylamide and bismethacrylamide cross-linkers produced polyacrylamide hydrogels with enough optical transparency in the aqueous media for spectroscopic characterization and measurements. Paracetamol, (*N*-(4-hydroxyphenyl)acetamide), a common analgesic and antipyretic drug, was adopted as the molecular template for the imprinting. Reorientation of the hydrated sulfonated azobenzene chromophores in the course of their trans−cis photoisomerization was found to be sterically demanding. When incorporated into the relatively confined and rigid hydrogel environment generated by the most commonly adopted polyacrylamide cross-linker, *N,N′*-methylenebisacrylamide (**1-C**), these chromophores were not able to undergo photoisomerization. Lengthening the spacers separating the polymerizable acrylamide/methacrylamide functionalities at both ends of the cross-linkers from ethylene (**2-C**) to octylene (**8-C**) enhanced the flexibility of the resultant hydrogel matrices and resumed the photoisomerization properties of the chromophores. The rate of photoisomerization gradually increased with spacer length. On the other hand, substrate binding strength of the imprinted receptors dropped with the increasing flexibility of the hydrogels. Balancing these factors, the cross-linker *N,N′*-hexylenebismethacrylamide (**6-C**) was subsequently selected as the optimal cross-linker for the fabrication of the photoresponsive imprinted hydrogel. Scatchard analysis revealed the specific and nonspecific binding strength of the resultant imprinted hydrogel to be 1.96×10^5 and 747.0 M^{-1}, respectively. The density of the imprinted receptors in the hydrogel was 0.47 μmol g^{-1}. The affinity of the hydrogel for paracetamol can be photoregulated. Upon irradiation at 353 nm, 83.6% of receptor-bound paracetamol was released from the imprinted hydrogel. Subsequent irradiation at 440 nm caused 94.1% of the released paracetamol to be rebound by the hydrogel again. Such a photoregulated release and uptake process is repeatable. Results of our work demonstrated the potential of stimuli-responsive molecularly imprinted materials as biocompatible smart chemicals and drugs transfer systems.

Introduction

Smart polymeric materials that are able to respond to specific environmental changes are the core of the emerging stimuli-responsive technologies in the biomedical, chemosensing, optoelectronics, transducers, and actuators fields.[1] One of the most promising areas of their applications is the controlled delivery of drugs and bioactive compounds in complex biological systems.[2] To achieve this goal, the "smart" materials involved have to be biocompatible and functional in the aqueous media. Hydrogels formed from cross-linked hydrophilic organic components are ideal polymer matrices for this purpose.[3] There are already numerous literature examples of hydrogels with characteristic discontinuous volume phase-transition behaviors that are able to release their trapped substrates upon phase transition induced by specific changes in the external environment.[4]

Recently, a new approach that makes use of the concept of molecular imprinting to manifest responsive behaviors in

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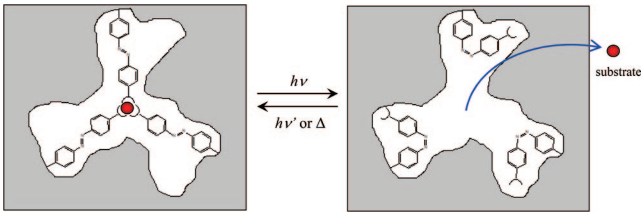
10.1021/cm7019526 CCC: $40.75

Scheme 1. Reversible Photoregulated Substrate Release and Uptake Process with Molecularly Imprinted Photoresponsive Materials



polymeric materials has received considerable attention.[5] Molecularly imprinted materials fabricated from tailor-made conformation-switchable functional monomers are found to be able to alter their substrate binding affinity upon specific external stimulus. A controlled release of bound substrates results when a specific external induction factor switches the imprinted binding sites to a configuration that is not favorable for substrate binding. The involvement of stimuli-responsive substrate binding sites in this kind of imprinted smart materials implies that the reversal of the release process can also be achieved when those transformed binding sites are switched back to their original configuration by a complementary stimulus. This reversible controlled release and uptake mechanism is fundamentally different from the responsive delivery process of stimuli-induced phase transition materials, where the substrate release process is generally irreversible, even after the phase transition is reversed when the specific induction factor is removed. In essence, molecularly imprinted responsive materials are able to provide additional degrees of control over the transfer of targeted substrates. One of the ways to demonstrate such a reversible controlled transfer process is via the use of photoresponsive molecularly imprinted materials.[6] With the incorporation of reversibly photoswitchable chromophores, such as azobenzenes, into the imprinted binding sites, photoregulated release and uptake of substrates can be obtained by photoexcitation (Scheme 1). Although there are already numerous linear polymers and hydrogels that can respond to photoexcitation in the aqueous media,[7] example of aqueous functioning photoresponsive molecularly imprinted hydrogel is scarce. In this work, we report the development of a water-soluble azobenzene-containing functional monomer, 4-[(4-methacryloyloxy)phenylazo]benzenesulfonic acid (MAPASA), and its incorporation into a polyacrylamide hydrogel matrix to produce a photoresponsive molecularly imprinted hydrogel for the photoregulated release and uptake of paracetamol (*N*-(4-hydroxyphenyl)acetamide), a common analgesic and antipyretic drug, in the aqueous media. To the best of our knowledge, this is the first demonstration of photoresponsive molecularly imprinted hydrogel material to regulate the transfer of chemical/drug in the aqueous media.

Experimental Section

Materials and Apparatus. Ammonium persulfate (99.0%) and sulfanilic acid (99.0%) were purchased from BDH. Phenol and sodium nitrite were purchased from Riedel-de Haën. Paracetamol, phenacetin, and antifebrin were kindly donated by Hong Kong Pharmical Company. Triethyamine was purchased from Aldrich. *N,N'*-Methylenebisacrylamide and 1,6-hexanediamine were purchased from Sigma. 1,2-Diaminoethane, 1,3-diaminopropane, and 1,4-diaminobutane were purchased from Merck. Methacrylic chloride was prepared from methacrylic acid (Aldrich) and thionyl chloride (Aldrich). 1,8-Diaminoctane, 1,12-diaminododecade, and HEPES (99.0%) were purchased from International Laboratory. All chemicals were used without further purification. All solvents used were of analytical reagent grade and obtained from Laboratory-Scan Analytical Sciences and BDH. Cross-linkers were prepared by a modified literature method from 1,2-diaminoethane, 1,3-diaminopropane, 1,4-diaminobutane, 1,6-hexanediamine, 1,8-diaminoctane, 1,12-diaminododecade, and methacrylic chloride, respectively.[8] ^{1}H NMR and ^{13}CNMR spectra were measured by a Varian YH300 300 MHz superconducting magnet high-field NMR spectrometer using tetramethylsilane (TMS) as internal reference. Infrared spectra in the range 500–4000 cm^{-1} were recorded on a Perkin-Elmer Model FTIR-1600 spectrometer. UV–vis spectra were measured by a Hewlett-Packard 8425A ultraviolet–visible diode-array spectrophotometer. Steady-state photolysis experiments at specific wavelengths were carried out with a Horiba Fluoromax-3 spectrofluorometer with a built-in magnetic stirrer. Mass spectra were measured by a PE SCIEX API365 LC/MS/MS system. HPLC analyses were performed with a Waters 600s controller coupled to a Waters 996 photodiode array detector monitored at 254 nm for paracetamol and 248 nm for phenacetin and antifebrin.

Synthesis of 4-[(4-Hydroxy)phenylazo]benzenesulfonic acid (PABSA). In a 250 mL flask, sulfanilic acid (7.1 g, 40 mmol) and K_2CO_3 (5.6 g, 40.6 mmol) were dissolved in 30 mL of deionized water. After the mixture was completely dissolved, 3.0 g of $NaNO_2$ in 20 mL of deionized water was added. The mixture was cooled to 0 °C over a salt−ice bath with constant stirring and 25 mL of 5 N HCl was added dropwise. The rate of addition of the HCl was adjusted so that the temperature of the suspension was maintained at <3 °C throughout the addition. A white crystal of the diazonium salt was obtained. The resultant slurry was stirred for another 1 h over the salt−ice bath and was added dropwise to a stirring mixture of phenol (3.9 g, 40 mmol) and K_2CO_3(9.0 g, 65 mmol) in 100 mL of deionized water at 0 °C. The rate of addition of the diazonium salt solution was adjusted so that temperature of the resulting mixture was maintained at <3 °C

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throughout the addition. A brownish-orange slurry was obtained. The slurry was stirred over the salt−ice bath for a further 3 h. Completion of the reaction was confirmed by the disappearance of phenol in the slurry monitored by TLC. The slurry was then neutralized by the addition of 2 N HCl. The red product that precipitated out was collected by filtration and washed with deionized water and dried over a freeze-drier. The crude product was recrystallized from aqueous ethanol to produce 9.0 g (yield 80.9%) of purified **PABSA** as red powder. ^{1}H NMR (300 MHz, d_6-DMSO): δ 10.4 (s, 1H), 7.83 (d, 2H), 7.76 (s,4H), 6.96 (d, 2H).

Synthesis of 4-[(4-Methacryloyloxy)phenylazo]benzenesulfonic acid (MAPASA). *N,N*-Dimethylaminopyridine (DMAP) (0.15 g, 1.0 mmol), triethylamine (4.30 g, 42 mmol) and **PABSA** (5.60 g, 20 mmol) were dissolved in 150 mL of THF, resulting in a dark red mixture. The mixture was cooled over an ice–water bath, and methacrylic chloride (4.5 g, 40 mmol) was added dropwise. After the addition, the resultant red solution was stirred at 40 °C for 24 h. After cooling to room temperature, 50 mL of saturated brine solution was added and the precipitate was collected by filtration and washed with 5 × 10 mL of 2 N HCl. The solid obtained was redissolved in 10 mL of DMF. The crude product was precipitated by the addition of saturated brine solution, collected by filtration, and dried over a freeze-drier. It was recrystallized from boiling glacial acetic acid to give 4.4 g of purified **MAPASA** as red crystals (yield 63.7%). MS-Q1:(*m/z*) 345.1. ^{1}H NMR (300 MHz, d_6-DMSO): δ 7.98 (d, 2H), 7.86 (dd, 4H), 7.42 (d, 2H), 6.32 (s, 1H), 5.94 (s, 1H), 2.02 (s, 3H). ^{13}C NMR (300 MHz, d6-DMSO), δ (ppm): 165.7, 153.6, 152.2, 151.6, 150.3, 135.8, 128.9,127.4, 124.6, 123.6, 122.8, 18.5. FTIR (KBr) (cm^{-1}): 3395.7 (ν_{OH} sulfonic acid), 2939 (ν_{CH} aliphatic), 1737.5 ($\nu_{C=O}$ methacrylic ester), 1644.4 ($\nu_{C=C}$ methacrylic), 1588 ($\nu_{C=C}$ aromatic).

Fabrication of Paracetamol-Imprinted Hydrogels by Precipitation Polymerization Using Various Bisacrylamide/Bismethacrylamide Cross-Linkers. **MAPASA**(0.345 g, 1.0 mmol) and paracetamol (31.0 mg, 0.2 mmol) were dissolved in 8.0 mL of DMF in a conical flask. Cross-linker (5.0 mmol) in 30 mL of deionized water was then added and the mixture was stirred in the dark at room temperature for 12 h. A 25% $(NH_4)_2S_2O_8$ solution (0.5 mL) was then added and the resultant mixture was degassed by bubbling with nitrogen for at least 20 min and sealed under a nitrogen atmosphere by a rubber cap. The mixture was then placed in a 60 °C oil bath for 24 h. The hydrogel obtained was filtered and washed with deionized water and methanol and dried over a freeze-drier. The resultant bulk hydrogel was crushed, milled, and wet sieved in methanol through a 48 μm sieve and was collected by centrifugation. Paracetamol in the hydrogel was removed by Soxhlet extraction with 200 mL of a methanol/acetic acid mixture (9:1 v/v) for 24 h followed by 200 mL of methanol for 24 h in dark. The resultant imprinted hydrogel material was dried to constant weight over a freeze-drier. Control hydrogel material was prepared and treated in exactly the same way as the paracetamol-imprinted hydrogel except that paracetamol was not used in the polymerization procedure. Both hydrogel materials were stored at room temperature in the dark. FTIR (KBr, cm^{-1}): 3384.5 (ν_{OH} sulfonic acid), 2939.3 (ν_{CH} aliphatic), 1741.8 ($\nu_{C=O}$ methacrylic ester), 1530 ($\nu_{C=C}$ aromatic).

Spectroscopic Characterization and Photoisomerization Studies. Spectroscopic characterization of the **MAPASA** monomer and the subsequent imprinted and control hydrogel materials were performed in an aqueous HEPES buffer of pH 7.16. Air-tight screw-capped quartz cells of 1.0 cm optical path length were used in all experiments. Suspension of the imprinted and control hydrogel materials were maintained with the help of a magnetic stirrer. Unless stated otherwise, all photoisomerization studies were performed with 1.5 mg of imprinted or control hydrogel material in 3.0 mL of aqueous HEPES buffer. Kinetics of the photoisomerization was analyzed by kinetic eq 1

$$\ln \frac{(A_0 - A_{ps})}{(A_t - A_{ps})} = kt \qquad (1)$$

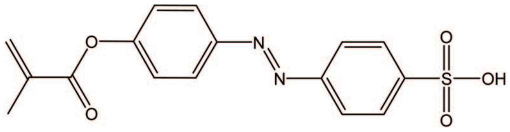
where A_0, A_t, and A_{ps} are the absorbance of the azobenzene chromophores at their correspondence wavelengths at time 0, *t*, and at the photostationary stage, respectively; *k* is the rate constant of the photoisomerization process.

Rebinding Assays. Binding properties of the imprinted and control hydrogel materials were studied by batch-type rebinding assays in aqueous HEPES buffers at pH 7.16 in the dark. The amount of analyte left after rebinding was determined by HPLC. Unless otherwise stated, all rebinding assays were performed with 10 mg of hydrogel material in 1.0 mL of aqueous HEPES buffer at room temperature. In a typical rebinding experiment, a known amount of paracetamol was spiked into aqueous suspensions of hydrogel material in 2.0 mL screw-cap vials. These suspensions were then sealed and agitated for 48 h. Each of the suspensions was then centrifuged at 10 000 rpm for 20 min, and 5.0 μL of the clear supernatant was analyzed by isocratic HPLC using a platinum EPS C18 100A, 3 μ column with a mobile phase of methanol/water/acetic acid at a volume ratio of 10:10:0.1 and a flowrate of 1.0 mL min^{-1}. Paracetamol was identified by a UV detector monitored at 254 nm. The HPLC was calibrated using standard solutions of paracetamol of concentration ranging from 0 to 100 μM.

Photoregulated Uptake and Release Studies. All steady-state photolysis experiments for the photoregulated release and uptake studies were performed with a Horiba Fluoromax-3 spectrofluorometer. In all experiments, 5.0 mg of hydrogel material in 3.0 mL of aqueous HEPES buffer at pH 7.16 were used. The initial concentrations of paracetamol, phenacetin, and antifebrin used were 3.0 μM. In a typical run, the hydrogel material was transferred into an aqueous HEPES buffer of the substrate in a quartz cell of 1.0 cm optical path length fitted with a magnetic stir bar. The quartz cell was then screw-capped to ensure air-tightness and was placed in the sample compartment of the spectrofluorometer. The suspension was stirred in the dark for 24 h. For the photoregulated release of paracetamol, phenacetin, and antifebrin, the mixture was stirred and irradiated at 353 nm by the excitation beam from the spectrofluorometer for 120 min. The stirring was then stopped and the mixture was allowed to settle for 2 min in darkness before 5.0 μL of the clear supernatant solution was taken out by a clean HPLC syringe. The level of substrate in the solution was monitored by HPLC. Stirring and irradiation was resumed after the sampling of supernatant. Each round of irradiation was 120 min, and substrate concentration was measured at the end of each irradiation round. For the photoregulated uptake of substrate, irradiation at 440 nm for 90 min was adopted. Besides this, all procedures were similar to those in the release experiments.

Results and Discussion

In an attempt to fabricate photoresponsive molecularly imprinted hydrogel materials, we have tried cross-linking various substituted azobenzenes possessing polymerizable methacrylate end-group with bisacrylamide/bismethacrylamide cross-linkers. Azobenzenes with carboxylate, amino, and pyridyl substituents were found to produce opaque hydrogels that eluded spectroscopic characterization in water. Eventually, a water-soluble azobenzene functional monomer, 4-[(4-methacryloyloxy)phenylazo] benzenesulfonic acid (**MAPASA**), was synthesized and found to produce polyacryla-



4-[(4-methacryloyloxy)phenylazo]benzenesulfonic acid (**MAPASA**)

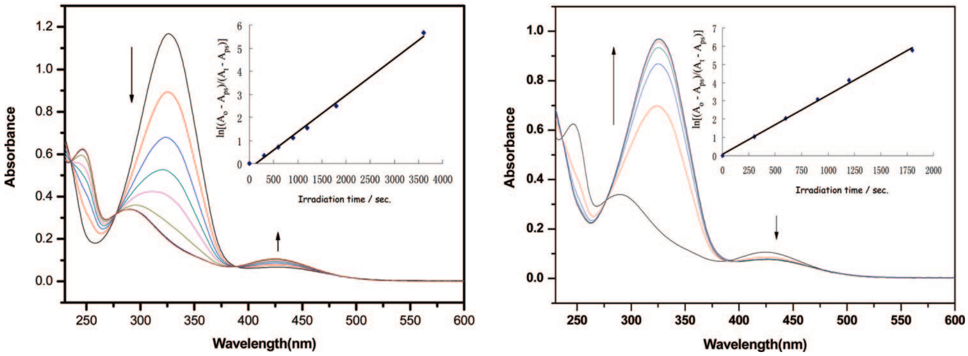


Figure 1. UV–vis spectral changes upon photoisomerization of **MAPASA** (50 μM) in aqueous HEPES buffer at pH 7.16: (a) trans → cis photoisomerization upon irradiated at 353 nm; (b) cis → trans photoisomerization upon subsequent irradiation at 440 nm. Kinetics of the photoisomerization processes are shown in the insets. Rate constants for the trans → cis and cis → trans photoisomerization are measured to be 1.59×10^{-3} s^{-1} and 3.24×10^{-3} s^{-1}, respectively.

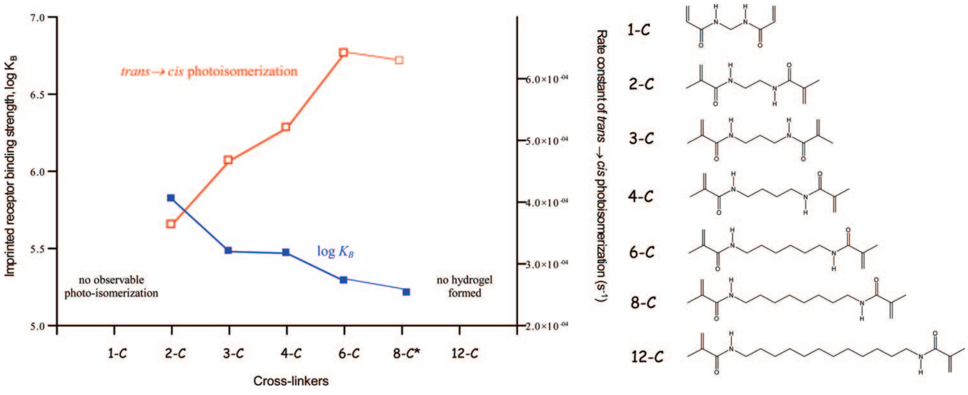


Figure 2. Effects of cross-linkers on the substrate binding properties of the imprinted photoresponsive polyacrylamide hydrogels. All imprinted hydrogels were fabricated in 3.75:1 v/v H_2O/DMF (except **8-C**) at a template:monomer:cross-linker ratio of 1:5:25. Binding strengths were determined by Scatchard analysis of rebinding assays performed in aqueous HEPES buffers of pH 7.16.

mide hydrogels that possessed adequate optical transparency for spectroscopic characterization and measurements in the aqueous media. Figure 1 shows the spectral changes of **MAPASA** upon photolysis at 353 and 440 nm in an aqueous buffer at neutral pH. Photoisomerization is clearly observed and the isomerization rate constants are comparable to other azobenzene chromophores in literature.[9] However, this photoisomerization behavior was obstructed when **MAPASA** was incorporated into the cross-linked polyacrylamide hydrogel matrices. With the most commonly used bisacrylamide cross-linker, *N*,*N*′-methylenebisacrylamide (**1-C**) (Figure 2), the **MAPASA**-containing polyacrylamide hydrogel formed was not able to show any spectral response resembling azobenzene photoisomerization. From the spectroscopic characteristics of the hydrogel, most of the azobenzene chromophores in the material were locked in the trans configuration and no trans → cis isomerization was observed even after prolonged photolysis at 353 nm. This was probably due to the confined and rigid environment created by the relatively small cross-linker moieties around the hydrated sulfonated azobenzene chromophores that prohibited their reorientation in the course of isomerization. This hypothesis was confirmed by the subsequent fabrication of suppler **MAPASA**-containing polyacrylamide hydrogels using specially synthesized bismethacrylamide cross-linkers of increased spacer length, from ethylene (**2-C**) to dodecylene (**12-C**), between the two methacrylamide end-groups (Figure 2). Starting from **2-C** to **8-C**, the **MAPASA** azobenzene chromophores in the resultant polyacrylamide hydrogels were able to undergo photoisomerization. In the case of *N*,*N*′-dodecylenebismethacrylamide (**12-C**), hydrogel was not formed because of the poor solubility of the cross-linker in the aqueous–organic porogen.

To further understand how the spacer chain length of the cross-linker in the hydrogel matrix affects the substrate binding properties of the imprinted receptor sites, we prepared a series of polyacrylamide hydrogels noncovalently imprinted with paracetamol (*N*-(4-hydroxyphenyl)acetamide) using various

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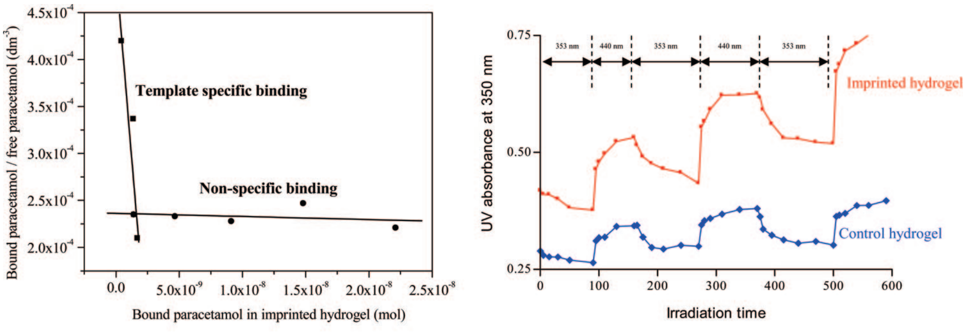
Table 1. Photorespond and Substrate Binding Properties of the Polyacrylamide Hydrogels Fabricated from Various Bisacrylamide/Bismethacrylamide Cross-Linkers

cross-linker for hydrogel fabrication	trans → cis photoisomerization rate of azobenzene chromophores in the resultant hydrogel $k_{trans\rightarrow cis}$ (s^{-1})	substrate binding strength of the imprinted binding sites in hydrogel log K_B	imprinted binding site density in the hydrogel B_{MAX} (μmol g^{-1} of hydrogel)
1-C	no photoisomerization observed		N.D.[a]
2-C	3.63×10^{-4}	5.82	0.20
3-C	4.67×10^{-4}	5.49	0.31
4-C	5.20×10^{-4}	5.47	0.24
6-C	6.41×10^{-4}	5.29	0.47
8-C	6.15×10^{-4}	5.12	1.73
12-C	no hydrogel formed		

[a] Not determined.



(a) (b)

Figure 3. Substrate binding properties and photoisomerization reversibility of the paracetamol imprinted **MAPASA**-containing polyacrylamide hydrogel (fabricated using cross-linker **6-C**) in aqueous HEPES buffer at pH 7.16: (a) Scatchard analysis of the rebinding assay revealed the specific and nonspecific receptor site binding constants of 1.96×10^5 and 747.0 M^{-1}, respectively; density of imprinted receptor sites in the hydrogel was 0.47 μmol g^{-1}-hydrogel; (b) spectral responses corresponding to the photoisomerization of azobenzene chromophore in the hydrogel.

bismethacrylamide cross-linkers. A template:functional monomer: cross-linker ratio of 1:5:25 was maintained throughout all the hydrogels. For most of the hydrogels, a porogen composition of 3.75:1 (v/v) H_2O/DMF was adopted. However, because of the poor water solubility of *N*,*N*′-octylenebismethacrylamide (**8-C**), a porogen composition of 1:1 (v/v) H_2O/DMF has to be used instead for the fabrication of the corresponding hydrogel. For *N*,*N*′-dodecylenebismethacrylamide (**12-C**), it was not soluble in aqueous DMF of any composition. Nevertheless, under the polymerization conditions adopted in this study, the imprinting process is likely to be achieved by hydrogen-bond interactions between the benzenesulfonate functionality of **MAPASA** and the phenolic and arylamide moieties on paracetamol. The quality of the hydrogels raised from different cross-linkers was comparable, except for that fabricated from **8-C**, which exhibited significantly lower optical transparency in aqueous buffer. Binding properties of the resultant imprinted hydrogels for paracetamol in aqueous media were analyzed by batch-type rebinding assays and Scatchard analysis and are summarized in Figure 2 and Table 1. The rate of trans → cis photoisomerization of the **MAPASA** azobenzene chromophores, $k_{trans\rightarrow cis}$, in all the imprinted hydrogels were 2.5- to 4.4-fold slower than that of the **MAPASA** monomer in the aqueous buffer solution. This is attributable to the comparatively rigid cross-linked hydrogel matrices, which have inevitably restricted the sterically demanding conformational switching of the hydrated azobenzene chromophores. Yet, among the various hydrogels, the rate of photoisomerization clearly increased with increasing spacer length of the bismethacrylamides from **2-C** onward and more or less leveled off beyond **6-C**. This indicates that a more flexible hydrogel matrix tends to impose less hindrance to the photoisomerization process. On the other hand, a more flexible polymer matrix seems to lower the affinity of the imprinted receptor for its substrate. From **2-C** to **8-C**, binding strength (K_B) of the receptor sites in the hydrogels for paracetamol has decreased 5-folds from log K_B = 5.82 to log K_B = 5.12. The density of the imprinted receptors in the hydrogels ranged from 0.20 to 1.73 μmol g^{-1} of hydrogel with a general increasing trend from shorter-spacer-length to longer-spacer-length cross-linkers. Although the highest imprinted receptor density was achieved by using *N*,*N*′-octylenebismethacrylamide (**8-C**) as cross-linker, because of the generally poor spectroscopic properties of the corresponding hydrogel, this cross-linker was not adopted in the subsequent preparation of imprinted hydrogel for photoregulated substrate transfer studies. Instead, *N*,*N*′-hexylenebismethacrylamide (**6-C**) was selected on the basis of the overall satisfactory performance of the hydrogels.

Figure 3a shows the substrate binding properties of the paracetamol imprinted **MAPASA**-containing polyacrylamide hydrogel fabricated from the cross-linker **6-C**. Figure 3b demonstrates that the azobenzene chromophores in both the imprinted and control hydrogels are able to undergo reversible photoisomerization in aqueous media. The extent of conformational switching of the chromophores is greater in the imprinted material than the control and is likely to be the result of the presence of imprinted binding cavities, which allows more room for the reorientation of the hydrated chromophores during their isomerization. Such photoinduced conformational changes of the azobenzene functionality in the imprinted receptor sites are able to greatly alter their binding affinity for paracetamol.

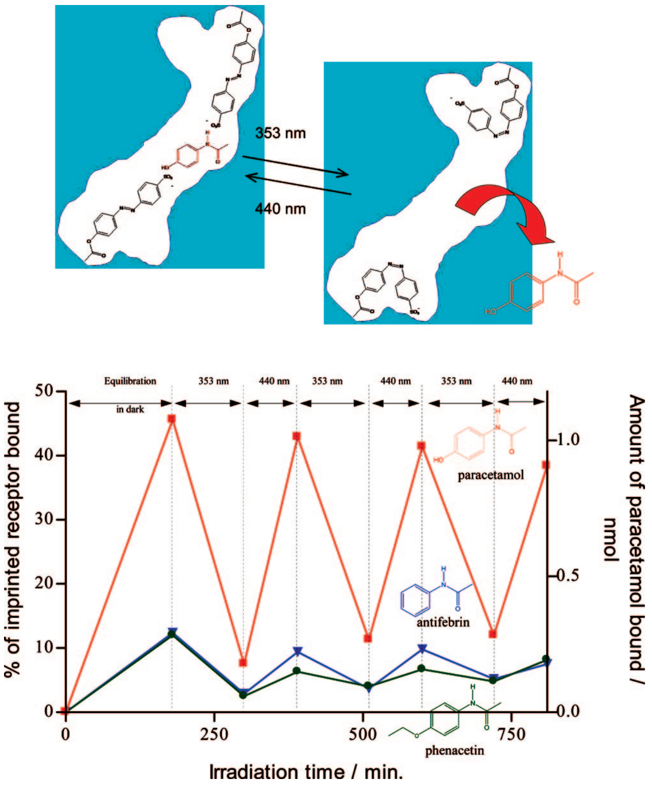


Figure 4. Photoregulated release and uptake of substrates by the paracetamol imprinted MAPASA-containing polyacrylamide hydrogel in aqueous HEPES buffer at pH 7.16. Concentration of substrates (paracetamol, phenacetin, and antifebrin) used in all experiments was 3.0 μM (in 3.0 mL solutions). Nonspecific binding of substrate (revealed by parallel experiments on control hydrogels) has already been subtracted from the binding data.

Figure 4 shows the change in the amount of imprinted receptors in the imprinted hydrogel occupied by paracetamol and its two structural analogs, phenacetin and antifebrin, in aqueous HEPES buffer of pH 7.16, under alternating irradiation at 353 and 440 nm. In the presence of excess paracetamol in the aqueous media, 45.6% of the imprinted receptors in the hydrogel were filled by the substrate. Irradiation at 353 nm caused the release of paracetamol from the hydrogel into the aqueous buffer. After 120 min of irradiation, a total of 83.6% of the receptor-bound substrate was released. The fraction of bound receptors in the hydrogel was reduced to 7.5%. Subsequent irradiation at 440 nm caused the fraction of occupied receptor sites to increase back to 42.9% in 90 min. This is equivalent to the uptake of 94.1% of the paracetamol that had been previously released into the buffer solution. This photoregulated release and uptake of paracetamol is repeatable. However, the amount of substrate involved is progressively reduced. Up to the third cycle, the imprinted hydrogel was able to retake only 70.9% of paracetamol that it had previously released. This phenomenon is probably caused by the gradual deformation of the imprinted receptors in the hydrogel upon repetitive photoswitching. The control hydrogel showed no photoregulated substrate release and uptake ability whatsoever, except the binding of a small amount of paracetamol bound at the initial stage of the experiment due to nonspecific binding. For the structural analogs of paracetamol, although the imprinted hydrogel was able to bring about some degree of photoregulated release and uptake, their extents were significantly smaller than that of paracetamol under similar experimental conditions. This demonstrates the substrate-specificity of the imprinted receptor sites in the hydrogel for paracetamol.

Conclusion

We have shown that efficient photoresponsive smart materials for repetitive controlled release and uptake of substrates can be easily achieved by molecular imprinting. In aqueous media, reorientation of the stimuli-responsive units within the imprinted receptor sites in the hydrogel matrix is sensitive to the nature of the cross-linker used. Cross-linkers with their polymerizable end-groups separated by longer spacers produce hydrogels that are less restrictive to the stimuli-induced conformation changes of the responsive elements within the materials. In the present case, the **MAPASA**-containing polyacrylamide hydrogel fabricated from the cross-linker *N*,*N*′-hexylenebismethacrylamide (**6-C**) was found to afford good optical transparency in the aqueous media, reasonable substrate binding affinity, and the fastest photorespond rate. Photoregulated release and uptake of paracetamol by this imprinted hydrogel in aqueous media has been demonstrated.

Acknowledgment. The work described in this paper was funded by the Research Grants Council of Hong Kong SAR, China (CityU 101407). K.-L.W. acknowledges the Research Enhancement Scheme of the City University of Hong Kong.

Supporting Information Available: Figures S1−S15 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

CM7019526